Searching for susceptibility genes for psychosis in late-onset Alzheimer's disease

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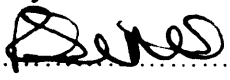
Signed  (candidate) Date 29.09.09

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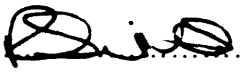
Signed  (candidate) Date 29.09.09

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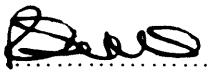
Signed  (candidate) Date 29.09.09

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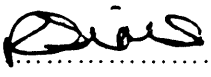
Signed  (candidate) Date 29.09.09

Contents

List of Figures

List of Tables

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1. General Introduction: Psychosis in Alzheimer's disease

A number of behavioural symptoms are commonly displayed by Alzheimer's disease (AD) sufferers. Behavioural disturbances in AD can include affective symptoms, agitation, aggression and psychosis (Burns et al. 1990a; Burns et al. 1990b; Burns et al. 1990c). Alois Alzheimer was the first to document psychosis in the disease bearing his name, during his description of the clinical presentation of a patient upon admission to the Frankfurt asylum in 1906 (Alzheimer 1995; Schneider and Dagerman 2004).

> *"Sometimes she greets the doctor as if he were a visitor...on other occasions she screams that he wants to cut her open...on others yet she fears him as a threat to her honour as a woman...she seems to have auditory hallucinations. Often she screams for many hours in a horrible voice"*

In this classic case of 51 year old Auguste D[eter], who presented with focal cognitive deficits, Alzheimer identifies both symptoms of psychosis, delusions and hallucinations, in addition to activity disturbances and aggression (Reisberg et al. 1987a). Psychosis is a mental state involving hallucinations and/or delusions (van Os et al. 2000). Hallucinations are disturbances of perception that occur without cause in the real world (Behrendt 1998). They can occur in any sensory form (sound, sight, touch, taste and smell). Delusions are irrational yet strongly held personal beliefs held despite contradictory evidence, which result from an inability to separate real from unreal experiences, and are unexplained by the person's cultural background (Manschreck 1996). Psychosis in Alzheimer's disease is common, often resulting in increased severity of functional and cognitive deficits (Jeste et al. 1992; Stern et al. 1994a), increased rate of decline (Neumann et al. 2001; Wilson et al. 2000), earlier institutionalisation (Borson and Raskind 1997; Steele et al. 1990) and increased caregiver distress (Donaldson et al. 1998; Craig et al. 2005). Evidence suggests that psychotic symptoms in AD are heritable and may characterise a distinct and more severe disease subtype. Identification of genetic variation associated with Alzheimer's disease with psychosis (AD+P) will

further our understanding of the disease mechanisms which underlie behavioural features of AD and may have implications for other complex genetic disorders where psychosis is common.

1.1 Alzheimer's disease

Alzheimer's disease is a devastating neurodegenerative disorder, afflicting approximately 30 million individuals worldwide (Sanchez 2009). AD accounts for 60-80% of late-onset dementia cases. In England and Wales there are thought to be approximately 700,000 AD cases (Comas-Herrera et al. 2007), with 180,000 new cases of dementia reported each year (Matthews and Brayne 2005). In addition to the detrimental effects to sufferers, AD also causes severe distress for family members and caregivers, and places a massive burden on the economy (Lowin et al. 2001). The direct cost of AD to the United Kingdom is over 17 billion pounds a year (Comas-Herrera et al. 2007). With the oldest sector of the population expected to increase by 68% in the developed world (United Nations 2008), current projections suggest that by 2020 more than a billion people over the age of 65 will live in Europe (The Harvard School of Public Health 1996). Given AD is an age dependent disease, the escalating growth of the elderly population means that the economic and societal costs of the disease will increase over the coming years (Souetre et al. 1999; Villareal and Morris 1999).

Histopathological examination of brain tissue at post mortem is the only definitive method of diagnosing AD. However, this is problematic, with only 50% to 60% of individuals meeting neuropathological criteria for AD having experienced cognitive decline (Knopman et al. 2003). Probable diagnosis of AD is generally based on physical examination, patient history and detailed cognitive assessment. The fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994) and the National Institute of Neurological and Communication Disorders and Stroke and the Alzheimer's disease and Related Disorders Associations (NINCDS-ADRDA) (McKhann et al. 1984) criteria are generally used in both research and clinical settings to make a diagnosis of Alzheimer's disease. Both require deficits in memory and one other area of cognition, including aphasia, apraxia, agnosia and executive functioning,

further stipulating that these difficulties should cause significant decline in functional abilities and activities of daily living. DSM-IV and NINCDS-ADRDA criteria also stipulate that the illness should be characterised by an insidious onset and gradual decline in cognitive and functional abilities, and that competing causes of dementia are ruled out. The reliability and validity of DSM-IV and NINCDS-ADRDA criteria for AD have been found to be good (O'Conner et al. 1996), with known validity for AD pathology (i.e. positive predictive value of 92–95%) (Becker et al. 1994; Foy et al. 2007; Gearing et al. 1995; Holmes et al. 1999). Neuroimaging may compliment the standard approaches, and serves to identify vascular contributions and other potentially treatable conditions (Kantarci and Jack 2003).

The chief symptoms of Alzheimer's disease are progressive decline of memory and other higher mental functions, such as cognitive decline, nominal and comprehensive dysphasia, dyspraxia, agnosia, impairment of executive functioning and behavioural disturbances (Desai and Grossberg 2005; White and Clare 2002). Although many of these symptoms will not be present at the time of diagnosis, most AD sufferers will experience the majority if not all of these symptoms during the course of their illness (Havard 2005). In the early stages of Alzheimer's disease when the cells in the hippocampus degenerate, short-term memory begins to decline accompanied by deficits in learning and recalling new information. The ability to perform routine tasks also declines. As Alzheimer's disease spreads through the cerebral cortex judgment declines, emotional outbursts may occur and language is impaired, from anomia in the early stages to complete aphasia as the illness progresses. Advancement of the disease leads to increased deficits in executive function (Baudic et al. 2006; Rainville et al. 2002), and behaviour changes such as wandering and agitation. Visuospatial difficulties can often become apparent, manifesting as geographical disorientation or difficulty with copying figures in cognitive testing (Cummings and Cole 2002). Motor disturbances are common in the later stages of disease development, including gait changes, rigidity and seizures (McKhann et al. 1984). The ability to recognize faces and to communicate is completely lost in the final stages of disease. Patients lose bowel and bladder control, and eventually need constant care, frequently being bed bound (Villareal and Morris 1999). Without treatment those with AD usually survive for between 7 and 10 years after the onset of symptoms (Bracco et al. 1994;

Larson et al. 2004), although it can take 20 years or more for the disease to run its course. A notable clinical variation in AD is age at onset (AAO). AD can present at anytime from 30 to 90+ years of age. A distinction is often made between those with disease onset before and after the age of 65, supported by both DSM-IV and NINCDS-ADRDA criteria. Those with a disease onset of less than 65 years are termed as early onset AD (EOAD); this form of the disease is rare, accounting for approximately 5% of AD sufferers. Both early and late onset cases share the clinical and neuropathological features of AD. However, early onset forms of the disease have been found to be characterised by shorter survival, more rapid cognitive deterioration, more severe language disturbances, and more severe AD related neuropathology (Koss et al. 1996; Sevush et al. 1993; Villareal and Morris 1999).The most compelling evidence for a distinction between early and late onset AD comes from genetic findings, discussed in section 1.1.1.

There is currently no cure for AD. Cholinesterase inhibitors and *N*-methyl d-aspartate receptor-targeted therapies are presently the only forms of medication available in the UK. Medication may provide modest benefits to cognition, activities of daily living and behaviour, and can provide temporary stabilisation of the rate of decline (Desai and Grossberg 2005). These treatments do not benefit all AD sufferers and their positive effects are usually temporary (Cummings and Cole 2002), meaning that there is an urgent need for more effective therapeutic interventions.

1.1.1. Genetics of Alzheimer's disease

AD is generally categorised as sporadic or familial (Ashford and Mortimer 2002). The term familial is reserved for cases in which a clear pattern of autosomal dominant inheritance is observed, or for cases carrying known EOAD causing genetic mutations. Familial cases usually present with the disease before 65 years of age. Sporadic cases have a complex polygenic mode of inheritance (Ashford and Mortimer 2002), constitute around 95% of AD cases, and usually present after the age of 65. To date, the most significant discoveries in the genetic aetiology of AD have come from the rare autosomal dominant forms of disease. Mutations in the genes encoding β-Amyloid Precursor Protein (APP) on chromosome 21 (Goate et al. 1991), presenilin 1 (PSEN1) on chromosome 14 (Sherrington et al. 1995) and

presenilin 2 (PSEN2) on chromosome 1 (Levy-Lahad et al. 1995; Rogaev et al. 1995; Sherrington et al. 1995) have been found to cause AD in families with early-onset autosomal dominant forms of the disease. Most of the pathogenic mutations in the APP and presenilin genes are associated with abnormal processing of APP, which leads to the overproduction of toxic amyloid-beta 42 peptide (Aβ42) found in senile plaques (Kamboh 2004). Together, mutations in these genes account for approximately 50% of early onset AD cases, with the main contribution from *PSEN1* (Tandon et al. 2000). These genes show no consistent evidence for involvement in the aetiology of late-onset AD (LOAD). Our understanding of the genetics of this more common form of AD remains much less complete (Lambert et al. 2004).Nu merous population based twin studies have been conducted and generally support heritability estimates for AD from 58-79% (Gatz et al. 2006), whilst family based studies consistently find the presence of one or more affected family members to be a strong risk factor for the disease (Fratiglioni et al. 1993; Jarvik et al. 1996; Martinez et al. 1998; Sleegers et al. 2004; van Duijn et al. 1991). To date, Apolipoprotein E (*APOE*) situated on chromosome 19 is the only gene to show consistent evidence of contributing to risk for both EOAD and LOAD. There are three common alleles of the *APOE* gene ε2, ε3 and ε4, resulting from amino substitutions (Arg and Cys) at positions 112 and 158 of the protein (Lambert et al. 2002). In normal populations the ε3 allele is the most frequent, whilst ε4 occurs slightly more often than the ε2 allele (Hendrie 1998). The ε4 allele of *APOE* displays replicable association with AD in hundreds of diverse case-control samples (Corder et al. 1993; Myers et al. 1996; Reiman et al. 2007), and pedigree cohorts (Saunders et al. 1993). The findings have been remarkably consistent with only a few studies, largely in selective populations, failing to find an association (Ashford et al. 2003; Raber et al. 2004). Carrying an ε4 allele increases the risk for AD in an allele dose-dependent manner and is associated with an earlier age at onset of the disease, with the strongest effect below 70 years (Corder et al. 1993). In Caucasian populations, individuals who carry the ε4 allele are three (heterozygotes) to eight (homozygotes) times more likely to develop AD than individuals who do not harbour the ε4 allele (Corder et al. 1993). Conversely bearing a ε2 allele confers protection against the disease (Farrer et al. 1997). This effect is not simply due to the absence of the ε4 allele (Talbot et al. 2004). In fact, Corder and colleagues (Corder et al. 1994) reported that up to 23% of the risk of

AD in their sample was attributable to the absence of *APOE* ε2. Thus, *APOE* genotype is an important biologic marker for AD susceptibility. However, possession of the ε4 allele is not sufficient to develop the disease. As many as 50% of people who have two copies of ε4 and survive to age 80 years are not cognitively impaired (Lambert et al. 2002). In fact, variation at the *APOE* locus accounts for less than half the genetic variation in liability to LOAD (Harold et al. 2003). Estimates of population risk attributable to *APOE* have ranged from 12% to 57% (Harold et al. 2009; Nalbantoglu et al. 1994; Seshadri et al. 1995; Slooter et al. 1998), with the most recent estimates lying at the lower end of the range (Harold et al. 2009). In addition to the ε2 and ε4 alleles, polymorphisms in the *APOE* promoter region have been implicated with susceptibility to AD, however the results of these associations are often contradictory (Bullido et al. 1998; Lambert et al. 2002; Lambert et al. 1998; Song et al. 1998; Wang et al. 2000). The biological mechanism by which APOE increases the risk of developing AD is not well understood. A number of studies have reported that the APOE ε4 allele is associated with increased senile plaque and neurofibrillary tangle formation (Ghebremedhin et al. 2001), and a reduction in choline acetyltransferase in brains of AD patients (Schmechel et al. 1993). Since APOE modulates clearance and aggregation of Aβ as well as other neuropathological changes, it has been difficult to elucidate the exact pathological mechanism behind the observed Aβ phenotypes in human and animal studies. Prevailing data suggest that the main effect of APOE isoforms on risk for AD is via the effect of APOE on Aβ metabolism, influencing the time of onset of Aβ deposition in both brain parenchyma and vasculature (Kim et al. 2009). Additionally, the ε4 isoform is not very efficient at catalyzing proteolytic breakdown of the Aβ peptide (Jiang et al. 2008), suggesting a mechanism to increase vulnerability to Alzheimer's disease in individuals with that gene variation (Jiang et al. 2008). Other potential mechanisms, such as the differential modulation of neurotoxicity and tau phosphorylation by APOE isoforms as well as its role in synaptic plasticity and neuroinflammation, have not been ruled out. Inconsistent results among studies have made it difficult to define whether the *APOE* ε4 allele represents a gain of toxic function, a loss of neuroprotective function, or both (Kim et al. 2009).

There remains a large percentage of the genetic risk for AD unaccounted for (Daw et al. 2000; Myers and Goate 2001). Over the past 16 years, since the

identification of the *APOE* locus, tremendous effort has been put into identifying these genes. Linkage analyses have generally proved inconclusive with most evidence implicating a region of chromosome 10 (Bertram et al. 2000; Ertekin-Taner et al. 2000; Farrer et al. 2003; Li et al. 2002; Myers et al. 2000).Findings from candidate gene association analyses have generally been negative, inconclusive or contradictory (Bertram and Tanzi 2004; Brookes and Prince 2005; Kamboh 2004). Most association studies have been restricted to genes which are both functional and positional candidates for AD (e.g. functional candidate genes within regions of linkage). However, very few of these associations have positively replicated when analysed in independent samples, increasing the possibility that these are 'false positive' findings (Brookes and Prince 2005). The recent evolution of genome-wide association studies (GWAS) has brought new hope to the field of AD genetics, with the identification and replication of one novel susceptibility locus and significant evidence for two additional loci (Harold et al. 2009; Lambert et al. 2009). The history of GWAS in AD is discussed more thoroughly in chapter 5. Despite these recent advances, a large amount of the genetic variation of AD is yet to be explained. Rare variation and/or alternative genetic elements such as copy number variation may explain a proportion of the missing genetics. However, it is possible that phenotypically distinguishable forms of AD, such as psychosis, could be linked to specific susceptibility loci. Therefore, utilising sample subsets with a narrow range of clinical variation may offer a suitable method to identify susceptibility loci for more homogenous forms of disease (Freimer and Sabatti 2003).

1.1.2. Neuropathology and Neurochemistry of Alzheimer's disease

Two kinds of microscopic damage develop in the AD brain; amyloid/senile plaques and neurofibrillary tangles. Neuron degeneration and synaptic loss are additional neuropathological hallmarks of the disease (Cummings and Cole 2002; Kamboh 2004). Amyloid plaques are abnormal extracellular structures which can be found throughout the AD brain, often particularly concentrated in the temporal lobes (Honig and Chin 2001). They consist predominantly of βeta-amyloid, a 40- to 42-amino-acid peptide derived from cleavage of the β-amyloid precursor protein (APP) (Abraham 2001). The APP protein is present in almost all tissues, and

undergoes three alternative steps of cleavage by α-, β- and γ-secretase enzymes. When cut by α-secretase and then γ-secretase, APP generates a harmless peptide. However, when cut by β-secretase and then γ –secretase, APP generates peptides of 39 to 43 amino acids, of which Aβ42 accounts for about 10%. Aβ42 is neurotoxic and involved in the formation of senile plaques in AD brains (Selkoe 2001).A dditional proteins, aberrant neuronal cell processes (neuritis), reactive astrocytes and microglial cells are also contained within the amyloid plaques. Neurofibrillary tangles are abnormal intracellular structures located in various parts of the brain, occupying the cell body and extending into the dendrites. They are composed of dense arrays of paired helical filaments which are composed of a hyperphosphorylated form of the microtubule-associated protein tau (Honig and Chin 2001). The number of neurofibrillary tangles seen post-mortem has been shown to correlate with the degree of dementia during life (Honig and Chin 2001). However, synaptic loss is currently the best pathologic correlate of cognitive decline, and synaptic dysfunction is evident long before synapses and neurons are lost (Coleman et al. 2004). Neuronal loss or atrophy in the nucleus basalis, locus ceruleus and raphe nuclei of the brainstem leads to deficits in cholinergic, noradrenergic and serotonergic neurotransmitters and neuromodulators (Cummings and Cole 2002). In particular, the level of choline acetyltransferase is markedly reduced, typically in the hippocampus, substantia inominata, locus cereleus, and tempoparietal and frontal corticies (Hauw and Duyckaerts 2001; White and Clare 2002). The current criteria for pathological diagnosis of definite AD require the presence of both amyloid plaques and neurofibrillary tangles (Reagan Institute Working Group 1997).

1.1.3. Environmental Risk Factors for Alzheimer's disease.

In addition to genetics, numerous other risk factors for AD have been reported, of which increasing age, female sex and low levels of education are the most consistent. Increasing age is the most notable non-genetic risk factor for AD. However, prevalence estimates have tended to vary across studies (Hebert et al. 1995; Ott et al. 1995). Multi centre meta-analyses have found the incidence of AD rises with age (Jorm and Jolley 1998; Launer et al. 1999; Matthews and Brayne 2005), with rates differing according to ethnicity and the diagnostic criteria used

(Jorm and Jolley 1998). Population based samples have also been used to determine age specific incidence rates for AD in a variety of populations of differing ethnicity (Bachman et al. 1993; Canadian Study of Health and Aging 1994; Di Carlo et al. 2002; Fillenbaum et al. 1998; Fratiglioni et al. 1997; Ganguli et al. 2000; Guo et al. 1999; Havlik et al. 2000; Hebert et al. 1995; Hendrie et al. 2001; Kawas et al. 2000; Newman et al. 2005; Paykel et al. 1998; Tang et al. 2001; von Strauss 1999; Waite et al. 2001; Yoshitake et al. 1995). On the whole most studies report incidence rates of AD between 1% and 3% in those aged around 65, rising to over 40% among those aged over 85. Across experimental designs, there is a general consensus that the risk of AD increases exponentially with age, with incidence rates approximately doubling every five years up to 90+ years of age (Kukull et al.2002).

Numerous studies report female sex as a risk factor for AD (Brayne et al. 1995; Fratiglioni et al. 1997; Hagnell et al. 1992). For example, in a longitudinal study by Brayne and colleagues, the incidence of Alzheimer's disease was increased 2.2-fold in females compared to males (Brayne et al. 1995). However, many findings are contradictory, and are likely to be imprecise (Ruitenberg et al. 2001), with prevalence estimates biased by the differential life expectancy of males and females (Bonsignore et al. 2002). Some large studies have suggested that the differential risk to males and females may be restricted to very old age (Launer et al. 1999; Ruitenburg et al. 2001). While several studies have reported no difference in the prevalence of dementia according to sex (Bachman et al. 1993; Ganguli et al. 2000; Letenneur et al. 1994a; Paykel et al. 1994; Rocca et al. 1998).

Low levels of education have been reported to increase the risk of developing AD in population (Ott et al. 1995; Qiu et al. 2001), longitudinal (Canadian Study of Health and Aging 1994; Di Carlo et al. 2002; Mortimer et al. 2003; Schmand et al. 1997; Zhang et al. 1990) and cross sectional studies (Hill et al. 1993; Katzman 1993; Mortel et al. 1995; Risch 2000; Stern et al. 1994b). The cognitive reserve hypothesis proposes that education/mental activity might enhance the brains reserves by increasing synaptic density in the neocortical cortex. (Katzman 1993; Stern et al. 1994b). However, a number of large studies have failed to find a relationship between education and AD (Cobb et al. 1995; Fratiglioni et al. 1991; Paykel et al. 1994). Numerous lifestyle factors differ according to education which are likely to bias findings if not successfully

accounted for (Winkleby et al. 1992). For example, socioeconomic status and disease diagnosis (Evans et al. 1997; Winkleby et al. 1992), as those with a higher education tend to perform better on neuropsychological tests (Qiu et al. 2001; Stern et al. 1994b). Other, less well supported risk factors include smoking (Bowirrat et al. 2001; Lee 1994; Letenneur et al. 1994b), head injury (Launer et al. 1999; Nicoll et al. 1995; Canadian Study of Health and Aging 1994), depression (Devanand et al. 1996; Speck et al. 1995), cardiovascular risk factors (Luchsinger and Mayeux 2004) and oestrogen replacement therapy (Henderson et al. 1994; Schmidt et al. 1996).

1.2. Psychosis in Alzheimer's disease

AD has long been known to be accompanied by a variety of behavioural and psychological symptoms (Reisberg et al. 1987b). The type, severity and prevalence of behavioural symptoms vary greatly and they are not generally believed to be an inevitable consequence of disease progression (Cummings 2000; Sweet et al. 2003). Psychotic symptoms in AD are defined as delusions and/or hallucinations that are not attributable to other causes such as intoxication or delirium (Mintzer and Targum 2003). Development of psychosis is common in patients with AD (Bassiony et al. 2000; Jeste et al. 1992; Paulsen et al. 2000b). With the estimated prevalence ranging from approximately 40% (Ropacki and Jeste 2005) to over 60% (Ballard et al. 1995; Cook et al. 2003). Delusions and hallucinations both seem to develop more readily within a 1 to 2 year span and incidence seems to plateau after 3 years (Paulsen et al. 2000b), with the development of psychosis subsiding before 5 years of follow-up (Chen et al. 1998). Psychotic symptoms rarely seem to persist after several months, however exceptions to this standard have been acknowledged (Ropacki and Jeste 2005), and once present psychosis may recur or persist for several years (Jeste and Finkel 2000). In contrast to the progressive worsening of cognitive and functional changes in AD, behavioural and psychological symptoms peak in occurrence at some point prior to the final stage of AD (Ropacki and Jeste 2005). One hypothesis is that non-cognitive symptoms decrease in severe dementia because the AD brain can no longer articulate the symptoms. Psychosis in AD has been found to associate with many serious

consequences including; severe functional (Stern et al. 1994a) and cognitive deficits (Jeste et al. 1992), increase in rate of decline (Wilson et al. 2000), earlier institutionalisation (Steele et al. 1990) and an increase in caregiver distress (Donaldson et al. 1998).

Delusions are the most common psychotic symptom in AD with a prevalence of around 36% in AD cases (Ropacki and Jeste 2005). The most frequent form of delusion in AD is misidentification. For example, the belief that one's home is not one's home, that a family member is someone else, that images on the television are actually people present in the house, or even that the person in the mirror is not oneself (Cook et al. 2003). Delusions of theft are also frequent. Delusions in AD are typically simple and non-bizarre and seem to differ somewhat from the more complex and bizarre delusions seen in patients with schizophrenia (Jeste and Finkel 2000). Hallucinations in AD occur less frequently, with an estimated prevalence of approximately 18% (Ropacki and Jeste 2005), and often co-occur with delusions. Hallucinations in AD are primarily visual and secondarily auditory in nature (Cook et al. 2003), with estimated prevalence of 18.7% and 9.2% respectively (Ropacki and Jeste 2005).

At post-mortem subjects with psychosis have been found to have increased amyloid plaque deposition and a 2.3-fold greater density of neocortical neurofibrillary tangles than subjects without psychosis (Farber et al. 2000; Marshall et al. 2006; Tekin et al. 2001; Zubenko et al. 1991). Increased levels of abnormal paired helical tau protein filaments in entorhinal and temporal cortices have also been documented in AD+P (Mukaetova-Ladinska et al. 1993). Although, a conflicting study showing no significant differences in neurofibrillary tangle or amyloid plaque density or distribution in AD+P has been reported (Sweet et al. 2002a). As the majority of studies report similar observations it is likely that AD patients experiencing psychosis do have a greater amyloid plaque and neurofibrillary tangle burden compared to AD patients without psychosis. Although the implications of these observations are unclear, it has been suggested that psychosis in AD represents a more severe form of disease (Sweet et al. 2002b).

Neuroimaging investigations have found interesting changes in brains of patients with AD+P compared to AD patients and controls. The frontal (Kotrla et al. 1995a; Lopez et al. 2001; Mega et al. 2000; Mentis et al. 1995; Staff et al. 1999;

Sultzer et al. 1995; Sultzer et al. 2003), caudal anterior cingulated (Mega et al. 2000; Mentis et al. 1995; Sultzer et al. 1995; Sultzer et al. 2003), temporal (Hirono et al. 1998a; Lopez et al. 2001; Mentis et al. 1995; Ponton et al. 1995; Starkstein et al. 1994; Sultzer et al. 2003), parietal (Kotrla et al. 1995a; Mega et al. 2000), and occipital (Hirono et al. 1998a) lobes and striatum (Mega et al. 2000) have demonstrated abnormalities in a number of metabolism or perfusion imaging studies (Meeks et al. 2006; Fukuhara et al. 2001). Moran and colleagues have reported a whole brain, voxel-based imaging study which finds sex differences in AD+P (Moran et al. 2008), supporting sex as a risk factor for AD and AD+P. However, these findings have not, to date, been replicated, and there is no structural imaging data to support the observations. Also, there are several reports of sex differences in healthy older subjects (Good et al. 2001; Gur et al. 1995; Jones et al. 1998; Li et al. 2004a; Slosman et al. 2001). Structural imaging of AD+P patients has revealed an increase in frontal and temporal asymmetry (Geroldi et al. 2002). For example, a magnetic resonance imaging (MRI) study indicated a relationship between delusions and white matter changes in LOAD (Lee et al. 2006). Significant associations with white matter changes and delusional misidentification were identified in frontal, right parieto-occipital, and left basal ganglia regions (Lee et al. 2006). While white matter changes are common in patients with AD (Scheltens et al. 1992; Scheltens et al. 1995; Fazekas et al. 1996; Filippini et al. 2009), the changes documented in psychosis appear distinct (Lee et al. 2006). Despite many studies using modest sample sizes (often less than 50 subjects), the results of the majority of imaging studies in AD+P appear robust with good consistency and reproducibility across groups. In many cases, the findings of these studies also appear to correlate well with the clinical presentation of a more severe form of disease. Therefore supporting the suggestion that psychosis in AD is a distinct subtype of AD and warrants independent investigation.

1.2.1. Development of Psychosis in Alzheimer's disease

There are two theories of psychosis development in AD (Hirono et al. 1998b). For both theories, the AD patient who develops psychosis may have an underlying predisposition to the psychosis phenotype. This psychosis has not

developed earlier in life due to avoidance of other 'risk' factors, or has been unmasked by the loss of compensatory mechanisms due to neuronal loss, amyloid plaque and neurofibrillary tangle formation and neurodegeneration. The first theory of psychosis development in AD suggests that psychosis develops on the 'background' of AD (Hirono et al. 1998b). As such, AD patients with psychosis develop AD by the same mechanism, and therefore through common genetic and environmental risk factors, as AD patients without psychosis. However, once AD has developed genetic and environmental risk factors for psychosis manifest in the presentation of a psychotic phenotype. The genes which promote psychosis in AD are modifying the disease phenotype and are thus referred to as 'modifier' genes. We would expect to identify psychosis modifier genes through comparison of allele and genotype frequencies in AD cases with psychosis (AD+P) against AD cases without psychosis (AD-P).

The second theory proposes that AD with psychosis is a divergent sub-form of AD which develops from risk factors for both psychosis and AD (Hirono et al. 1998b). Thus, the genetic risk factors involved in the aetiology of AD+P are distinct from the genetic risk factors involved in aetiology of AD *per se*, although both forms of disease may share common risk factors. This may be termed the 'disease subtype' theory. Due to the possible overlap in the genetic aetiology of AD+P and AD, the 'disease subtype' theory is best analysed by comparison of allele and genotype frequencies in AD cases with psychosis against control individuals.

1.2.2. Classification of Alzheimer's disease with Psychosis

In general, there is controversy about how best to categorise behavioural symptoms in AD. DSM-IV guidelines do assess behavioural symptoms in AD, but suggest the use of additional coding to fully encapsulate non-cognitive phenotypes. However, specific guidelines for the diagnosis and classification are not available. Numerous rating scales have been created to assess behavioural symptoms in AD. For example, the Present Behavioural Examination (Hope and Fairburn 1992), the Columbia University Scale for Psychopathology in AD (Devanand et al. 1992a), the Manchester and Oxford University Scale for the Psychopathological Assessment of Dementia (Allen et al. 1996), the Neurobehavioural Rating Scale (Levin et al. 1987), Behave-AD (Reisberg et al. 1987b), the Brief Psychiatric Rating Scale

(Overall and Gorham 1962) and the Neuropsychiatric Inventory (NPI) (Cummings 1994). The NPI is probably the most widely used and regarded of these classification systems for both research and clinical settings (Cummings 1997) and was the system of choice for this study. Classification criteria were determined following extensive literature searches of previously employed thresholds for both linkage and association studies of AD+P (Bacanu et al. 2002; Sweet et al. 1998). The classification procedures used in non-genetic longitudinal studies (Ballard et al. 1995; Lyketsos et al. 2001; Mintzer and Targum 2003) were also evaluated before the threshold for AD+P and AD-P classification were decided upon. The NPI and the threshold criteria utilised for sample classification are fully described in chapters 2.2.1, 2.2.3 and 5.2.1.

1.3. Environmental Risk Factors for Alzheimer's disease with Psychosis

Non-genetic risk factors for AD+P have not been studied to any great extent, and those studies that have been conducted show conflicting results.
Psychosis in AD has been reported to associate with many clinical and biological factors, such as age (Binetti et al. 1995; Förstl et al. 1993; Gilley et al. 1991; Hirono et al. 1998b; Kotrla et al. 1995b; Swearer et al. 1988), sex (Burns et al. 1990a; Devanand et al. 1992b; Hirono et al. 1998b; Reisberg et al. 1987a), and education (Devanand et al. 1992b). For example, one of the larger studies of over 110 cases with psychosis, conducted by Hirono and colleagues (Hirono et al. 1998b), found that sex and age were each significantly and independently associated with psychosis, and that the level of education was not a significant predictor of the presence of psychosis. Despite general consensus as to the environmental risk factors for AD+P, there is contradiction in the direction of effect across a number of studies. For example older age was shown to be a significant risk factor for the development of psychosis by several investigators (Binetti et al. 1995; Förstl et al. 1993; Hirono et al. 1998b; Kotrla et al. 1995b; Swearer et al. 1988), whereas Gilley and colleagues (Gilley et al. 1991) reported that early onset was significantly associated with psychosis. Devanand and colleagues (Devanand et al. 1992b) maintained that age at examination was associated with paranoid delusions and inversely correlated with hallucinations. While, many studies failed to show a

significant relationship between age and psychosis (Binetti et al. 1993; Doody et al. 1995; Jeste et al. 1992; Lehtovirta et al. 1996; Migliorelli et al. 1995; Patterson et al. 1990; Reisberg et al. 1987a; Rosen and Zubenko 1991; Rubin et al. 1993; Starkstein et al. 1994; Teri et al. 1988).Fe male sex has been suggested as a risk factor for Alzheimer's disease (Hirono et al. 1998b). However, few studies have considered whether sex specifically predisposes patients to the development of psychotic symptoms in Alzheimer's disease. Reisberg and colleagues (Reisberg et al. 1987a) found that women tended to exhibit behavioural symptoms more often than men and Devanand and colleagues (Devanand et al. 1992b) reported that women's scores of delusion were significantly higher than those of men. On the other hand, Burns and colleagues (Burns et al. 1990a) reported a higher prevalence of delusions of theft in men. Other studies have failed to show an association of psychosis with sex (Becker et al. 1994; Binetti et al. 1993; Binetti et al. 1995; Doody et al. 1995; Migliorelli et al. 1995; Rosen and Zubenko 1991; Starkstein et al. 1994) A 2005 study by Ropacki and Jeste (Ropacki and Jeste 2005), which reviewed data from 55 studies investigating risk factors associated with psychosis in AD, found positive association with African American ethnicity in 5 studies, with negative findings reported in 2 studies (Ropacki and Jeste 2005). Education, sex, and family history of dementia or psychiatric disorder were weakly associated with increased risk for psychosis in the majority of reviewed studies. While the relationships between psychosis and patients' age and age at onset of Alzheimer's disease were generally equivocal (Ropacki and Jeste 2005). From this data it is clear that further studies are needed to convincingly test the myriad of potential environmental risk factors for AD+P and elicit their role in disease aetiology.

1.4. Mapping Complex Traits

1.4.1. Familiality

Measures of familiality compare the rate of a disorder in relatives of an affected person to a baseline rate found in the general population (Falconer and Mackay 1996). Quantifying familiality is the initial step in determining whether a disorder has a genetic aetiology. However, familiality alone is not sufficient to

confirm that genes are important in the development of a disorder since families also share common environments.

1.4.2. Heritability

The heritability of a trait or disease is defined as the proportion of the total variance which is genetic (Strachen and Read 2003).Heritability may be estimated by the use of twin studies which offer a unique design for teasing apart the relative importance of genetic and environmental influences. Monozygotic (MZ) twins are genetically identical clones and will be concordant for any genetically determined character (Strachen and Read 2003). This is true regardless of the mode of inheritance or number of genes involved; the only exceptions are for characters dependent on postzygotic somatic genetic changes (Strachen and Read 2003). Dizygotic (DZ) twins share half their genes on average, the same as any full siblings (Strachen and Read 1999). Genetic characters should therefore show a higher concordance in MZ than DZ twins (Strachen and Read 2003). However, higher concordance in MZ twins compared to DZ twins may also be affected by sex, which can be discordant in DZ twins, and environmental factors (Strachen and Read 2003).

1.4.3. Linkage Analysis

Historically the starting point in determining the genetic aetiology of both simple and complex disease has been linkage analysis. It can be used to identify broad genomic regions that might contain a disease gene, in the absence of previous biologically driven hypotheses (Teare and Barrett 2005). Linkage analysis detects departure from independent inheritance (Teare and Barrett 2005) by testing co-segregation of a genetic marker and disease phenotype within many independent families or over many generations in an extended pedigree. Parametric linkage analysis analyses the co-segregation of genetic loci in pedigrees. This requires specification of a genetic model, making this analysis a powerful method for detecting loci segregating in Mendelian (simple) diseases (Teare and Barrett 2005). For multifactorial traits, such as AD+P, there is no clear mode of inheritance. Therefore, non-parametric linkage analyses are used. These

methods examine allele sharing identity by descent (IBD) in affected relatives. Allele sharing IBD increases above the level expected by chance when a marker is linked to a variant influencing susceptibility to the phenotype, irrespective of the mode of inheritance (Teare and Barrett 2005). In practice, IBD sharing between relative pairs is rarely known with complete certainty (Teare and Barrett 2005), meaning that non-parametric analysis is less powerful than conventional parametric analysis.

To allow for imperfect informity the genome is scanned with a dense collection of genetic markers, typically 300-600 microsatellite markers, corresponding to marker-marker intervals of 10-5 centimorgans (cM) respectively (Lander and Kruglyak 1995). Linkage analysis results are generally shown as a logarithm of the odds (to the base 10) (LOD) score function, that is the logarithm of the odds that the locus is linked to the trait compared to the odds that the locus is not linked to the trait. It is a function of the recombination fraction (θ) (the probability of recombination between two loci at meiosis) or chromosomal position measured in cM (Teare and Barrett 2005). The best (maximum likelihood) estimate of θ or position which maximises the lod score function is named the maximum LOD score (MLS) (Teare and Barrett 2005). Interpretation of the statistical data produced by these analyses is contentious (Teare and Barrett 2005). In general, the higher the LOD score, the greater the evidence for linkage. The level of significance can be assessed according to standards devised by Lander and Kruglyak (Lander and Kruglyak 1995). Classification is based on the number of times that one would expect to see a result at random in a dense, complete genome scan (Table 1.4). It is important to note that prohibitively large samples are required to detect effects of a low magnitude in linkage analyses. Therefore, most linkage studies are only capable of detecting loci with moderate to large effects (Feingold 2001).

Table 1.4. Linkage analysis criteria and classification as defined by Lander and Kruglyak (Lander and Kruglyak 1995). LOD scores quoted refer to those for an affected sibling pair (ASP) study.

Classification	Criteria (LOD>)
Suggestive	2.2; statistical evidence that would be expected to occur one time at random in a genome scan
Significant	3.6; statistical evidence expected to occur 0.05 times in a genome scan (that is, with probability 5%)
Highly significant	5.4; evidence expected to occur 0.001 times in a genome scan
Confirmed	significant linkage from one or a combination of initial studies that has been confirmed in a further sample, preferably by an independent group of investigators (a nominal *P* value of 0.01 is required)

1.4.4. Association Analysis

Association is a statistical statement referring to the co-occurrence of a particular allele(s) and a phenotype above a level expected by chance (Strachen and Read 2003). Both linkage and association rely on co-inheritance of DNA variants adjacent to each other. However, linkage looks at the information by identifying haplotypes that are inherited intact over only a few generations (where little recombination has occurred) and association looks at the retention of adjacent variants over many generations (where much recombination may have occurred). Association analyses are less sensitive to misdiagnosis, decreased penetrance and genetic heterogeneity than linkage analyses (Hodge 1993) and have been able to detect loci that linkage has failed to identify. Association studies can be subdivided into direct or indirect models. Direct association studies assume that if they exist in a gene, susceptibility or casual alleles themselves are evaluated. Indirect association studies assume that the actual susceptibility allele may not be genotyped, but that it is located near to a genotyped marker so any association detected is due to linkage disequilibrium (LD). Recent technological advances have significantly advanced the through-put of association analyses and have brought about the era of genome-wide association studies. A genome-wide association study (GWAS) involves rapidly scanning markers across the complete genomes of many people to find genetic variations associated with a particular disease. Such studies are particularly useful in finding genetic variations that contribute to common, complex diseases, and have proved successful in investigating numerous conditions including type II diabetes (Zeggini et al. 2008) and Alzheimer's disease (Harold et al. 2009).

1.4.5. Replication of Association Studies

The majority of associations reported do not replicate in secondary samples and contradictory findings are common in complex diseases. For replication studies it should only be necessary to genotype the marker or markers that have been reported to be associated with the disease. However, it is important when designing a replication study to ensure numerous variables are controlled; 1) population differences are common and may be the result of real biological differences or differences in allele frequencies and varying LD in differing

populations. Testing for genetic heterogeneity between sample groups and stratifying by ancestry and demographic location can help control type I (false positive) and type II (false negative) errors. 2) Complex diseases can vary in severity and type of symptoms resulting in difficulties defining the global phenotype. The genetic aetiology of each subtype of a complex phenotype may differ, therefore, using distinct phenotypes, even those highly correlated, to study the same gene can result in different observations (Recker and Deng 2002). This problem is compacted by the frequency of differing measures being used to phenotype samples. 3) Differences in gene-gene and gene-environment interactions within samples could also account for non-replication of associations.

It is vital to replicate a positive finding before accepting an association as definitive. It is possible that the initial association finding may be a type I error, as there is a 1 in 20 chance of rejecting the null hypothesis when it is in fact true given a significance level of $\alpha=0.05$. To avoid inflation of these chance findings significance thresholds should be adjusted appropriately whenever multiple statistical tests are performed on the same dataset. The degree to which data should be corrected for multiple testing is a source of much debate. The Bonferroni correction (which approximates by dividing the required significance level by the number of independent null hypotheses being tested) is highly regarded to be overly conservative (Sha et al. 2006). Throughout this thesis multiple testing was corrected for by a permutation calculation which is discussed more fully in chapter 2.9. There are other methods of multiple testing correction in genetic association analyses most of which, like the Bonferroni correction and permutation analysis, control the overall error rate. Alternative forms of multiple testing correction include the false discovery rate (FDR), which is defined as the expected number of false rejections divided by the number of rejections (Benjamini et al. 2001). Controlling the FDR automatically controls the overall error rate in the special case that all the null hypotheses are true. (Devlin et al. 2003).

Type II errors are inflated when type I errors are decreased, increasing the likelihood of a false negative association can be as erroneous as a false positive. A replication study may fail to detect association when variation at that locus does in fact cause susceptibility to the disease. A likely reason is that the replication study is insufficiently powered to detect the effect size. Many studies are underpowered to detect even moderate odds ratios. Especially, as power calculations assume that

either the susceptibility allele itself or a marker in complete LD with the susceptibility allele is being tested. Therefore, a well designed study needs to utilise both a suitably sized sample and appropriately informative markers.

1.4.6. Linkage Disequilibrium

If loci do not segregate independently, more often than can be accounted for by chance, they are said to be in linkage disequilibrium (LD). Any new mutation arising on a chromosome will be in complete LD with all the other polymorphisms carried by that chromosome at that time. LD varies across the genome, therefore it must be considered locally for informative studies to be undertaken. The extent of LD is affected by numerous factors. The primary drive in the breakdown of LD is meiotic recombination (Tsunoda et al. 2004). As recombination occurs the LD between variations decays. The closer two loci are on a chromosome the less likely it is that crossover will separate them (Strachen and Read 2003). Thus, the recombination fraction is a measure of the distance between two loci, this equates to genetic distance which is measured in cM. The recombination rate may vary from 0 to as high as 5 cM/Mb (5% chance of recombination at each meiosis per megabase) (Kong et al. 2002). Nonreciprocal exchange of short tracts of DNA between homologous chromosomes can result in the breakdown of LD, as can recurrent mutation and gene conversion (Hallast et al. 2005). Conversion rates in humans are high and become relatively important for very tightly linked markers (Collins 1999; Frisse et al. 2001; Quintana et al. 2001), as gene conversions disrupt LD between closely linked markers faster than may be predicted by the recombination rate.

Several factors add to the accumulation of LD. Inversion polymorphisms suppress recombination (Martin et al. 2000; Pritchard and Przeworski 2001), and slow LD breakdown. Assortative mating violates the assumption that two genes segregate independently by increasing the likelihood of offspring being homozygous (Hasstedt 1995). A reduction in population size (i.e. a population bottleneck) reduces the number of haplotypes in a population. Therefore, if a bottleneck has occurred recently (in terms of generations) there may be extensive LD (Reich et al. 2001).Gen etic drift is relevant in small populations; rapid changes in gene frequencies result in one allele at a locus becoming fixed inflating LD in this

region. Natural selection can also lead to gene fixation (Wang and Rannala 2004). Greater levels of LD are seen in populations where there has not been a recent demographic expansion i.e. founder populations, than in exponentially expanded populations (Terwilliger et al. 1998; Terwilliger and Weiss 1998). The introduction of genes from one distinct population into another can create LD. The resulting offspring will have linked and unlinked pairs of loci. Over time LD decays, but much more rapidly for the unlinked loci than the linked pairs.

Under a uniform recombination rate of 1 cM/Mb and a simple model of human demography, LD in the human genome is unlikely to extend further than 3 kilobases (kb) (Kruglyak 1999). However, LD may extend further if the assumptions of the model are relaxed, and the average extent of 'useful' LD for disease marker association has been estimated to be 11 kb in African populations and 22 kb in other populations (Gabriel 2002). Measures of linkage disequilibrium quantify how frequently two alleles are found on the same chromosome in a certain population. The statistical measures D' and r^2 commonly summarise the LD between two markers. Both measures are based on the basic pairwise-disequilibrium coefficient D, the difference between the probability of observing two markers alleles on the same haplotype and observing them independently in the population. D has the disadvantage of depending on the frequency of the alleles. D' is the normalisation of D by dividing it with the theoretical maximum for the observed allele frequencies (Hedrick and Kumar 2001). Thus,

$$D' = \frac{D}{D_{\max}} \qquad \text{when } D \geq 0$$

$$D' = \frac{D}{D_{\min}} \qquad \text{when } D < 0$$

When D' is 0 the two loci are deemed to be independent. A value of 1 implies all copies at one locus occur exclusively with one of the two possible alleles at the second marker. r^2 is the square of the correlation coefficient, r, that measures the association between alleles (Hedrick and Kumar 2001);

$$r = \frac{D}{\sqrt{p_1 p_2 q_1 q_2}}$$

where; p_1= frequency of allele 1 at locus 1, p_2 = frequency of allele 2 at locus 1, q_1 = frequency of allele 1 at locus 2, q_2 = frequency of allele 2 at locus 2

r is commonly squared to remove the arbitrary sign (Hedrick and Kumar 2001). If r^2 equals 0, independence is implied. r^2 equals 1 when the occurrence of an allele at one marker perfectly predicts which allele will be present at a second locus.

1.5. Genetics of Alzheimer's disease with Psychosis

1.5.1. Familiality of Alzheimer's disease with Psychosis

There is evidence that familial factors play an important role in the aetiology of AD with psychosis (AD+P). Analysis of 280 siblings of AD+P probands versus 91 siblings of AD probands without psychosis (AD-P) showed an increase in familial risk when AD+P was defined by multiple symptoms ($P < 0.0001$; OR = 3.2, CI 95%; 2.2-4.7) compared to AD+P defined by a single symptom ($P = 0.0006$, OR = 2.4, CI 95%; 1.5-4.0) (Sweet et al. 2002b). This finding was replicated in two additional studies (Eror et al. 2005; Hollingworth et al. 2007), meaning that there is strong support for a genetic element to AD+P.

1.5.2. Heritability of Alzheimer's disease with Psychosis

Using a pedigree of 826 subjects Bacanu and colleagues (Bacanu et al. 2005) employed affected sibling pair (ASP) analysis to estimate heritability of AD+P. The heritability estimate assumes uncorrelated environmental effects and that occurrence of psychosis is independent of ascertainment on AD (Bacanu et al. 2005). Heritability of AD+P defined by multiple psychotic symptoms was estimated at 61% ($P = 0.02$; *half* $X^2_1 = 5.18$); and 30% for AD+P defined by any occurrence of psychosis ($P = 0.04$; *half* $X^2_1 = 3.25$) (Bacanu et al. 2005). The former estimate

compares favourably with generally accepted heritability estimate of 58-79% for LOAD (Gatz et al. 2006), and approximately 80% for schizophrenia (Owen 2005).

1.5.3. Linkage Analyses of Alzheimer's disease with Psychosis

The AD with psychosis phenotype demonstrates linkage to specific chromosomal regions (Avramopoulos et al. 2005; Bacanu et al. 2002, Hollingworth et al. 2007). The first AD+P linkage study was conducted by Bacanu and colleagues (Bacanu et al. 2002). They selected a subset of 65 pedigrees containing at least two siblings diagnosed as having AD+P from those collected as part of the National Institute for Mental Health (NIMH) AD genetics initiative. Data from 237 markers previously genotyped by Kehoe and colleagues (Kehoe et al. 1999) was analysed. A secondary analysis was performed restricted to the 42 families in which both siblings also possessed at least one *APOE* ε4 allele (LOAD+P+ε4). This secondary analysis produced significant linkage on chromosome 2p at approximately 64.3 cM (MLS = 3.52) (Figure 1.5a). Some support for the region can be gathered from schizophrenia literature (Coon et al. 1998). However, the increase in MLS to reach statistical significance appears to be solely due to the influence of the *APOE* ε4 allele. Under both models suggestive linkage on chromosome 6 at approximately 112cM was identified (AD+P; MLS = 2.51 and AD+P+ε4; MLS = 2.01) (Figure1.5b) (Bacanu et al. 2002). Chromosome 6 is an interesting candidate region for psychosis as numerous schizophrenia (Cao et al. 1997; Levinson et al. 2000; Martinez et al. 1999) and bipolar disorder (Dick et al. 2003; Lambert et al. 2005; Pato et al. 2004) studies have identified evidence for linkage in this region. Thus, suggesting that this region harbours a locus (or loci) that influences susceptibility to a broad psychosis phenotype. Bacanu and colleagues (Bacanu et al. 2002) have also reported suggestive linkage on chromosome 21 under a AD+P+ε4 model (36.8 cM; MLS = 1.94) (Figure 1.5c) (Bacanu et al. 2002).L inkage of chromosome 21q to AD was reported in the original Kehoe study (Kehoe et al. 1999), although this evidence comes from the ε4-negative families, making the finding of linkage to LOAD+P+ε4 at this region questionable.

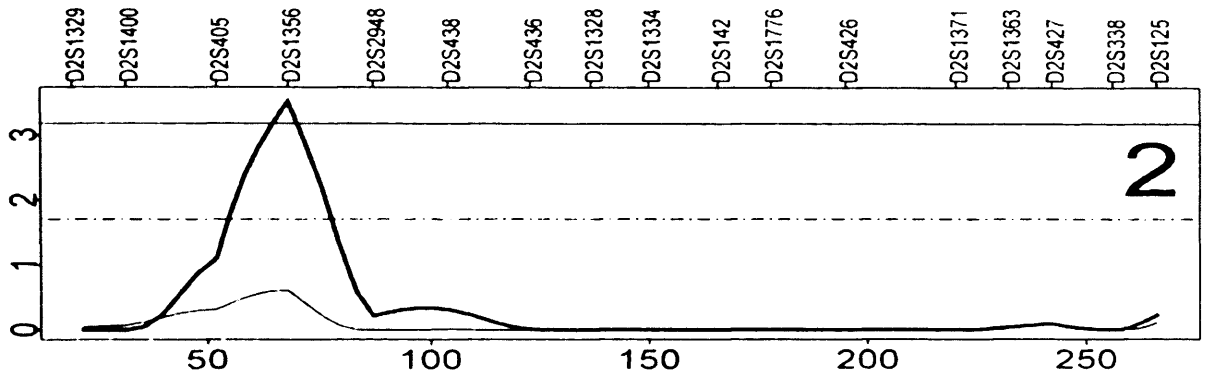


Figure 1.5a. Chromosome 2. Multipoint MLS for families with AD+P (solid, thin line) and AD+P with *APOE* ε4 allele (Solid, thick line). Map distance on x-axis (in centimorgans) as given by NIMH. Reference lines on y-axis indicate critical values for genome-wide significant (solid) and suggestive (dashed) linkage. [Source Bacanu et al. (2002).]

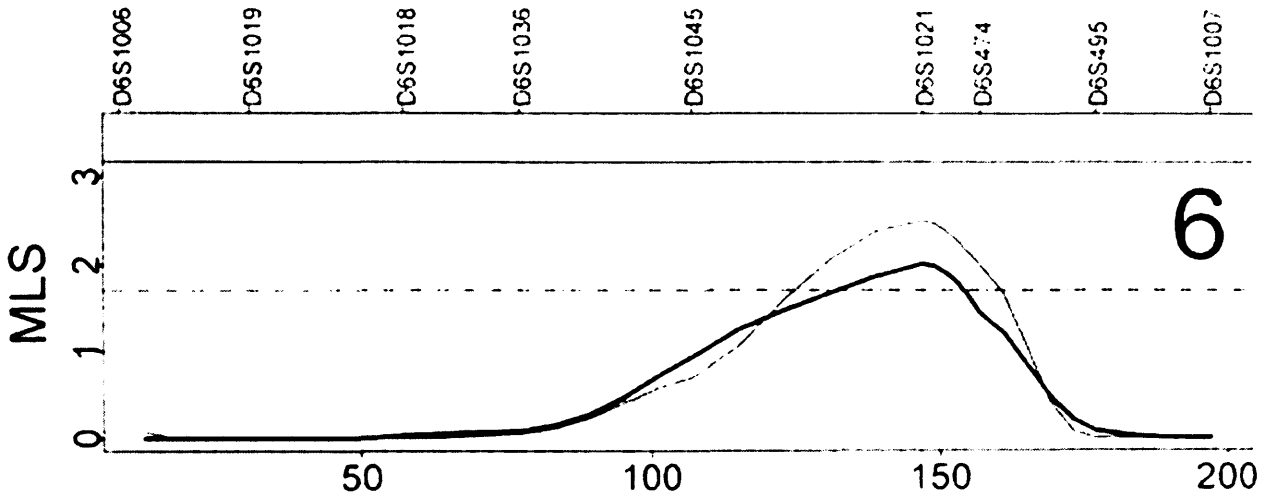


Figure 1.5b. Chromosome 6. Multipoint MLS for families with AD+P (solid, thin line) and AD+P with *APOE* ε4 allele (Solid, thick line). Map distance on x-axis (in centimorgans) as given by NIMH. Reference lines on y-axis indicate critical values for genome-wide significant (solid) and suggestive (dashed) linkage. [Source Bacanu et al. (2002).]

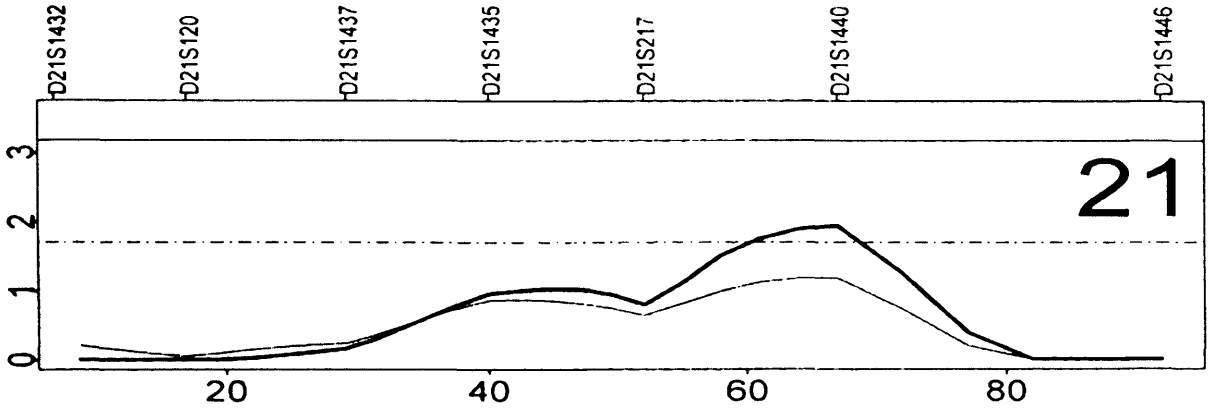


Figure 1.5c. Chromosome 21. Multipoint MLS for families with AD+P (solid, thin line) and AD+P with *APOE* ε4 allele (Solid, thick line). Map distance on x-axis (in centimorgans) as given by NIMH. Reference lines on y-axis indicate critical values for genome-wide significant (solid) and suggestive (dashed) linkage. [Source Bacanu et al. (2002).]

Avramopolous and colleagues (Avramopolous et al. 2005) performed a regression based covariate analysis (Goddard et al. 2001; Olson 1999) using genotypes from the AD genome screen reported by Bassett and colleagues (Bassett et al. 2002). Analysis was based on the presence or absence of hallucinations and delusions using data from 381 markers (average spacing 9 cM) through 148 NIMH families having at least two affected siblings (ages of onset more than 50 years). Their most significant evidence for linkage was on chromosome 14q24.3 (LOD = 3.91; genome-wide empirical $P = 0.052$ at D14S540), near *PSEN1*. This effect was derived from individuals that did not have co-morbid hallucinations, a lower LOD score was observed for individuals without delusions. Linkage appeared to be stronger in families containing individuals with an age at onset < 65 years, with a LOD score empirically shown to approach genome-wide significance (LOD = 5.74; genome-wide empirical $P = 0.048$) (Avramopoulos et al. 2005). Evidence for linkage was observed on chromosome 1 for the hallucinations covariate in discordant siblings (LOD = 2.26) and approximately the same region showed linkage for the delusions covariate (Avramopolous et al. 2005). Moderate evidence for linkage on chromosome 3 was observed, with a LOD score of 2.75 detected at marker D3S4523 for delusions, compared to a LOD score of 2.1 for hallucinations (Avramopolous et al. 2005). Avramopolous and colleagues (Avramopolous et al. 2005) showed linkage to a region of chromosome 7 in sibling pairs discordant for hallucinations (LOD = 2.14). There is also evidence for linkage to delusional symptomatology at approximately the same position.

The final study by Hollingworth and colleagues (Hollingworth et al. 2007) combined microsatellite marker genotypes from three LOAD genome screens (Blacker et al. 2003; Kehoe et al. 1999; Myers et al. 2002) utilising data from 321 affected relative pairs (ARPs) from the USA and UK. This provided data from 610 markers with an average between marker spacing of 6cM. Logistic regression was employed to model the probability of allele sharing, which then allowed psychosis to be incorporated into the model as a categorical variable (Hamshere et al. 2005a; Risch 1990). This methodology also incorporates information from affected relatives who have not displayed psychotic symptoms. A MLS of 2.84 (chromosome-wide p-value = 0.012, genome-wide p-value = 0.242) was identified on chromosome 7, at 91 cM (nearest marker: D7S2204 at 78.0 Mb) for ARPs who

were concordant for the absence of psychotic symptoms (Hollingworth et al. 2007). This region of chromosome 7 is approximately that identified by Avramopolous and colleagues (Avramopolous et al. 2005), who saw evidence for linkage in AD cases discordant for psychosis. *APOE* ε4 status had a minimal effect on linkage evidence on chromosome 7 with a MLS increase of 0.06 after its inclusion as a covariate (Figure 1.5d) (Hollingworth et al. 2007). Hollingworth and colleagues (Hollingworth et al. 2007) observed their most significant increase in LOD score on chromosome 15 (MLS = 3.16, chromosome-wide $P = 0.001$, genome-wide $P = 0.039$) (Hollingworth et al. 2007). ARPs who were concordant for the absence of psychotic symptoms provided the most evidence for linkage, and *APOE* ε4 status had a minimal effect (Figure 1.5e). A MLS of 1.07 was observed close to the linkage region on chromosome 6 reported by Bacanu and colleagues (Bacanu et al. 2002) (Figure 1.5f). A secondary analysis was performed adding *APOE* ε4 status to the logistic regression model, this increased the observed LOD score to 2.98 (Figure 1.5f). Evidence for a potential locus at 35 to 36 Mb on chromosome 21 was observed when psychosis and *APOE* ε4 status were included in the analysis (Figure 1.5g) (Hollingworth et al. 2007). However, this locus was not in the same region as that identified by Bacanu and colleagues (Bacanu et al. 2002) and the majority of the increase in the maximum LOD score was accounted for by *APOE* ε4 status, agreeing with previous findings in studies using part of this sample (Kehoe et al. 1999; Olson et al. 2001).

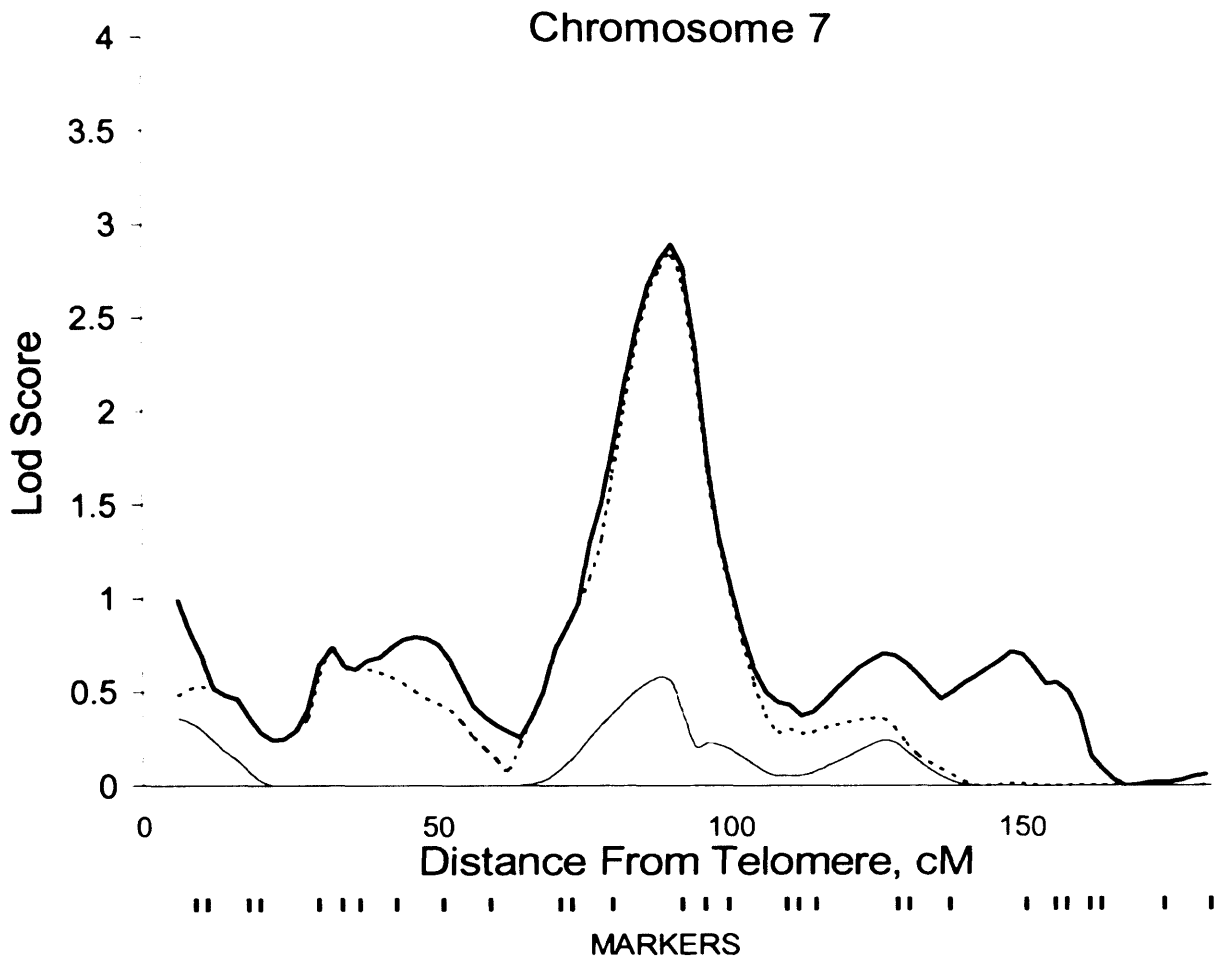


Figure 1.5d. Covariant linkage analysis of LOAD-P and LOAD-P+ε4 on chromosome 7q. LOD score for ARPs with LOAD (solid, thin line), LOAD-P (dashed line) and LOAD-P with *APOE* ε4 allele (solid, thick line). [Source Hollingworth et al. (2007).]

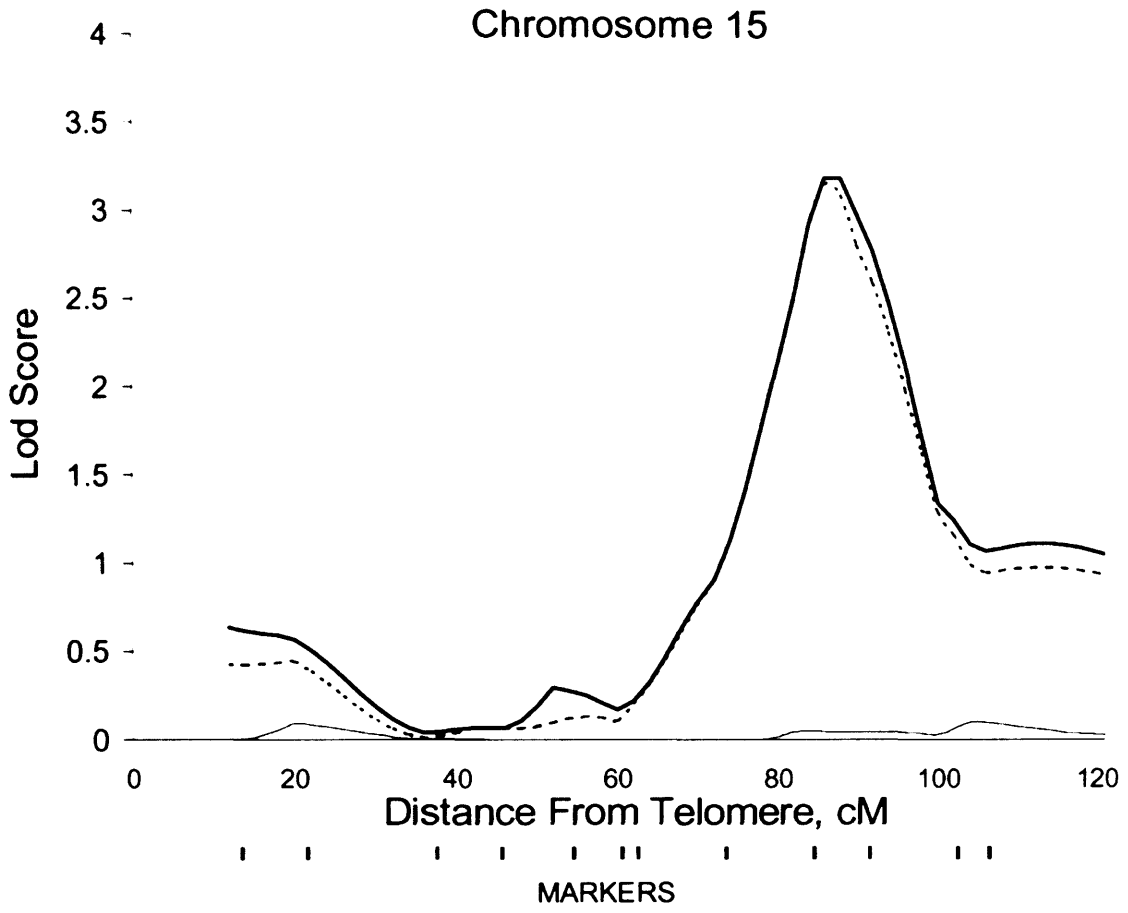


Figure 1.5e. Covariant linkage analysis of LOAD-P and LOAD-P+ε4 on chromosome 15q. LOD score for ARPs with LOAD (solid, thin line), LOAD-P (dashed line) and LOAD-P with *APOE* ε4 allele (solid, thick line). [Source Hollingworth et al. (2007).]

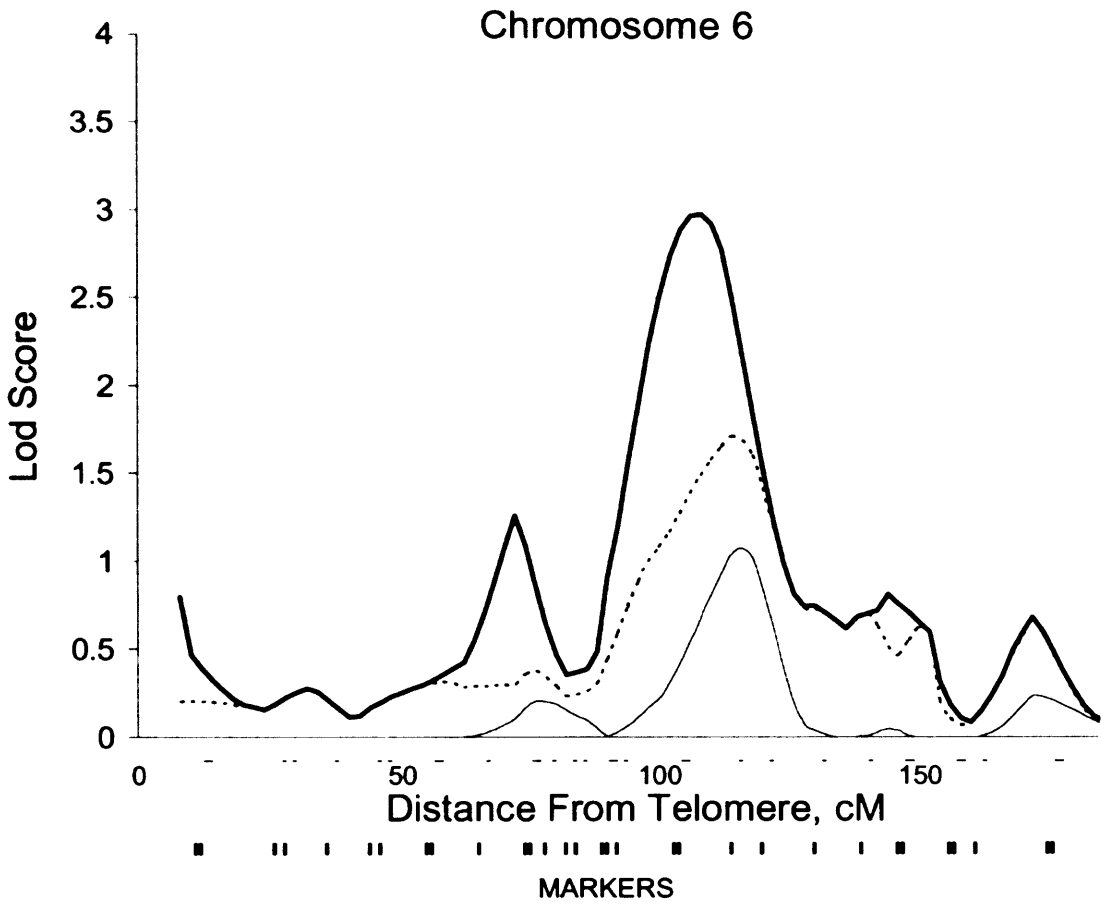


Figure 1.5f. Covariant linkage analysis of LOAD+P and LOAD+P+ε4 on chromosome 6q. LOD score for ARPs with LOAD (solid, thin line), LOAD+P (dashed line) and LOAD+P with *APOE* ε4 allele (solid, thick line). [Source Hollingworth et al. (2007).]

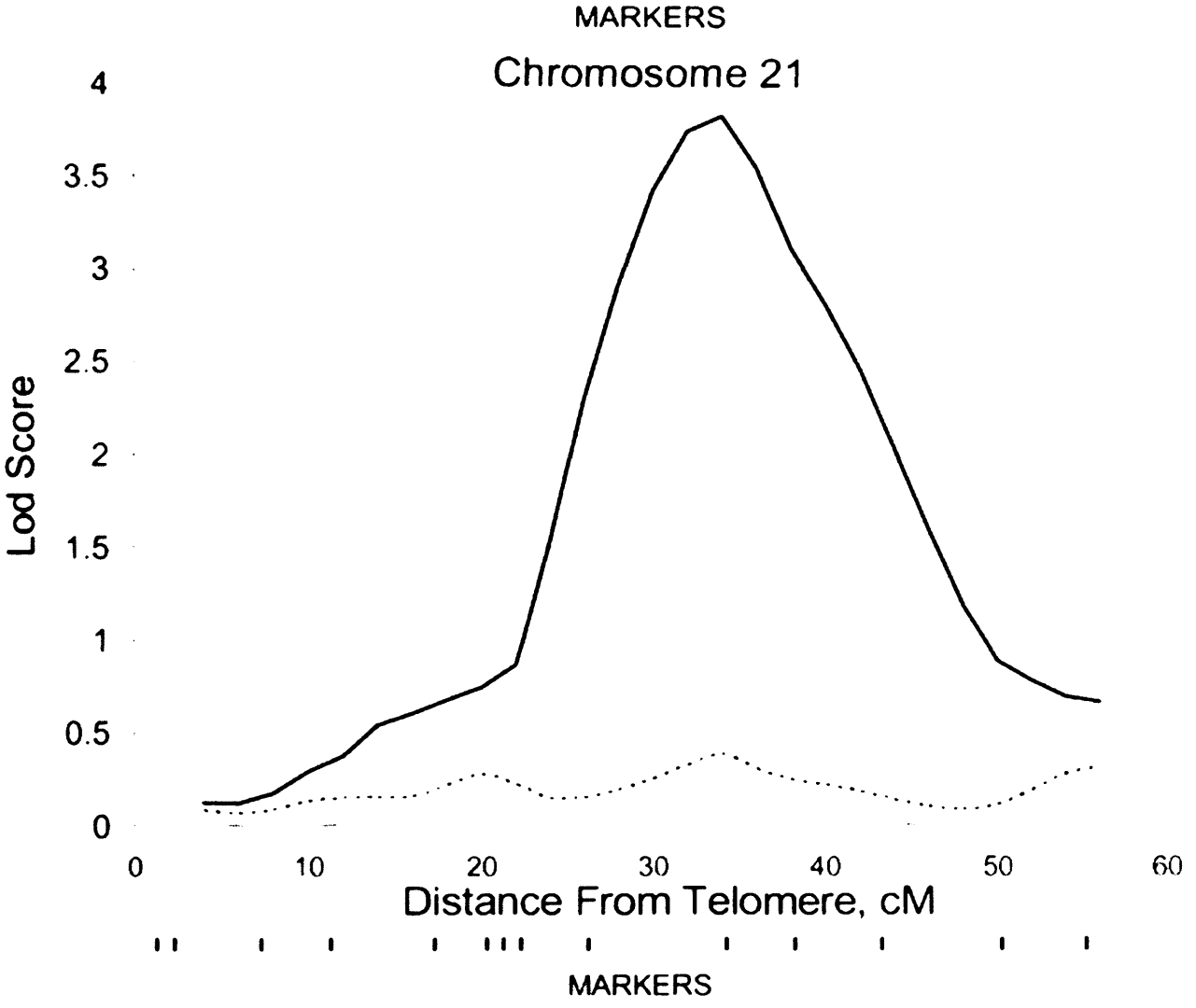


Figure 1.5g. Covariant linkage analysis of LOAD+P and LOAD+P+ε4 on chromosome 21. LOD score for ARPs with LOAD (solid, thin line), LOAD+P (dashed line) and LOAD+P with *APOE* ε4 allele (solid, thick line). [Source Hollingworth et al. (2007).]

None of the AD+P linkage analyses are conclusive, as none show significant, replicable evidence for linkage. However, there is overlap in the identified linkage regions between studies suggesting that susceptibility loci for psychosis in AD may reside at these locations, and that loci within these regions should be prioritised for future investigations. The sample used in the study of Bacanu and colleagues (Bacanu et al. 2002) was small in comparison to the other linkage analyses, with the study of Hollingworth and colleagues (Hollingworth et al. 2007) by far the largest of the 3 published analyses. The Avramopolous study (Avramopolous et al. 2005) utilised a design with a broad age range which is not conducive to the identification of linkage regions for LOAD. Indeed, the linkage observed on chromosome 14 in early onset patients without psychosis is within 40 Mb of the *PSEN1* gene, mutations of which are known to cause EOAD. The evidence for linkage identified by analysis of AD+P ARPs (chromosomes 2, 6 and 21) appears to be largely due to *APOE* ε4 status. Bacanu and colleagues (Bacanu et al. 2002) showed significant linkage on chromosome 2p at approximately 64.3 cM as a result of inclusion of *APOE* ε4 status into the analysis. Hollingworth and colleagues (Hollingworth et al. 2007) saw a small increase in linkage evidence for LOAD+P on chromosome 2p but this failed to reach chromosome-wide significance. Bacanu and colleagues (Bacanu et al. 2002) detected evidence for suggestive linkage on chromosome 6 at approximately 112cM (Bacanu et al. 2002). A region close to this showed evidence for linkage in the study by Hollingworth and colleagues (Hollingworth et al. 2007). Although, the LOD score increase attributable to psychosis, after controlling for APOE ε4 status did not meet criteria for chromosome-wide significance in either study. This region of chromosome 6 remains an interesting candidate region for psychosis as numerous schizophrenia (Cao et al. 1997; Levinson et al. 2000; Martinez et al. 1999) and bipolar disorder (Dick et al. 2003; Lambert et al. 2005; Pato et al. 2004) studies have identified evidence for linkage in this region. The overlap of linkage in AD+P, schizophrenia and bipolar disorder suggests that this region harbours a locus that influences susceptibility to psychotic symptoms across disorders. Both Bacanu and colleagues (Bacanu et al. 2002) and Hollingworth and colleagues (Hollingworth et al. 2007) have reported suggestive linkage on chromosome 21 under a AD+P+ε4 model, with the increase in MLS attributable to psychosis not meeting criteria for suggestive linkage. Thus, suggesting that a gene residing on chromosome 21 may

have a role in the aetiology of LOAD+P dependent on *APOE*. However, the linked regions identified differ between studies and replication is warranted. Linkage regions identified by analysis of LOAD+P and LOAD without psychosis (LOAD-P) ARPs (chromosomes 1, 3, 7, 14 and 15) largely show evidence of linkage in AD patients without psychosis irrespective of *APOE* ε4 status. Chromosomes 1 and 3 provide poor evidence of linkage (Avramopoulos et al. 2005), with no replication of these regions (Hollingworth et al. 2007). Hollingworth and colleagues (Hollingworth et al. 2007) showed suggestive evidence for linkage of LOAD+P to chromosome 7 as do Avramopoulos and colleagues (Avramopoulos et al. 2005) at a similar position, although under a different psychosis model; Avramopolous and colleagues in discordant sibling pairs (Avramopolous et al. 2005) and Hollingworth and colleagues in pairs concordant for the absence of psychosis (Hollingworth et al. 2007). Despite this, the concurrence across studies as to the linked region suggests that this region of chromosome 7 harbours variant(s) associated with psychotic symptomology in AD. Additional chromosomal regions identified as showing linkage to AD patients without psychosis are chromosome 14 (Avramopolous et al. 2005) and chromosome 15 (Hollingworth et al. 2007), indicating that these regions may harbour gene(s) protecting against the psychotic phenotype of AD, independent of *APOE* status. A notable limitation of the linkage analyses of AD+P is that all studies have used overlapping subsets of the NIMH sample; therefore no finding in any study has been independently replicated. The study by Hollingworth and colleagues (Hollingworth et al. 2007) in particular incorporates a substantial number of additional pedigrees and marker genotypes. Thus, suggesting that while independent replication is essential, the evidence for linkage at chromosomes 6 and 7 is probably robust. The convergence of linkage findings on chromosomes 2p in schizophrenia and AD+P, and 6q in schizophrenia, bipolar disorder and AD+P is consistent with the hypothesis that multiple genes, no single one of which is a major locus for idiopathic psychoses, could increase the risk for psychosis onset in the context of neurodegenerative and neurodevelopmental conditions (Bacanu et al. 2002). However, this interpretation should be made cautiously as despite the common psychosis phenotype, there are substantial clinical and neurobiological differences between the disorders, and the chromosomal region in which linkage has been detected in these conditions is broad. Moreover, alternative models are possible. For example, variants in genes

may contribute major risk for idiopathic psychoses such as schizophrenia, and may also increases risk for AD+P. Or variants in genes that increase the liability to onset of AD could also increase risk for psychosis.

1.5.4. Association Analyses of Alzheimer's disease with Psychosis

Relatively few association studies have been performed in AD+P. Of those that have, none provide consistent or compelling evidence for association with any gene. Studies investigating the association between *APOE* genotype and psychotic symptom in AD have reported contradictory results. At least 20 studies have examined *APOE* genotype in respect to psychosis in AD. Eight have reported that the ε4 allele increases the risk for psychosis (Ballard et al. 1997; Chang et al. 2004; Harwood et al. 1999; Ramachandran et al. 1996; Scarmeas et al. 2002; Spalletta et al. 2006; Weiner et al. 1999; Zdanys et al. 2007).For example, *APOE* ε4 was significantly associated with psychotic symptoms ($P = 0.029$, OR = 1.87) in the study by Zdanys and colleagues (Zdanys et al. 2007). The effect seemed to be specific to patients with severe-stage AD and primarily from an association between ε4 and delusions (Zdanys et al. 2007). In a longitudinal study of 171 AD patients *APOE* ε4 allele possession was associated with increased levels of delusions ($P < 0.05$, OR = 1.23) (Spalletta et al. 2006). Both hallucinations and delusions showed association to *APOE* ε4 in the 2004 study by Chang and colleagues (Chang et al. 2004). Fifty six patients without psychotic symptoms were followed to identify incident psychotic symptoms. The presence of the *APOE* ε4 allele carried a 19-fold risk for developing hallucinations and a 3.4-fold risk for delusions (Chang et al. 2004). The studies which have identified a positive association between psychosis in AD and *APOE* ε4 possession do not show association to the same phenotype of AD+P. For example 2 longitudinal studies show association to delusions only (Scarmeas et al. 2002; Spalletta et al. 2006) while Chang and colleagues show an association to both delusions and hallucinations, with the strongest effect seen with hallucinations (Chang et al. 2004). Eleven studies have found no effect of ε4 on AD+P risk (Borroni et al. 2006; Cacabelos et al. 1997; Gabrvelewicz et al. 2002; Hirono et al. 1998a; Hirono et al. 1999; Hollingworth et al. 2006; Lehtovirta et al. 1996; Levy et al. 1999; Lopez et al. 1997; Lyketsos et al. 1997; Quaranta et al. 2009; Sweet et al. 2002c), including a

study of *APOE* genotype in the MRC LOAD sample (Hollingworth et al. 2006). The MRC genetic resource for LOAD is the largest of the sample-sets used to study the potential *APOE* ε4 association with AD+P. The study included 1120 subjects, 879 of which were genotyped for *APOE* ε4 (Hollingworth et al. 2006). The number of *APOE* ε4 alleles was not associated with any behavioural component (Hollingworth et al. 2006). Therefore, despite a number of studies reporting a relationship between *APOE* genotype and psychosis in AD, the majority have found no relationship. The results of the studies which do identify association of ε4 with AD+P should be received with caution. All of these studies are based on small sample sets, of less than 300 AD cases (40% to 60% of which will experience psychosis), and different populations and statistical methods have been used across studies to detect association. It therefore seems unlikely that APOE is implicated in the prevalence or severity of psychosis in AD.

The catechol-O-methyltransferase (*COMT*) gene is a putative susceptibility candidate for schizophrenia owing to its role in dopamine metabolism, and location within the deleted region in Velocardiofacial Syndrome (VCFS), a disorder associated with high rates of schizophrenia (Craddock et al. 2006). Numerous studies have found a highly significant association between schizophrenia and *COMT* (Lee et al. 2005; Palmatier et al. 2004; Shifman et al. 2002), and also an association with lower *COMT* mRNA expression (Bray et al. 2003a). Genetic variation at multiple *COMT* polymorphisms has been investigated in AD+P. A significant association with the val/met variant rs4680 has been observed (Borroni et al. 2004) and replicated (Borroni et al. 2006; Borroni et al. 2007; Sweet et al. 2005); this association was found to be specific to hallucinations (Borroni et al. 2006). Haplotype analysis of *COMT* markers revealed a highly significantly associated 4-locus haplotype in two studies of AD+P (Borroni et al. 2006; Borroni et al. 2007; Sweet et al. 2005). The association with *COMT* and AD+P appears robust although only nominally significant; the studies have been well constructed despite small sample sizes. The identification of association with AD+P to the val/met variant and haplotypes at the *COMT* locus by 2 independent groups suggests these findings are not false positives. Although, it would be interesting to determine if the associations seen would replicate in a suitably powered sample-set.

Polymorphisms of the serotonin receptor *5HT-2A* have been shown to associate with hallucinatory symptoms and delusions in demented and non-demented cohorts (Holmes et al. 1998). In schizophrenic cohorts the most clearly linked serotonergic genotypic variant is the *5HT-2A* T/C 102 polymorphism (Williams et al. 1997; Prasad et al. 2002). Numerous groups have reported the influence of the C variant in producing psychotic symptomatology in AD (Holmes et al. 1998; Nacmias et al. 2001; Rocchi et al. 2003), other groups have reported significant under representation of 5HT-2A 102C homozygotes in AD+P (Lam et al. 2004; Assal et al. 2004). One study of this polymorphism in AD+P found no significant genotypic or allelic association (Craig et al. 2007). However, a recent meta-analysis of the *5HT-2A* T/C 102 polymorphism in AD+P showed a significant effect of both the C allele (OR = 2.19) and the CC genotype (OR = 5.14) (Ramanathan and Glatt 2009), strongly supporting the action of this variant in the aetiology of AD+P. Borroni and colleagues (Borroni et al. 2006) found that AD+P patients carrying the serotonin gene-linked promoter region (*5-HTTLPR*) long variant experienced fewer delusions (OR = 0.38, CI 95%; 0.01-0.90). An independent study of 148 AD patients found significant association of the long variant of *5-HTTLPR* with psychosis in AD (Quaranta et al. 2009). However, the association was in the opposite direction to the study of Borroni and colleagues (Borroni et al. 2006), with the percentage of AD+P increasing with the number of copies of the long-allele: 13% among short allele homozygotes; 36% among heterozygotes (P = 0.018, OR = 3.91); 51% among long allele homozygotes (P = 0.003, OR = 7.25) (Quaranta et al. 2009). Thus, providing strong support for the action of this variant in the aetiology of AD+P. The long variant of the polymorphism increases the transcriptional efficiency of the *5-HTT* gene promoter resulting in enhanced 5-HTT expression and 5-HT uptake in lymphoblasts (Lesch et al. 1996). The cumulative effect of *COMT* and *5-HTTLPR* polymorphisms was investigated in AD+P using previously reported data (Borroni et al. 2004; Borroni et al. 2006). The findings claim a synergic effect of *COMT**G and *5-HTTLPR*_short variant polymorphisms on the risk of psychosis in AD. Carriers of one polymorphism showed a 2-fold increase in risk, and carriers of both polymorphisms showed a 5-fold increased risk for psychosis, compared with patients bearing no polymorphisms (Borroni et al. 2006). This cumulative association has not been

tested in independent populations, but due to the reported associations of both polymorphisms independently, warrants investigation.

Neuregulin-1 (*NRG1*) is a strong functional candidate for psychosis with isoforms involved in generation of neurons and development of Schwann cells (Falls 2003). Numerous studies in schizophrenia (Li et al. 2004b; Stefansson et al. 2003; Williams et al. 2003a; Zhao et al. 2004) and bipolar disorder (Green et al. 2005; Thomson et al. 2007) have shown positive association of the gene with disease. Following linkage analyses of 65 families enriched for AD+P, Go and colleagues (Go et al. 2005) demonstrated a significant association with a *NRG1* SNP (rs3924999) and AD+P ($P = 0.008$). Despite requiring replication, the linkage and association findings at first appear convincing. However, it is unclear whether the 65 families used to identify linkage contained both early-onset and late-onset AD pedigrees. The group had at their disposal 437 pedigrees, of which 65 were enriched for psychosis and 320 of the families were late-onset AD, it is unclear which of these sample sets was used for the association analysis.

Dopamine receptors are a class of metabotropic G protein-coupled receptors that are prominent in the central nervous system (CNS). They have key roles in many processes including the control of motivation, learning, and fine motor movement, as well as modulation of neuroendocrine signaling. Abnormal dopamine receptor signaling and dopaminergic nerve function is implicated in several neuropsychiatric disorders (Girault and Greengard 2004). The dopamine receptor D2 (*DRD2*) has shown association with schizophrenia in a Japanese population (Arinami et al. 1994), but not in a European population (Sobell et al. 1994). In contrast, a persistent association of the dopamine receptor D3 (*DRD3*) *BalI* polymorphism with schizophrenia has been reported (Jönsson et al. 2003; Nimgaonkar et al. 1996). Polymorphisms in the dopamine receptors *DRD1*, *DRD2*, *DRD3*, and *DRD4* have been investigated for association with AD+P (Sweet et al. 1998). Among Caucasian patients psychosis was significantly more frequent in *DRD1* B2/B2 homozygotes ($P < 0.02$) and *DRD3 BalI* 1/1 or 2/2 homozygotes ($P < 0.05$) (Sweet et al. 1998). The joint risk for psychosis due to the *DRD1* and *DRD3* polymorphisms exceeded the risk due to either locus alone, suggesting an interaction (Sweet et al. 1998). Neither the *DRD2* S311C polymorphism nor the presence of long alleles for the *DRD4* exon III repeat sequence were associated with presence of psychosis (Sweet et al. 1998). The associations between the

variation in the *DRD*1 and *DRD*3 receptor genes and psychosis in Alzheimer's disease have replicated (Holmes et al. 2001). Although, the replication study was performed by the authors of the original finding, and a negative finding of the *DRD3 BalI* polymorphism and the presence or absence of psychotic symptoms in AD has been reported (Craig et al. 2004a).

Glutamate is an excitatory neurotransmitter in the central nervous system (CNS). The D-amino acid oxidase activator (DAOA) gene, plays a key role in the glutamate pathway regulated through the NMDA receptors. Hypofunction of the NMDA glutamate receptor is a well regarded model in the aetiology of psychosis and polymorphisms in this gene have been implicated in susceptibility to schizophrenia and bipolar affective disorder, (Farber 2003). A recent study genotyped a panel of *DAOA* SNPs in a cohort of 185 Alzheimer's disease patients, investigating these variants with respect to the occurrence of delusions and hallucinations in AD (Di Maria et al. 2009). The analysis demonstrated a nominally significant association with the delusion phenotype ($P < 0.05$) with one SNP (rs2153674). Multivariate regression showed that the rs2153674 genotype accounts for up to 15% of the variance in delusions severity, as assessed by using the Neuropsychiatric Inventory (Di Maria et al. 2009). This study seems well constructed and if replicated, the glutamate hypothesis could be invoked to explain the occurrence of psychosis in neurodegenerative. However, the association identified is not striking and the sample utilised is small.

Regulation disturbances of cytokines, such as interleukins, have been noted in schizophrenia (Gaughran 2002) and AD (Tarkowski et al. 2003) and the low expression variant (C) of interleukin-1β (*IL-1β*) has been implicated in producing an increased risk for schizophrenia (Katila et al. 1999). Craig and colleagues (Craig et al. 2004b) investigated the -511 promoter polymorphism of *IL-1 β* in 424 LOAD patients. The CC genotype and C allele was significantly over represented in those LOAD patients experiencing delusions ($P = 0.002$; OR = 1.49), hallucinations ($P = 0.014$; OR = 1.6), and the combined phenotype ($P = 0.048$; OR = 1.62) (Craig et al. 2004b). This study seems to be well executed and the findings warrant replication in independent samples.

Finally, the alpha 7 nicotinic acetylcholine receptor gene (*CHRNA7*) which has been associated with schizophrenia in linkage and association studies (Stephens et al. 2009; Xu et al. 2001), has shown association with AD+P in a

Northern Ireland population. The most significant association was seen between delusions and the T allele of rs6494223 (P = 0.014, OR = 1.63) (Carson et al. 2008). This study appears robust with the utilisation of a more adequately sized cohort of over 900 individuals. This novel finding requires independent replication.

To date, variants in 11 genes have been tested for association with AD+P. A number of studies have reported a relationship between *APOE* genotype and psychosis in AD, however the results of the studies should be received with caution. The majority of comprehensive studies have found no relationship with *APOE* ε4 and AD+P. It therefore seems unlikely that APOE is involved in the manifestation of psychosis in AD. Polymorphism rs4680 and a 4-marker haplotype of *COMT* have both shown association with AD+P which replicates in independent samples (Borroni et al. 2004; Borroni et al. 2006; Sweet et al. 2005). However, the significance levels seen are underwhelming and the sample sets utilised are underpowered to identify such an association. Further analysis of these variants would eliminate the possibility that these findings are false positive associations. Studies of serotonin genes have given conflicting results (Craig et al. 2007; Holmes et al. 1998; Nacmias et al. 2001; Rocchi et al. 2003). The most consistent findings appear to be with the *5-HTTLPR* long variant (Borroni et al. 2006; Quaranta et al. 2009), which has also shown significant association to LOAD+P in conjunction with *COMT* (Borroni et al. 2006). The *NRG1* marker rs3924999 has shown significant association with AD+P, but it is unclear whether this finding is exclusive to late-onset AD+P (Go et al. 2005) and requires replication. The *DRD1* and *DRD3* genes show association to AD+P which replicate, but the discovery and replication studies have been performed by the same group (Holmes et al. 2001; Sweet et al. 1998), and a negative association of *DRD3* with AD+P has also been published (Craig et al. 2004a). Nominal associations of AD+P to *IL-1β* (Craig et al. 2004b) and *CHRNA7* (Carson et al. 2008) have been identified but not replicated. Most, of these association studies have only utilised a small number of samples, meaning that they are underpowered to confidently accept or decline a putative variant as being associated to disease. An excellent and comprehensive examination of practical sample size requirements is provided by Wang and colleagues (Wang et al. 2005). The positive associations that are seen are generally only nominally significant and most have not replicated. It is also of note that comparison of AD+P studies is confounded by a number of factors including; variability in the definitions

of delusions and hallucinations (Scarmeas et al. 2002), analysis of hallucinations and delusions separately in some studies but jointly in others, and use of differing classification systems and threshold criteria to quantify psychosis. However, these analyses have prioritised interesting candidate genes and variants for future investigations and highlighted the need for a uniform classification system to accurately assess and define AD+P.

1.6. Study Design

This thesis is based on the wealth of evidence that psychosis in AD is a distinct form of AD showing a more severe phenotype (Sweet et al. 2002b). The AD+P phenotype displays familiality (Eror et al. 2005; Hollingworth et al. 2007; Sweet et al. 2002b), heritability (Bacanu et al. 2005), linkage (Avramopoulos et al. 2005; Bacanu et al. 2002; Hollingworth et al. 2007), and association (Borroni et al. 2004; Borroni et al. 2006; Borroni et al. 2007; Go et al. 2005; Holmes et al. 2001; Sweet et al. 1998; Sweet et al. 2005), making the phenotype an excellent candidate for gene mapping efforts. The findings from previous linkage and association studies predominantly highlight putative psychosis susceptibility regions and genes in the aetiology of LOAD+P, supporting the theory that psychosis modifier genes act across schizophrenia, bipolar disorder and AD+P. There are clinical differences in the manifestation of psychosis observed across neurodegenerative and neurodevelopmental disease. Psychosis in AD develops within a few years of dementia onset and usually persists only a matter of months before ceasing (Chen et al. 1991; Paulsen et al. 2000b; Ropacki and Jeste 2005). Psychosis in Parkinson's disease (PD), vascular dementia and Huntington's disease (HD) also develops post disease onset, while in dementia with lewy bodies (DLB) psychosis is one of the first symptoms of disease. The prevalence of psychosis is increased in DLB subjects (McKeith et al. 2005) and decreased in vascular dementia (Ballard et al. 2000) and HD patients compared to those suffering with AD (Paulsen 2001). Delusions are the most common psychotic symptom in AD (Jeste and Finkel 2000), with visual hallucinations occurring less frequently, and often co-occurring with delusions (Cook et al. 2003). The presence

of psychosis in vascular dementia follows a similar pattern to that seen in AD. In DLB (McKeith et al. 2005) and PD (Goetz et al. 2005) visual hallucinations are the most common form of psychosis. Whereas, auditory hallucinations are the most predominant psychotic symptom in HD (Correa et al. 2006). Psychotic symptoms in neurodevelopmental conditions have a more common clinical presentation. Hallucinations are the most common psychotic symptom which are predominantly auditory in nature (Böcker et al. 2000). Delusions of persecution, grandiosity and paranoid or bizarre delusions are frequent (Corner 2004; Tasman 1997). Schizophrenia is a psychosis, a disorder of thought and sense of self (Andreasen 1995). Although it affects emotions, it is distinguished from mood disorders in which changes in emotion are primary. Psychosis in bipolar disorder usually occurs during a manic or depressed episode (Goodwin and Jamison 1990) and tends to reflect the extreme mood state at the time (Tasman 1997). While, psychosis in schizoaffective disorder commonly occurs after depressive, mixed and/or manic episodes (Blacker and Tsuang 1992). However, in neurodevelopmental and neurodegenerative disease the core symptoms of psychosis, delusions and hallucinations are observed. Therefore, the prioritisation of putative schizophrenia and bipolar disorder susceptibility polymorphisms and genes for investigation in LOAD+P seems a logical starting point on the quest to identify genetic variants involved in the aetiology of this form of LOAD.

1.7. General Aims

The aim of the research presented within this thesis is to detect a novel locus or loci conferring susceptibility to LOAD+P. Using aspects of clinical variation to identify sub-phenotypes has proved successful in identifying genes for other complex disorders such as Crohn's disease (Rioux et al. 2001), and asthma (Van Eerdewegh et al. 2002). In the analysis of complex genetic traits there are a number of sample designs. Cross-sectional studies utilise a specific population, cohort studies study outcome and exposure in a longitudinal fashion, family based studies investigate disease in selected pedigrees, and case-control studies define the outcome before measuring the exposure. This study utilises a case-control

sample design. A number of affected individuals (cases) and unaffected individuals (controls) are genotyped. Controls are carefully selected to avoid population stratification, by matching with cases for age, sex and ethnicity. Thus, preventing false representation of the population the sample group is estimating and reducing the probability of false findings. Implicit in a case-control approach is the concept that the phenotype studied is clinically homogeneous and can be defined categorically as either present or absent. However, LOAD is a clinically heterogeneous illness and increasing attention is now being paid to utilizing defined subgroups in the hope of unpicking the complex aetiology of the illness (Olson et al. 2001; Pericak-Vance et al. 2000; Sweet et al. 2003). Currently, little is known about the underlying causes of the clinical differences observed in AD. Gaining a more comprehensive understanding of LOAD+P may aid both the study of this form of clinical heterogeneity and AD as a whole, providing a strong platform for the development of future preventative and therapeutic strategies.

1.8. Thesis Outline

This thesis aims to investigate the underlying genetic aetiology of psychosis observed among AD sufferers. Psychotic variation will be considered from two distinct standpoints. First, psychosis could identify a distinct 'sub-phenotype', or more homogeneous form of the disease. Under such a model, genetic variation would increase disease risk but only within those sufferers experiencing AD with psychosis. Alternatively, genetic variants may act as 'disease modifiers'. Under such a model, genetic variation would not increase the risk of developing AD, but could influence the presentation of psychosis in the presence of neurodegeneration. In this thesis both theories as to the development of psychosis in AD will be explored. Initial work focuses on exploration of the current literature to determine the criteria used to classify psychosis presence or absence in AD. Following extensive literature searches a threshold criterion is determined. This is comprehensively described in chapter 2.2.3. A 2 stage approach to identify genes implicated in the aetiology of AD+P is then undertaken. As discussed the region of chromosome 6q previously identified by linkage analyses of LOAD+P (Bacanu et al. 2002; Hollingworth et al. 2007) overlaps with linkage regions identified for

schizophrenia (Cao et al. 1997; Levinson et al. 2000; Martinez et al. 1999) and bipolar disorder (Dick et al. 2003; Lambert et al. 2005; Pato et al. 2004). Also association analyses in LOAD+P have identified positive associations to putative schizophrenia and bipolar disorder candidate genes. In chapter 3 of this thesis 73 variants from 11 psychosis candidate genes are tested for association with LOAD+P in the large MRC genetic resource for LOAD sample which comprises a cohort of 1205 AD sufferers. The aim of this study is to test if any 'historical' putative psychosis candidate genes or variants highlighted by studies of psychotic disorders such as schizophrenia and bipolar disorder, contribute to the aetiology of LOAD+P. This analysis is based on the hypothesis that these genes may affect psychosis across disorders through a common underlying neuropathology. The same design approach is used in chapter 4. In this chapter 'novel' variants and genes identified by a recent genome-wide association study of schizophrenia (O'Donovan et al. 2008) are investigated for association with LOAD+P in the MRC genetic resource for LOAD sample. In chapter 5 the second method of identifying susceptibility loci for LOAD+P is employed. In this chapter a hypothesis free approach is utilised by analysis of genome-wide association data for AD conditioning on the presence or absence of psychosis. This data consists of genotypes for over 500,000 markers. This study examines 4957 LOAD cases, 1671 with information on their experience of psychosis during disease, and 9682 controls, including 2486 elderly controls matched for age, sex and ethnicity. The prevalence of psychotic symptoms in the combined sample is 39.9%. This is the first genome-wide association study of LOAD+P. The identification of loci which increase susceptibility to LOAD+P will be the first step in understanding the causes and pathogenesis of a distinct sub-phenotype of AD.

2. Materials and Methods

2.1. Sample Ascertainment

2.1.1. MRC Genetic Resource for LOAD Sample

Clinical data and DNA samples were collected by members of the Alzheimer's field team from 1,205 individuals (71% females) with late onset Alzheimer's disease (LOAD) and 1,361 control subjects (62% females). Age at onset (AAO) ranged from 60 to 95 years (mean = 75.84 years, SD = 6.79). Controls were matched for age (mean = 76.53 years, SD = 6.33), sex and ethnicity. The sample consisted of individuals ascertained from both community and hospital settings in the UK collected as part of the Medical Research Council (MRC) genetic resource for LOAD. AD cases and controls described here were ascertained by three collaborating centres: Department of Psychological Medicine, Cardiff University, Cardiff (co-ordinating centre); Institute of Psychiatry, London; and Cambridge University, Cambridge, as previously described (Morgan et al. 2007). Ethical permission was obtained from the Multi-centre Research Ethics Committee, relevant local ethics committees and NHS trusts.

All cases were Caucasian, of UK origin (parents born in the UK) and diagnosed with probable AD in accordance with the National Institute of Neurological and Communication Disorders and Stroke and the Alzheimer's disease and Related Disorders Associations (NINCDS-ADRDA) clinical diagnostic criteria for AD (McKhann et al. 1984). All diagnoses were made based on a semi-structured interview with known validity for AD pathology (i.e. positive predictive value of 92-95% (Foy et al. 2007; Holmes et al. 1999)), which included: (1) The Mini Mental State Examination (MMSE) (Folstein et al. 1975); (2) The Cambridge Mental Disorders of the Elderly Examination (CAMDEX; informant interview) (Roth et al. 1986); (3) The Blessed Dementia Scale (Blessed et al. 1968); (4) The Bristol Activities of Daily Living Scale (Bucks et al. 1996); (5) Webster Rating Scale (Webster 1968); (6) Global Deterioration Scale (GDS) (Reisberg et al. 1988); (7) Cornell Scale for Depression in Dementia (Alexopoulos et al. 1988); (8)

Neuropsychiatric Inventory (NPI) (12 Item version) (Cummings 1997). Interviews were primarily conducted with the AD sufferer's next of kin by members of the Alzheimer's field team.

MRC control subjects were either spouses of AD patients or selected from primary-care practices situated in the same geographical areas as AD patients. All controls were 60 years or above and of UK origin. Control individuals were screened for cognitive decline using the MMSE, and a cut-off score of 28 or above was adopted. Assessment also included a section of the Cambridge Mental Disorders of the Elderly Examination and the Geriatric Depression Scale (Sheikh and Yesavage 1985). Exclusion criteria included the presence of dementia, depression, delirium or other illnesses likely to significantly reduce cognitive function.

2.2. Behavioural Assessment

2.2.1. The Neuropsychiatric Inventory

The Neuropsychiatric Inventory (NPI) (Cummings 1997) was used to assess the prevalence and severity of behavioural and psychological symptoms in all participants. The NPI is an informant-based rating scale which evaluates 12 common behavioural and psychological symptoms in AD including delusions, hallucinations, agitation, depression/dysphoria, anxiety, euphoria, apathy, disinhibition, irritability, aberrant motor behaviour, night-time behaviour disturbances, and appetite/eating abnormalities. The severity of each symptom is rated categorically from 0-3, with anchor points for 'does not occur' (0), 'mild' (1), 'moderate' (2) and 'severe' (3). The frequency with which the symptom occurs is rated categorically from 0-4, with points for 'never' (0), 'less than once per week' (1), 'about once per week' (2), 'several times per week' (3) and 'once or more per day' (4). Frequency and severity scores are then multiplied to give an overall *domain score* for each symptom ranging from 0 to 12. Content validity, concurrent validity, inter-rater reliability, and test-retest reliability of the NPI are well

established and it is commonly used in both research and clinical settings (Cummings 1997).

2.2.2. Principle Components Analysis

Behavioural and psychological symptoms in AD had been previously assessed under the hypothesis that these symptoms represent fewer underlying components, and that symptoms of the same component are more likely to co-occur (Hollingworth et al. 2006). The identification of behavioural and psychological components in complex diseases may allow identification of more homogeneous samples with sufficient power (through relatively large sample sizes) to detect susceptibility variants for disease. The assessment used NPI data from a total of 1,120 individuals collected as part of the MRC genetic resource for LOAD (Hollingworth et al. 2006). Domain scores for each section of the NPI were used to test the component structure of the NPI and determine clusters of symptoms which occur together, the resulting correlation matrix was submitted to principal components analysis. Four interpretable components were identified: behavioural dyscontrol (euphoria, disinhibition, aberrant motor behaviour sleep, and appetite disturbances), psychosis (delusions and hallucinations), mood (depression, anxiety, and apathy), and agitation (aggression and irritability) (Hollingworth et al. 2006).

2.2.3. Sample Classification Criteria

Samples were classified based on the scores of the NPI, rated to reflect the worst episode of each symptom over the lifetime of the illness. Individuals classified as having late-onset Alzheimer's disease with psychosis (LOAD+P) first had to meet a frequency threshold for presence of symptom(s) ≥ 2, this minimum criterion aimed to exclude those patients experiencing very few psychotic episodes which maybe attributable to another cause.I ndividuals classified as LOAD+P had to experience both hallucinations and delusions or experience hallucinations or delusions singly with a NPI domain score > 3. These criteria were designed after

extensive literature searches and were based on similar classification definitions of NPI data being implicated in AD (Cummings et al. 2005). Three hundred and seventy nine individuals were categorised as LOAD+P (78% females, mean AAO = 76.07 years). AD cases who had not displayed any hallucinations or delusions were coded as having late-onset Alzheimer's disease without psychosis (LOAD-P). The prevalence and severity of psychosis in AD has been shown to increase with advancing disease progression (Paulsen et al. 2000b; Ropacki and Jeste 2005). Therefore, individuals with mild-moderate AD (GDS < 5) were considered at unknown risk of developing psychosis and excluded from the LOAD-P subgroup. Two hundred and sixty nine individuals were categorised as having late-onset Alzheimer's disease without psychosis (LOAD-P) (70% females, mean AAO = 75.97 years).

2.3. Marker Selection

Three approaches to gene investigation were undertaken in this study. The first was to genotype specific markers and haplotypes previously shown to be associated with some form of psychosis. Where no individual marker or haplotype was specifically associated with disease state, the locus was "tagged". Functional variants were analysed at those loci that were practically and financially too large to be "tagged" to a satisfactory standard. Following extensive literature searches using PubMed (see section 2.9.1.) and consultation with senior members of the department (Professor's Michael Owen. Michael O'Donovan, Nick Craddock and Dr Nigel Williams), who are experts in the genetics of schizophrenia and bipolar disorder, markers and haplotypes were chosen that were deemed to be putatively associated with psychosis. Polymorphisms chosen to tag a specific gene were identified using the tagger tab within the HAPLOVIEW software version 4.0 (Barrett et al. 2005) with input data from the HapMap project (www.hapmap.org) (see sections 2.9.2 and 2.10.3 respectively), or in house CEPH genotypes. SNPs were chosen to tag the gene of interest plus an additional 40 kb (20 kb of flanking sequence either side of the gene of interest) using the pairwise tagging algorithm, meaning that all tag SNPs act as direct proxies to all other untyped SNPs, with a r^2 threshold of 0.8, the minimal coefficient of determination r^2 at which all alleles are

to be captured. Functional variants included SNPs within coding regions (exons), un-translated regions, putative promoter regions, evolutionary conserved regions and transcription factor binding sites. These markers were identified using the UCSC genome browser (see section 2.10.2) and their surrounding sequences downloaded from the database.

2.4. DNA Extraction, Quantification, Storage and Sample Preparation

2.4.1. DNA Extraction

All MRC DNA samples were obtained from venous blood taken by members of the Alzheimer's field team from participants willing to give blood. DNA was extracted as high molecular weight fractions from lymphocytes using standard phenol-chloroform procedures followed by ethanol precipitation.

2.4.2. DNA Quantification

Following DNA extraction DNA concentration was calculated. Firstly, by ultraviolet (UV) absorbance using an uQuant spectrophotometer (Biotek® Instruments Inc, Winooski, Vermont, USA). An aliquot of extracted DNA was diluted to 5% its original concentration. UV light absorbance (A) at 260 nm and 280 nm wavelength (λ) were measured and the ratio between $A_{260\ nm}$ and $A_{280\ nm}$ calculated. Assuming that an $A_{260\ nm}$ of 1 is equivalent to 50 µg of DNA, a ratio above 1.8 indicated a suitable level of clean DNA and the absence of contaminating RNA or protein. An aliquot of extracted DNA was diluted to approximately 20 ng/µl in sterile water (Fresenius Kabi, Paris, France) based on the concentration calculated by spectrometry.

The Quant-iT™ PicoGreen® double-stranded (ds) DNA Quantification kit (Molecular Probes®, Carlsbad, California, USA) and a Fluoroskan Ascent® fluorometer (LifeSciences International Ltd, Singapore) with Ascent® Software (Thermo Labsystems, Altrincham, UK) were used to calculate more accurately the concentration of DNA. The Quant-iT™ PicoGreen® reagent specifically interacts with dsDNA and is therefore a more accurate method of quantification than

spectrometry. The 20 ng/µl DNA aliquot was further diluted to 1% concentration in 1x TE buffer in a 96 well labsystems cliniplate (Thermo Labsystems, Altrincham, UK). A working concentration of Quant-iT™ PicoGreen® reagent was produced by adding 5 µl Quant-iT™ PicoGreen® at 200x to 995 µl of 1x TE buffer. One hundred microlitres of Quant-iT™ PicoGreen® working concentration was dispensed into each sample by the fluorimeter and the DNA concentration measured using a UV excitation wavelength of 485 nm and an emission wavelength of 538 nm. DNA was quantified by comparison to the gradient of a standard curve produced by using the calibrant standard provided. Using this accurate measure of dsDNA concentration, working concentrations of 5 ng/µl genomic DNA were prepared by sterile water dilutions in 96-shallow-well boxes (ABgene, Epsom, UK) using a liquid handling system (Biomek® FX^P Laboratory Automation Workstation, Beckman Coulter Inc, Fullerton, California, USA).

2.4.3. Liquid Handling

All large scale liquid processing was undertaken using semi-automated robotic liquid handling systems. DNA samples and pre-PCR reagents were aliquoted using a Biomek® FX^P Laboratory Automation Workstation (Beckman Coulter Inc, Fullerton, California, USA). Post polymerase chain reaction (PCR) reagents were aliquoted using a Biomek® NX^P Laboratory Automation Workstation (Beckman Coulter Inc, Fullerton, California, USA). All liquid handling programs utilised during this study were written by other members of the department.

2.4.4. DNA Storage

All DNA stocks were kept at -20°C in water in individual microcentrifuge tubes. All DNA dilutions were prepared in 96-shallow-well boxes (ABgene, Epsom, UK) and stored at -20°C.

2.4.5. Sample Preparation

3 µl of 5 ng/µl genomic DNA was aliquoted into 384-well PCR plates using a Biomek® FX^P Laboratory Automation Workstation. Sample plates were dried on 384 well tetrad thermocyclers (MJ Research Genetic Research Instrumentation, Rayne, UK) at 55°C for 30 min in preparation for PCR amplification.

2.5. Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a well established method of amplifying a specific DNA sequence that lies between two regions of known sequence. The technique is dependent upon the thermostable enzyme *Thermus Aquatics* (Taq). Taq polymerase performs the synthesis of the complementary DNA strand from a denatured DNA template in the presence of a suitable buffer containing $MgCl_2$, deoxy-nucleotide triphosphates (dNTPs), and forward and reverse oligonucleotide primers. The oligonucleotide primers are designed to anneal to the known sequence and flank the region to be amplified. The Taq polymerase uses these primers as a double stranded initiation point for 5' to 3' synthesis of DNA. The PCR reaction involves the denaturation of the double stranded DNA at a high temperature to produce a single stranded DNA template, the annealing of the oligonucleotide primers to their complementary sequence, and the enzymatic extension of the DNA product. Each stage of the PCR reaction, denaturation, annealing and extension is repeated in a cyclic manner by alteration of reaction temperature using a MJ Tetrad thermocycler (MJ Research, Rayne, UK) (see Figure 2.5), the template and synthesised product is sequentially amplified for approximately 35 cycles.

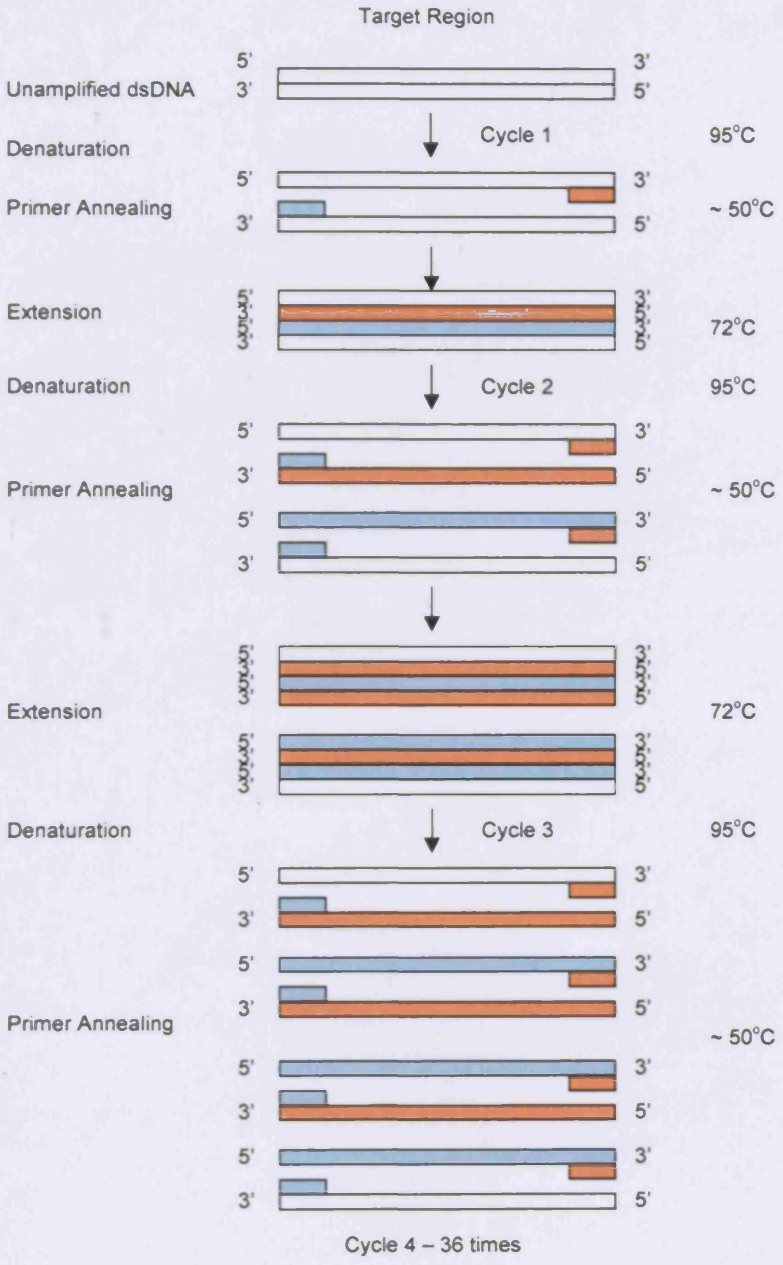


Figure 2.5. Schematic outline of DNA amplification by Polymerase Chain Reaction. Extended primers are complementary to their template.

2.5.1. Oligonucleotide Primer Design

Oligonucleotide primers for PCR reactions used in this study were designed using the online Primer3 (v4.0) software (Rozen and Skaletky 2000) (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), the Amplifluor™ AssayArchitect™ software (https://apps.serologicals.com/AAA/) and the Sequenom™ MassARRAY™ Assay Design software. Flanking sequences were obtained from the 'get DNA' function of the UCSC genome browser (http://genome.ucsc.edu/) with surrounding polymorphisms highlighted. Primer sequences were designed to not overlap with any polymorphism to ensure optimum primer binding and subsequent product amplification. Oligonucleotides used in this thesis were synthesised by Eurogentec (Eurogentec Ltd, Southampton, UK) (www.eurogentec.com), Invitrogen™ (Invitrogen Ltd, Paisley, UK) (www.invitrogen.com), Metabion (Metabion International AG, Martinsried, Germany) (www.metabion.com), or Sigma-Aldrich® (Sigma-Aldrich Company Ltd, Dorset, UK) (www.sigmaaldrich.com).

2.5.2. PCR Reagents

Two types of Taq polymerase were used for PCR; HotStar Taq (Qiagen, Valencia, California, USA) and Titanium Taq (BD Biosciences) the latter of which was used exclusively for Amplifluor genotyping (see section 2.7.2). HotStar Taq is chemically modified to prevent enzymic activation before heating at 95°C for 15 minutes preventing non-specific elongation. HotStar Taq based PCR was performed in a 5 µl volume containing 5 pmol of each primer, 20 µM dNTPs (Amersham Biosciences, UK Ltd), 1 x PCR buffer (Qiagen, Valencia, California, USA) and 0.5 units Hotstar taq Polymerase (Qiagen, Valencia, California, USA)(see Table 2.5a), which was aliquoted into sample plates containing pre-dried DNA.

Table 2.5a. Reagents used for PCR reactions (note: volume quoted per single PCR reaction).

Reagent	Company	Volume
Buffer (10x containing 15 mM $MgCl_2$)	Qiagen, USA	0.5 µl
dNTPs (2.5 mM)	Amersham, UK	0.1 µl
Primer (10 pmol/µl)-Forward	Eurogentec, UK	0.2 µl
Primer (10 pmol/µl)- Reverse	Eurogentec, UK	0.2 µl
ddH_2O	UHW, UK	3.9 µl
Hot Start Taq Polymerase (5 Units/µl)	Qiagen, USA	0.1 µl

2.5.3. PCR Condition Optimisation

Where possible the PCR cycling conditions followed a standard three step method. Assays that failed to optimise under such conditions were optimised using a "touch-down" PCR. To find the optimum temperature (Tm) for primer annealing optimisation reactions were undertaken for each primer pair on control DNA using a temperature gradient (52°C, 53°C, 56°C, 60°C, 64°C and 66°C) on MJ Tetrad thermocyclers (MJ Research, Rayne, UK).

Standard Three-step Cycling Conditions;

1. 94°C - 96°C for 15 minutes
2. 94°C - 96°C for 20 - 30 seconds
3. Tm°C for 20 - 30 seconds
4. 72°C for 30 - 45 seconds
5. Repeat steps 2 – 4 for 34 - 36 cycles
6. 72°C for 10 minutes
7. 15°C for 10 minutes

Touch-down PCR Conditions;

1. 94°C - 96°C for 15 minutes
2. 94°C - 96°C for 5 seconds
3. Tm°C + 6°C for 5 seconds (-0.5 °C per cycle)
4. 72°C for 10 seconds
5. Repeat steps 2 – 4 for 11 - 15 cycles
6. 94°C for 5 seconds
7. Tm°C for 5 seconds
8. 72°C for 10 seconds
9. Repeat steps 6 – 8 for 19 - 25 cycles
10. 72°C for 10 minutes
11. 15°C for 10 minutes

2.6. Agarose Gel Electrophoresis

Agarose gel electrophoresis was undertaken to check PCR product production. DNA fragments are fractionated according to their size and conformation when an electrical potential difference is applied through a porous substance such as an agarose gel. The negatively charged phosphate group of DNA allows the migration of the fragment towards the positive anode. Hence, allowing fragments of differing sizes and shapes to be separated. Analysis of post PCR samples was performed using 2% agarose gels. The reagents to construct a 2% agarose gel are listed in Table 2.6a. The agarose – TBE mixture was heated to ensure complete liquefaction and mixing. The solution was allowed to cool before the Ethidium Bromide solution was added. The molten gel was poured into a gel casting tray and appropriate well former combs were added before the gel was allowed to set at room temperature for approximately 30 minutes.

Table 2.6a. Reagents to prepare a 2% Agarose Gel.

Reagent	Supplier	Quantity
ddH2O	UHW, UK	95 ml
TBE (x10)	National Diagnostics, USA	5 ml
Agarose	AGTC Bioprod Ltd, UK	2 g
Ethidium Bromide (10 mg/ml)	Fischer Scientific, UK	1 µl

After PCR, 2 µl of PCR product was mixed with 2 µl of loading buffer (see Table 2.6b). Loading buffer allows the PCR product to "fall" into the wells and also enables tracking of DNA migration through the agarose gel. The resulting mix was aliquoted into the formed wells of the solidified gel, along with a 4 µl aliquot of size standard (1 kb plus DNA ladder, see Table 2.6c). This ladder constitutes DNA fragments of known size and was used to assess the size of PCR amplified fragments. The gel was immersed in an electrophoresis tank (Thermo Scientific, Northumberland, UK) containing 0.5 x TBE buffer. Gel electrophoresis was performed at 120 volts for 45 minutes. DNA products assayed by agarose gel electrophoresis were visualised using an UV transilluminator (UVP, Upland, California, USA) and photographs taken using an attached Kodak Electrophoresis Gel documentation and analysis system (Eastman Kodak Company, Rochester, New York, USA).

Table 2.6b. Reagents for Loading Buffer.

Reagent	Supplier	Quantity
ddH2O	UHW, UK	20 ml
EDTA (0.5 M)	Sigma, UK	4 ml
Ficoll	BDH, USA	3 g
Orange G/Bromophenol Blue	Sigma, UK	0.01 g

Table 2.6c. Reagents for 1 kb+ ladder.

Reagent	Supplier	Quantity
ddH2O	UHW, UK	140 µl
1 kb+ Ladder	New England Biolabs, USA	30 µl
Loading Buffer	Prepared Above	30 µl

2.7. Genotyping Methods

Individual SNP genotyping was performed using either MassARRAY™ (Sequenom™, San Diego, California, USA) or Amplifluor™ (Millipore, Billerica, Massachusetts, USA) platforms. The iPlexGOLD™ assay for the MassARRAY™ system allows multiplexing of SNP assays up to the 39-plex level (Oeth et al. 2005) and is explained in detail in section 2.7.3. Microsatellite markers and repeat elements were also investigated in this study and were genotyped by the genescan fluorescent PCR method.

2.7.1. Genescan Fluorescent PCR

The genescan fluorescent PCR method is often used to genotype insertions/deletions and repeat elements. The methodology is based on 5' fluor-labelled oligonucleotide PCR primers (the 5' primer is labelled and the 3' primer is unlabelled). The fluorescent labels used were FAM and HEX which fluoresce at different wavelengths. A panel of markers was designed so that the size of amplimer for each marker (and all allelic variants) did not overlap with the size of any other marker amplimer (and that markers alleles) labelled with the same fluorophore. All marker PCRs used in this study had a size greater than 100 bp and smaller than 400 bp.E ach fluorescent PCR was performed and purified separately. Unincorporated dNTPs and unextended primers were disabled using;

0.5 µl Shrimp Alkaline Phosphatase (SAP) (GE Biosci, Bucks, UK)
0.1 µl Exonuclease I (exo I) (GE Biosci, Bucks, UK)
4.4 µl ddH_2O

Incubated at;

1. 37°C for 1 hr
2. 80°C for 15 min

The independent products were pooled at an appropriate ratio to allow genotyping to be performed (as determined by a pre-test on optimisation DNA). For analysis 4 µl clean multiplexed PCR product was added to 9 µl HiDi formamide (Applied Biosystems, Foster City, California, USA) and 0.5 µl fluorescent size standard. The size standard used was Genescan™ -500 ROX™ (Applied Biosystems, Foster City, California, USA) which is designed for sizing DNA fragments in the 35-500 bp range, and provides 16 single-stranded fragments of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500 bases.

The fluorescent labelled products were analysed using an automated ABI3100 PRISM Genetic Analyser (Applied Biosystems, Foster City, California, USA). The ABI3100 PRISM Genetic Analyser (Applied Biosystems, Foster City, California, USA) loads the samples by electrokinetic injection, the samples are then electrophoretically moved through a polyacrylamide filled capillary where the fluorophore is excited by a laser; the resultant fluorescence is measured by a Photo-Multiplier-Tube. The PCR samples were run through a 36 cm long capillary using POP4 polyacrylamide (Applied Biosystems, Foster City, California, USA). Analysis of the fluorescent fragments was performed automatically using Genescan Analysis version 3.7 software (Applied Biosystems, Foster City, California, USA). These data were then analysed using Genotyper software (Applied Biosystems, Foster City, California, USA), which allows the user to delineate the size of the fluorescently labelled product and judge whether alleles are present for size-polymorphic markers. Genotype results were exported to a Microsoft excel file for further analysis. If alleles were genotyped in samples that contained water instead of DNA then a PCR contamination had occurred and the experiment was re-

performed. Experiments were also re-performed for markers where more than two alleles were present.

2.7.2. Amplifluor™ UniPrimer Chemistry

Amplifluor™ UniPrimer Chemistry is based on PCR amplification of genomic DNA using two allele-specific tailed primers that introduce priming sites for universal energy transfer labelled primers. The reaction requires no post-PCR handling (Myakishev et al. 2001). The assays were designed by the Amplifluor™ AssayArchitect™ software freely available online at the Amplifluor™ website (https://apps.serologicals.com/AAA/). The design process entails the input of the SNP details and the flanking DNA sequence into the design software. The technique utilises five primers: two forward allele-specific tailed primers, a single universal anti-sense reverse primer and two "universal" energy-transfer labelled primers (UniPrimers). Each UniPrimer contains a 3' sequence of approximately 15-20 bp, which is complimentary to the 5' end of one of the allele specific tailed primers and is labelled with either a green dye (fluorescein, FAM) or a red dye (sulforhodamine, SR) as well as a quencher (Dabsyl) at the 5' hairpin structured end. In this hairpin state the primers emit very low levels of fluorescence because the energy from the fluorophore is transferred to the nearby quencher.

The allele-specific primers initiate a competitive allele-specific PCR and these amplimers serve as templates for the binding of the UniPrimers. The incorporation of the UniPrimer into the allele-specific amplimer linearises the hairpin structure of the UniPrimer, separating the fluorophore from its quencher and enhancing the level of fluorescence. The resultant levels of red or green fluorescence can distinguish the levels of each allele. The aim of the assay is to have one homozygote yield green fluorescence only, the other homozygote red only, and the heterozygote a mix of red and green fluorescence. The step by step reaction is displayed in Figure 2.7a.

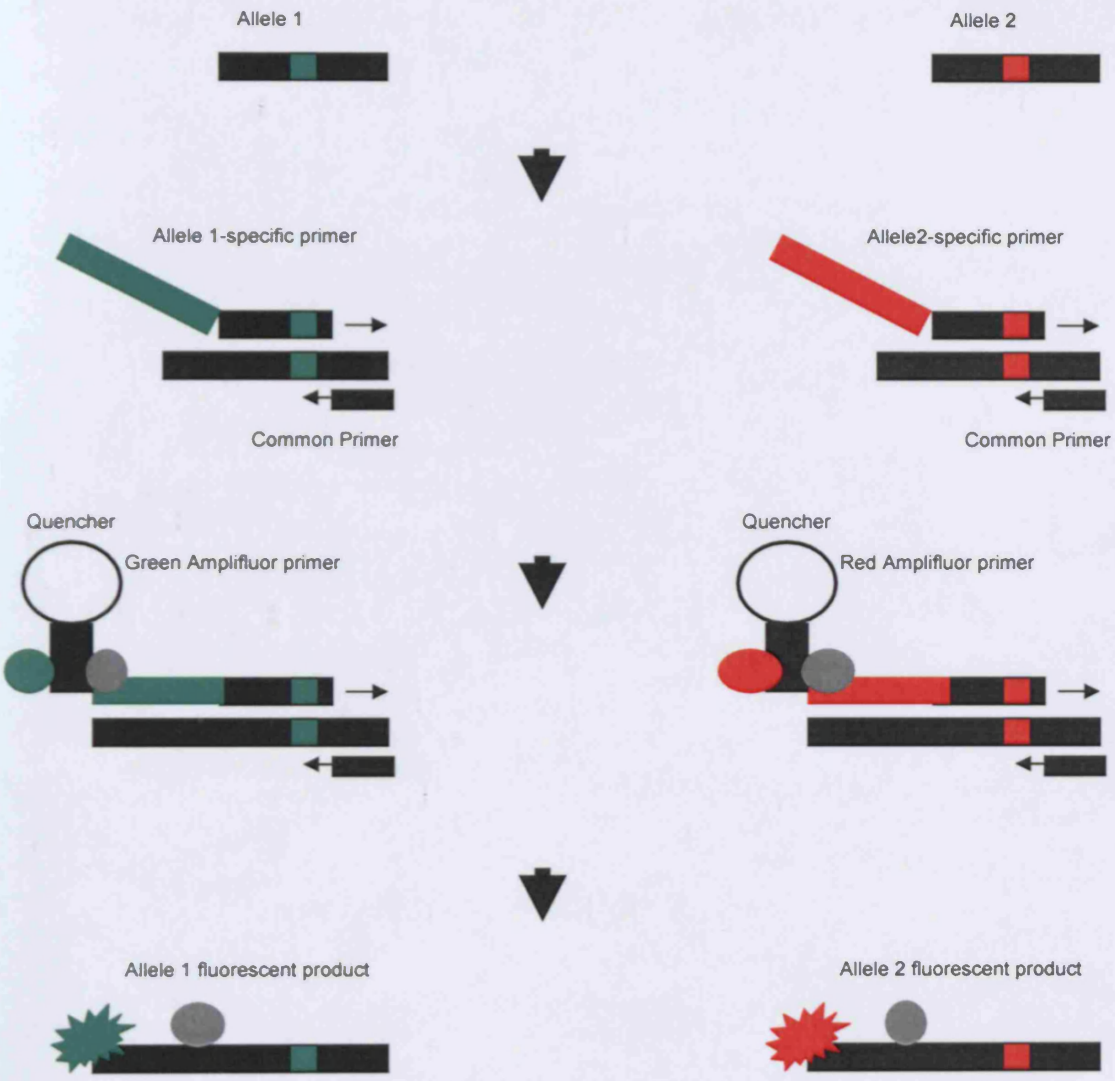


Figure 2.7a. Schematic of the step-by-step Amplifluor™ reaction.

The primer specific PCR primer mix for the reaction (primers were obtained at 100 pmol/µl);

Forward primer (Allele 1)	2.5 µl
Forward primer (Allele 2)	2.5 µl
Reverse primer	25 µl
ddH_2O	470 µl

The reagent mix for the Amplifluor™ reaction;

10x Titanium Taq Buffer (BD Biosciences, NJ, USA)	0.5 µl
dNTPs (2.5 mM each) (Amersham Biosciences, UK)	0.4 µl
Primer mix (as prepared above)	0.07 µl
SR labelled primer (BD Biosciences, NJ, USA)	0.07 µl
FAM labelled primer (BD Bioscience, NJ, USA)	0.07 µl
Titanium Taq Polymerase (BD Biosciences, NJ, USA)	0.05 µl
Reaction Mix S (BD Biosciences, NJ, USA)*	0.0625 µl*
ddH_2O	3.84 µl (3.2 µl*)

* Reaction Mix S is an optional additive which may improve assay clusters.

The total volume of the reaction mix is 5 µl. The mix was added to 15 ng of dried sample DNA in a black 384 well microtitre plate (ABgene, Epsom, UK). For large scale genotyping the reaction mix was added using a Biomek® FX^P Laboratory Automation Workstation (Beckman Coulter Inc, Fullerton, California, USA). The cycling conditions for the reaction on a MJ thermocycler (MJ Research, Rayne, UK) are as follows;

1. 96°C for 4 minutes
2. 96°C for 10 seconds
3. 58°C for 5 seconds*
4. 72°C for 10 seconds
5. Repeat steps 2 – 4 for 20 cycles
6. 96°C for 10 seconds
7. 55°C for 20 seconds
8. 72°C for 40 seconds
9. Repeat steps 6 – 8 for 15 - 22 cycles*
10. 68°C for 7 minutes
11. 15°C for 10 minutes

*These steps require optimisation for each specific polymorphism reaction.

The Amplifluor™ reaction takes place in two stages. The first stage (steps 1-5) involves the denaturation of the DNA sample, the annealing of the allele specific primers to the DNA template and the elongation of the fragments which include the complimentary tails for the universal UniPrimer. In the second stage (steps 6-11) the PCR product is denatured and the UniPrimers anneal to their complimentary sequence and then subsequently extend to unfold their hairpin structures and allow a fluorescent signal to be generated. The fluorescence of each sample was analysed using an Analyst HTS Assay Detection Platform (LJL Biosystems, Sunnyvale, California, USA) at the wavelengths shown in Table 2.7a.

Table 2.7a. Excitation and Emission wavelengths for the each fluorophore (FAM and SR) used in this study.

	Excitation	**Emission**
FAM	485 nm	520 nm
SR	580 nm	620 nm

The results are given as signal intensities for the two fluorophores which were plotted on a graph program (http://apps.serologicals.com). The clusters of sample fluorescent points correspond to the three genotype classes. The output of the program is in the form of 11, 12 or 22 where 1 corresponds to the FAM allele and 2 the SR allele.

2.7.3. Sequenom™ iPlexGOLD™ MassARRAY™

The Sequenom™ MassArray™ genotyping system allows the highly accurate genotyping of simple polymorphisms by combining primer extension chemistry with MALDI-ToF (Matrix Assisted Laser Desorption Ionisation – Time of Flight) Mass Spectrometry (MS). Sequenom™ iPlexGOLD™ MassARRAY™ genotyping involves primer extension over the polymorphism of interest and the examination of the mass of the extended product to discern the genotype of a sample. The main advantages of the MassARRAY™ genotyping system is its accuracy and high assay multiplexing level (up to 39-plex) which increases throughput and minimises cost.

2.7.3.1. Multiplex Assay Design

The initial step of MassARRAY™ genotyping involves the design of the multiplex assay. For each polymorphism the flanking DNA sequence was obtained, from the UCSC browser 'get DNA' feature, with any DNA sequences or variants that may confound PCR amplification (e.g. known SNPs and repetitive sequence) highlighted to prevent assay design over these regions. The highest multiplex assay possible was then designed using the Sequenom™ MassARRAY™ Assay Design 3.1 software, which analyses the flanking sequences, primer parameters and secondary structures for each polymorphism to create an optimum set of multiplex reactions. Details of the PCR primers and an appropriate MassEXTEND™ extension primer for each polymorphism are provided in an output file (.trs) on design completion. Where necessary the design software adds a 10 bp non-specific "tag" sequence to the 5' end of PCR primers, to ensure they are detected later in the MALDI-ToF mass spectrum. The software designs the PCR oligonucleotides to create the shortest amplimer possible to allow efficient PCR,

and an annealing temperature as close to 56°C as possible to match the universal PCR conditions detailed below. The extension is terminated after a single base during the iPlex™ reaction. The mass is resolved either by the extended primer or by the addition of a non-specific sequence to the extension primer by the MassARRAY™ Assay Design software, allowing for better size discrimination between allele masses of different assays.

2.7.3.2. Multiplex Assay PCR

The forward and reverse PCR primers for all SNPs were combined into a primer mix with a final concentration of 1 pmol/µl for each of the separate primers. The PCR was performed on 15 ng of each sample aliquoted into a 384 well PCR plate and dried down by incubating at 55°C for 30 minutes. The PCR mastermix was made and 5 µl added to each of the 384 samples.

Multiplex PCR Reaction Mix;

ddH_2O	3.39 µl
PCR Buffer 10x (Qiagen, USA)	0.625 µl
$MgCl_2$ (25mM) (Qiagen, USA)	0.325 µl
dNTPs (25mM) (Amersham Biosciences, UK)	0.1 µl
Hotstar Taq (Qiagen, USA)	0.06 µl
F and R PCR primers (1 pmol/µl)	0.5 µl

The PCR reaction was then performed on a MJ thermocycler (MJ Research, Rayne, UK) using the listed conditions:

1. 95°C for 15 minutes
2. 94°C for 20 seconds
3. 56°C for 30 seconds
4. 72°C for 1 minute
5. Repeat steps 2-4 for 44 cycles
6. 72°C for 3 minutes

The PCR products were examined by electrophoresis on an agarose gel (as described in section 2.6) to check that the correct sized products had been generated and there was no contamination present. Two genomic DNA positive samples and two negative water samples per 384-well PCR plate plus two reaction mix blanks were electrophoresed. Due to the large amount of primers being added to the reaction it was important to recognise any primer dimers that were present in either the DNA samples or the negative controls and not mistake them for either PCR product or contamination.

2.7.3.3. PCR Purification

If no PCR contamination was identified by the agarose gel electrophoresis, the PCR product was cleaned using the shrimp alkaline phosphatase enzyme (SAP) to remove any unincorporated dNTPs and unused primers from the reaction. A 2 µl mix was added to each PCR product. The mix contained;

Shrimp alkaline phosphatase (SAP)	0.3 µl
SAP buffer	0.17 µl
ddH_2O	1.53 µl

The samples were then incubated to allow PCR clean up, before the enzyme is denatured. The thermocycler conditions for this reaction are as follows;

1. 37°C for 30 minutes
2. 85°C for 15 minutes
3. 95°C for 5 minutes

2.7.3.4. iPlexGOLD™ Primer Extension

The iPlex™ extension primers are designed to extend over the polymorphic base to one base past the SNP before termination. This allows un-extended and extended primer to be separated by sufficient mass for accurate genotyping of alleles. The MALDI-ToF system can resolve masses that differ by as little as 3 Daltons. Regardless of the number of assays, the reaction conditions are universal for all stages, the only optimisation required is the adjustment of extension primer concentration. This optimisation was achieved by amplification of 18 control DNA

samples and 6 negative controls. The procedure split the extension primers into four groups dependent on their masses, which were initially diluted to final concentrations of 0.9375 µM, 1.17 µM, 1.425 µM and 1.875 µM (lowest mass to highest mass). Extension primers are divided into mass groups because lower mass products generate a lower signal-to-noise ratio when detected by MALDI-ToF MS. After the initial test run extension primers were diluted according to the following equation:

$$DilutionFactor = \frac{OptimalPeakHeight}{(ActualPeakHeight/10)}$$

The optimum peak height (user detected ~ 30-50) and actual peak height are obtained from the raw MassARRAY spectra. At the optimisation stage, failed or anomalous assays (e.g. self-priming) are removed.

Once the multiplex PCR has been SAP treated the optimised iPlex extension reaction can be performed. The extension reaction involves the addition of the unextended iPlex™ primer, the specific 3 base termination mix (two ddNTPs and one dNTP corresponding to the polymorphic base plus the extra non-polymorphic base) and thermosequenase in the following mix;

iPlexGOLD™ Buffer Plus	0.2 µl
iPlexGOLD™ Termination Mix	0.2 µl
Extension Primer Mix	1.559 µl
iPlex™ Enzyme	0.041 µl

2 µl of the iPlex™ extension reaction mix was added to each 7 µl of clean PCR product and thermocycled following the conditions detailed below;

1. 94°C for 30 seconds
2. 94°C for 5 seconds
3. 52°C for 5 seconds
4. 80°C for 5 seconds
5. Repeat steps 3-4 for 4 cycles
6. Repeat steps 2-5 for 39 cyles
7. 72°C for 3 minutes
8. 4°C for 10 minutes

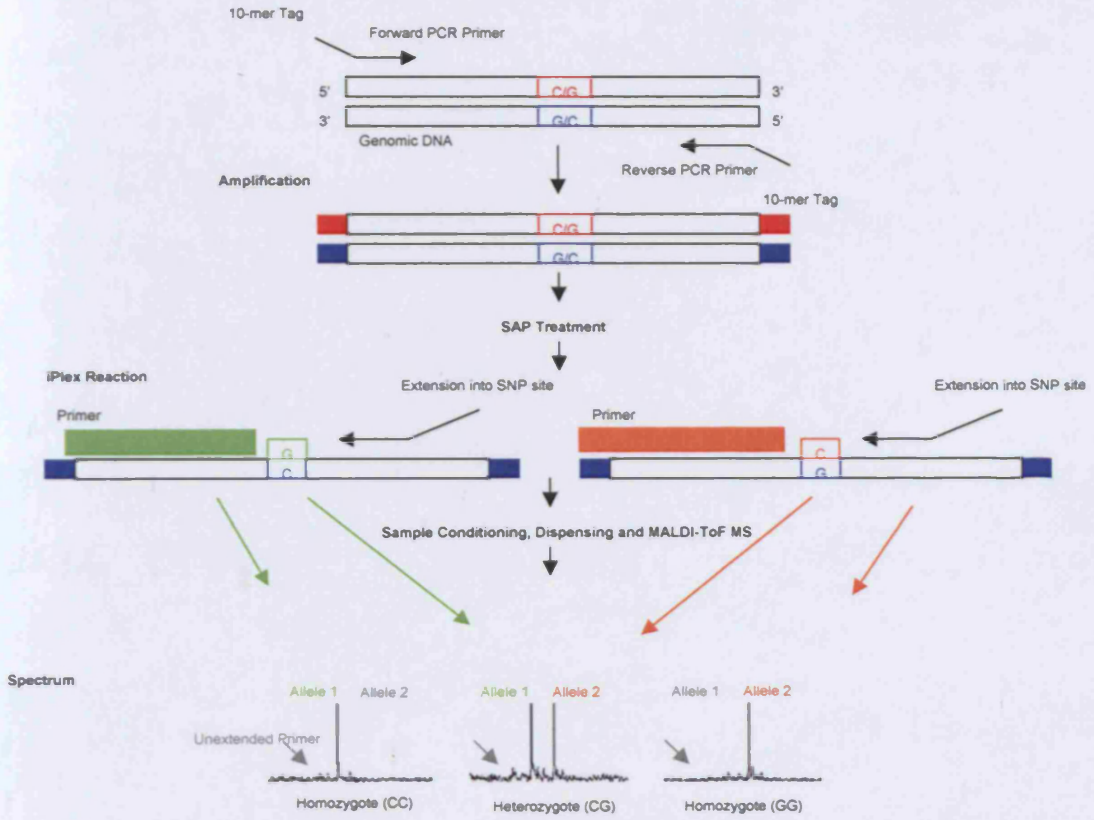


Figure 2.7b. Schematic of the Sequenom™ iPlex™ Assay (The scheme depicts a single assay).

2.7.4. Quality Control

With all genotyping methods it is vital that standard quality control procedures are adopted to ensure accurate genotype calls. On all genotyping plates a number of wells were "blank" meaning that they were empty of DNA. An aliquot of reaction mix was added to these wells, and post-PCR an aliquot from at least two blank wells was run on agarose gel electrophoresis. This procedure checks for assay contamination. A number of case and control samples were duplicated across sample plates as one method of calculating genotype accuracy and correlation. Assays were optimized in the Centre d'Etude du Polymorphisme Humain (CEPH) sample and where possible, the in house genotype calls were compared to those freely available from the HapMap database (www.hapmap.org). CEPH samples were also included on every sample plate. In house CEPH genotypes and HapMap CEPH genotypes were compared using a command script operated program written by Dobril Ivanov. Markers were assessed to ensure they followed the law of Hardy-Weinberg Equilibrium (HWE). This states; 'If an infinitely large, randomly mating population is free from outside evolutionary forces (i.e. mutation, migration and natural selection), then the gene frequencies will not change over time'. Using a chi squared distribution the observed genotype frequencies obtained from the data were compared for deviation from the expected genotype frequencies as defined by the law of HWE.

2.8 Data Storage

Following genotyping and QC checks, raw data were formatted so that it could be uploaded into in-house PsychMed database (created by Dr. Ivan Nikolov). Numerous formats are acceptable for upload, dependent on the genotyping system. Sequenom™ data is acceptable in its output format. The PsychMed database contains all the phenotypic data for all the samples in the study. Once all the genotypic data had been imported, the database was used to store data and construct the PLINK ready files for statistical analysis.

2.9. Statistical Analysis

Following genotyping and providing markers passed QC, the numbers of individuals homozygous for allele 1, heterozygous, and homozygous for allele 2 was determined; from these figures 2 × 3 Chi-square tables were constructed to calculate genotypic chi square (χ^2) and p-values for differences between groups. Allele frequencies in each comparison group, relative allelic χ^2 using 2 x 2 tables, p-value and odds ratio were calculated using the PLINK software (section 2.9.1). Polymorphisms with allele or genotype frequency differences at $P \leq 0.05$ were considered as significantly associated with disease after permutation correction for multiple testing. Permutation correction was performed using 10,000 permutations via the PLINK software. This corrects for multiple testing but accounts for correlation between markers and so is less conservative than a Bonferroni correction, which is appropriate for independent tests. To perform the permutation test, the case and control status are reassigned. In each replicate, all the selected markers are analysed and the most significant p-value stored, so that the permutation procedure gives a significance level corrected for the multiple markers tested. The D' and r^2 estimates of linkage disequilibrium (LD) were calculated using various versions of HAPLOVIEW (http://www.broad.mit.edu/personal/jcbarret/HAPLOVIEW/index.php) (section 2.9.2).Most haplotype frequency analysis was performed using PLINK with a permutation test. Some early haplotype analyses were performed using UNPHASED (v3.0) (Dudbridge 2003). All polymorphisms typed individually were tested for deviation from Hardy-Weinberg equilibrium (HWE) using a goodness of fit test using the PLINK software (Purcell et al. 2007).

2.9.1. PLINK

PLINK (v1.05 and v1.06) is freely available for online download (http://pngu.mgh.harvard.edu/purcell/plink/). The software is a whole genome association analysis toolset, designed to perform a range of basic, large-scale analyses in a computationally efficient manner (Purcell et al. 2007). There are five main functional domains to PLINK; Data management, which allows data recoding and reformatting, file merging and compressing and subset extraction amongst

other operations; Summary statistics for quality control, which include functions such as HWE calculations, missing call rates, inbreeding and non-Mendelian transmission; Population stratification detection; Association analysis, in both case/control and familial samples using multiple methods and including multiple testing correction by permutation analysis; and Identity-by-descent estimation. Other functions include haplotypic tests and imputation methods, copy number variant analysis and epidemiologic analyses. PLINK data can be integrated into HAPLOVIEW using the gPLINK function. gPLINK is a freely-available, Java-based software package that is a graphical user interface (GUI) allowing construction of many common PLINK operations. In this thesis, the summary statistics; association analysis, haplotype testing, logistic regression, multidimensional scaling, identical by state calculation and set-based analysis functions of PLINK were utilised. These were run using cygwin, a Linux-like environment for Windows freely available at http://www.cygwin.com/.

2.9.2. HAPLOVIEW

HAPLOVIEW is designed to simplify and expedite the process of haplotype analysis by providing a common interface to several tasks relating to such analyses (Barrett et al. 2005). HAPLOVIEW supports LD & haplotype block analysis, haplotype population frequency estimation, single SNP and haplotype association tests and permutation testing for association significance. HAPLOVIEW accepts input in a variety of formats. Pedigree data can be loaded as partially or fully phased chromosomes or as unphased diplotypes in a standard linkage/PLINK format. These formats allow the user to specify family structure information as well as disease affection or case/control status. Marker information, including name and location is loaded separately. HAPLOVIEW directly accepts genotype data 'dumped' from the Human HapMap website (http://www.hapmap.org) detailed in section 2.10.3 or can retrieve data directly from the HapMap website providing an internet connection is in place. The software presents a series of marker genotyping quality control measures which include a check for conformance with HWE, a tally of Mendelian inheritance errors and the genotyping success rate for each marker. The program filters out markers which fall below a preset threshold for these tests which can be defined by the user, who can also add or remove

markers from the analysis. The HAPLOVIEW program calculates several pairwise measures of LD, which it uses to create a graphical representation, examples of which are presented throughout the thesis. A number of different colour schemes are available to represent the LD relationships in either D' or r^2. HAPLOVIEW uses a two marker expectation-maximization (EM) algorithm (ignoring missing data) to estimate the maximum-likelihood values of the four gamete frequencies, from which the D', LOD and r^2 calculations are derived (Barrett et al. 2005). Hedrick's multiallelic D', is also used, which represents the degree of LD between two blocks, treating each haplotype within a block as an 'allele' of that region (Barrett et al. 2005). Several block definitions are available to partition the region into segments of strong LD; alternatively manual selection of groups of markers for haplotype analysis is possible. If affection status is included in the input file, HAPLOVIEW calculates the standard transmission disequilibrium test (TDT) statistic (for trio data) or simple χ^2 (for case/control data) for each marker that can be used for association studies. Haplotype and pairwise-tag SNP selection methods are included in the software, as well as haplotype-based association testing and evaluation of significance using permutation testing. The information produced by HAPLOVIEW may be exported to a portable network graphics for use in presentations or publications or dumped to a text file. Through-out this project HAPLOVIEW was frequently used to calculate the LD between variants and create graphical representations of these LD measures. The software was also used to choose tag SNPs for a locus using the pair-wise tagging approach ($r^2 > 0.8$; MAF > 0.05) and to estimate the coverage of a locus by previously genotyped markers.

2.9.3 UNPHASED

UNPHASED (v3.0) is a versatile application for performing genetic association analysis. Its features include; analysis of nuclear families and unrelated subjects, and combinations of the two, analysis of discrete or quantitative traits, maximum likelihood treatment of missing genotype data and uncertain haplotypes, global association tests and tests of individual haplotypes, conditioning tests that allow for previous associations of linked loci, inclusion of information from additional tag markers, support for non-genetic covariates including parent-of-

origin, and permutation tests. For this thesis, the association tests of haplotypes function were used for some of the haplotypes investigated in chapter 3.

2.9.4. R

R (v2.9.1) is a language and environment for statistical computing and graphics. It is available as free Software (http://www.R-project.org) and runs on a wide variety of UNIX platforms, Windows and MacOS. R includes; a data handling and storage facility, a suite of operators for calculations on arrays, a large integrated collection of intermediate tools for data analysis, graphical facilities for data analysis and display either on-screen or on hardcopy, and simple and effective programming language which includes conditionals, loops, user-defined recursive functions and input and output facilities (R Development Team 2008). In this thesis, R was used to create the quantile-quantile plots for the LOAD+P GWAS in chapter 5.

2.9.5. WGAViewer

WGAViewer (v1.26A) is a free software tool (http://people.genome.duke.edu/~dg48/WGAViewer/download.php) developed in JAVA language that is designed to annotate, visualize, and help interpret the full set of p-values indicating evidence of association resulting from a Whole Genome Association (WGA) study (Ge et al. 2008). The software includes; chromosome view of WGA results, genetic annotation of WGA results, SNP annotation, gene/SNP finding, comparison of multiple genome scans, and uploading of supporting/QC data. The WGAViewer was extensively used in chapter 5 of this thesis to visualise the LOAD+P GWAS data. Specifically to annotate top hits, find SNPs of interest or relevant proxies using the WGAViewer's link to the HapMap database, find genes of interest, create a gene SNP list, and to use the databases external links to define SNP and gene functions.

2.9.6. Power Calculations

Power estimations and sample size requirements for case-control samples were calculated using the downloadable Power and Sample Size Calculation (PS) (v3.0) program provided by the Department of Biostatistics, Vanderbilt University (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). PS is an interactive program for performing power and sample size calculations. The program can determine the sample size needed to detect a specified alternative hypothesis with the required power, the power with which a specific alternative hypothesis can be detected with a given sample size, or the specific alternative hypotheses that can be detected with a given power and sample size (Dupont and Plummer 1998). The PS program can evaluate multiple study designs as listed in Table 2.9a. For this study the Dichotomous tab was used to compare case-control sample designs analyzed by chi-squared or Fisher's exact-test. The PS program can produce graphs to explore the relationships between power, sample size and detectable alternative hypotheses and multiple curves can be plotted on a single graphic allowing easy comparison. PS graphics may be saved as Windows Metafiles, printed, or copied and pasted into other programs. A useful introduction to the program and instruction on its use is provided within the software.

Table 2.9a. The PS program tab labels and the study designs provided by each tab. The program provides an additional tab which summarises the users' calculations and graphics.

Program Tab	Study Design
Dichotomous	Comparing matched or independent dichotomous outcomes
Manten-Haenszel	Test for common odds ratio within multiple 2 x 2 tables
Regression 1	Testing the slope of a simple linear regression line
Regression 2	Comparing two linear regression lines
Survival	Comparing survival data for two groups
t-test	Comparing matched or independent continuous outcomes

2.10. Bioinformatics

Numerous web-based resources allow the user to search for and retrieve desired information from the wealth of available data on the human genome and the research that has been conducted in the field of genetics/genomics. These resources database and correlate this information behind user-friendly interfaces for quick and easy access. Details of the bioinformatics resources predominantly used during this study are provided in this section.

2.10.1. National Center for Biotechnology Information

The National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) was established in the USA in 1988 as a resource for molecular biology information; NCBI creates public databases, conducts research in computational biology, develops software tools for analyzing genome data, and disseminates biomedical information to improve understanding of molecular processes affecting human health and disease. For this thesis, the public databases of the NCBI were utilised, primarily the PubMed database. PubMed is a service of the US National Library of Medicine (http://www.nlm.nih.gov/), it includes over 18 million citations from the MEDLINE database (http://medline.cos.com/) and other life science journals for biomedical articles back to 1948. PubMed provides an advanced search facility to link to full text articles and other related resources. Other databases utilised include; 1) The Online Mendelian Inheritance in Man® (OMIM®) database which is a comprehensive compendium of human genes and genetic phenotypes. The full-text, referenced overviews in OMIM® contain information on all known Mendelian disorders and over 12,000 genes. OMIM® focuses on the relationship between phenotype and genotype, it is updated daily, and the entries contain links to other genetics resources. 2) The Entrez Single Nucleotide Polymorphism (SNP) database catalogues all known genetic variants allowing one to search for a polymorphism/groups of polymorphisms across the genomes of numerous organisms. The SNP record contains substantial information for each marker, including; the allelic variation seen, chromosomal position, base position, gene name (if intragenic), polymorphism function, assay method used to

detect polymorphism and genotyping success rate, heterozygosity in genotyped samples, date of publication, all polymorphism IDs used, validation status, and polymorphism class (e.g. SNP, microsatellite). The SNP database is regularly updated and provides links to other useful resources, both internal and external to NCBI. 3) The Entrez Gene database which is a searchable database of genes; from Reference Sequence (RefSeq) genomes (http://www.ncbi.nlm.nih.gov/RefSeq/) defined by sequence and/or located in the NCBI Map Viewer (http://www.ncbi.nlm.nih.gov/mapview/). The RefSeq collection aims to provide a comprehensive, integrated, non-redundant, well-annotated set of sequences, including genomic DNA, transcripts, and proteins. It provides a stable reference for genome annotation, gene identification and characterisation, mutation and polymorphism analysis, expression studies, and comparative analyses. Entrez Gene aims to supply key connections in the nexus of map, sequence, expression, structure, function, citation, and homology data. Unique identifiers are assigned to genes with defining sequences, genes with known map positions, and genes inferred from phenotypic information. These gene identifiers are used throughout NCBI's databases and tracked through updates of annotation and related information.

2.10.2. University of California Santa Cruz Genome Browser

The University of California Santa Cruz (UCSC) Genome Browser website (Kent et al. 2002; Karolchik et al. 2008) (http://genome.ucsc.edu/) contains the reference sequence and working draft assemblies for a large collection of genomes. It also provides a portal to the ENCyclopedia Of DNA Elements (ENCODE) project (Kent 2007) (http://www.genome.gov/10005107). The website provides a number of applications. The Genome Browser was intensively used during this project. This allows the user to zoom and scroll over chromosomes, showing the work of annotators worldwide and providing detailed information of each known or hypothesised element (e.g. gene, polymorphism, repeat element). The Table Browser (Karolchik et al. 2004) provides convenient access to the underlying database, this function of the UCSC Genome Browser was also heavily used, specifically to identify conserved regions and is detailed further in section

2.11.2. The basic local alignment tool (blat) (Kent 2002) maps your sequence to the genome, while In-Silico PCR searches a sequence database with a pair of PCR primers, using an indexing strategy for fast performance. Both these functions were utilised during this project to ensure amplimers and PCR primers utilised were region specific. The Gene Sorter shows expression, homology and other information on groups of genes that can be related in many ways. VisiGene lets you browse through a large collection of *in situ* mouse and frog images to examine expression patterns. While, Genome Graphs allows you to upload and display genome-wide data sets.

2.10.3. The International HapMap Project

The International HapMap Project (The International HapMap Consortium 2003; The International HapMap Consortium 2004; The International HapMap Consortium 2005; Frazer et al. 2007; Thorisson et al. 2005) aims to develop a haplotype map of the human genome. The Project is a collaboration among scientists in Japan, the UK, Canada, China, Nigeria, and the US. Due to haplotype frequency differences between populations several populations (30 trios of Yoruba people in Ibadan, Nigeria; 45 unrelated Japanese individuals from Tokyo; 45 unrelated Han Chinese individuals from Beijing and 30 trios from the US with ancestry from Northern and Western Europe which were collected in 1980 by the Centre d'Etude du Polymorphisme Humain (CEPH)) are being genotyped by the HapMap project. Phase I and II of the HapMap data were used in this thesis which contains data on approximately 3.4 million SNPs the majority of which were identified by the project. Aliquots of the CEPH DNA samples were used to optimise assays and as a quality control measure on each sample plate. The HapMap project data can be directly downloaded or 'dumped' from the website providing genotype data of all genotyped SNPs in a particular gene or region. These data were used to choose tag SNPs for genes/regions and to define the degree of LD between linked variants using the HAPLOVIEW software (section 2.9.2).

2.10.4. Ensembl Genome Browser

Ensembl began in 2000 as a browser for the human genome, but has expanded to host the genomes and associated information for more than 40 species of vertebrates and other eukaryotic species (Flicek et al. 2008). Ensembl (v52) automatically annotates the genome, integrates this annotation with other available biological data and makes this data publicly available via the web (http://www.ensembl.org/index.html). The range of available data has expanded to include comparative genomics, variation and regulatory data, in addition to the long established location, gene, transcript and variation views previously available. The Ensembl database allows the user to add and remove tracks, configure graphical displays and upload and manage your own data. The database also provides links to other useful web-based resources. The BioMart function of Ensembl is a search engine that can find multiple terms and put them into a table format. For example, human gene IDs, chromosome and base pair position.

2.10.5. SchizophreniaGene

The SchizophreniaGene (szgene) database (http://www.schizophreniaforum.org/res/sczgene/default.asp) is a comprehensive, unbiased, publicly available and regularly updated collection of published genetic association studies performed on schizophrenia (Allen et al. 2008). Eligible publications are identified following systematic searches of scientific literature databases, as well as the table of contents of journals in genetics and psychiatry (Allen et al. 2008). This database calculates crude odds ratios (ORs) from the reported allele distributions of SNPs in four or more independent samples. The most promising meta-analysis results indicate the most strongly associated genes ('Top Results'), which are ranked by effect size. In this thesis, the szgene database's 'Top Results' list was used to define genes investigated in secondary analyses in chapter 5. The SNPs which led these genes to be considered as 'Top Results' were also investigated in the LOAD+P GWAS data.

2.10.6. SNP Annotation and Proxy Search

The SNP Annotation and Proxy Search (SNAP) (v2.1), provided by the Broad Institute (http://www.broadinstitute.org/mpg/snap/), finds proxy SNPs based on linkage disequilibrium, physical distance and/or membership in selected commercial genotyping arrays (Johnson et al. 2008). Pair-wise linkage disequilibrium is pre-calculated based on phased genotype data from the HapMap (Johnson et al. 2008). The software can also generate linkage disequilibrium plots and allows the user to map SNP IDs. For this thesis the proxy search facility was used to identify SNPs present on the Affymetrix 5.0 array which act as proxies for SNPs present on the Illumina HumanHap 610quad array. The proxy search application allows batch upload of SNPs of interest and filtering of the proxy search by either Affymetrix or Illumina arrays.

2.11. Conservation and Transcription Factor Binding Site Prediction

Programs to identify evolutionary conserved regions (ECRs) such as transcription factor binding sites, or putative binding motifs are a useful tool to decipher the functionality of an association. ECRs correspond to protein coding exons which are generally under strong selection pressure to stay unchanged. Other ECRs can be identified, including promoter and enhancer elements which are usually located upstream of a gene; functional elements that lie far away from genes and regulate the spatial and temporal transcription patterns of neighbouring genes, and active regulatory elements that can be located hundreds of kb away from the genes that they control, especially in regions of low gene density. Three software packages were used in this study to identify such regions.

2.11.1. Cluster Buster

Cluster Buster (Frith et al. 2003) is a program for finding clusters of pre-specified motifs in nucleotide sequences. The program is freely available and may be used via a web server (http://zlab.bu.edu/cluster-buster/cbust.html) or downloaded for use on a local computer. Genomic sequences greater than

100,000 kb can only be analysed via the downloaded program. The main application of Cluster Buster is detection of sequences that regulate gene transcription, such as enhancers and silencers, but other types of biological regulation may be mediated by motif clusters. The Cluster Buster program provides numerous motifs for the user to select and identify within their. These motifs are; TATA boxes (bound by TATA binding protein, a component of the transcription factor binding complex TFIID), CCAAT boxes (found in gene promoters and bound by the transcription factor NF-Y), Sp1 (found in gene promoters and bound by transcription factors), AP-1 (Activator Protein 1), CRE (cAMP Response Element), Ets, ERE (Estrogen Response Element), Myc, NF1, GATA, E2F, LSF, Mef-2 (Myocyte Enhancer Factor 2), SRF (Serum Response Factor), Myf (Myogenin / MyoD family) and Tef (Transcription Enhancer Factor) sequences. It is also possible to enter your own motifs or upload predicted or verified motifs from other genetic resources into the Cluster Buster program. The software parameters can be set by the user and include; gap parameter, cluster score threshold, motif score threshold, residue abundance range, and pseudocount, details of which can be found at the webpage listed above. All analyses reported in this thesis utilised the default parameter settings and selection to search for all motifs provided by the program. The Cluster Buster output provides a diagram of motif cluster locations within the input sequence, along with protein-coding regions (CDS) annotated on the GenBank record. Detailed information for each cluster is also supplied. This includes a comprehensive cluster diagram and the sequence position, DNA strand, log likelihood ratio and nucleotide bases of each motif identified within the cluster. The position of the identified clusters and the motifs within can then be compared to the position of known polymorphisms within the nucleotide sequence of interest.

2.11.2. University of California Santa Cruz Most Conserved Track

The University of California Santa Cruz (UCSC) Most Conserved Track is a facility within the Table Browser (Karolchik et al. 2004) option of the UCSC web based database (http://genome.ucsc.edu/). The Table Browser provides convenient access to the underlying database and enables the user to retrieve the data associated with a track in text format. The Most Conserved Track is part of the

Comparative Genomics group within the Table Browser. For this thesis the *clade* Vertebrate, *genome* Human, *assembly* Mar. 2006 and *table* Mammal (phastConsElements28wayPlacMammal) were utilised on a specified chromosomal position with *output format* all fields from the selected table. The data output of the chromosomal positions of the most conserved regions corresponds to those depicted on the genome browser track. Using this output it is possible to accurately align polymorphisms within your nucleotide sequence of interest to the most conserved mammalian regions according to the UCSC genome browser.

2.11.3. Evolutionary Conserved Region Browser

The Evolutionary Conserved Region (ECR) browser is a web based software package that allows comparison of Human, Mouse, Chimpanzee, Dog, Opossum, Rat, Cow, Chicken, Frog, Blowfish, Zebrafish and Pufferfish genomes (Ovcharenko et al. 2004) (http://ecrbrowser.dcode.org/). The conservation profile is created by aligning one "base" sequence with all others in a pair-wise fashion, utilizing the Basic Local Alignment Tool (BLAT) (http://genome.brc.mcw.edu/cgi-bin/hgBlat) and Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Finally a pair of sequences from every homology block is aligned to establish nucleotide level match-mismatch similarity profiles using BLASTZ (Schwartz et al. 2003). ECRs are identified as regions of high sequence identity against a neutrally evolving background. By scanning an alignment the browser detects and highlights sequence elements of significant length that are conserved above a specified level of sequence identity between the two genomes (user-defined parameter). Visually ECRs are represented as coloured peaks on a graph, with the x-axis representing positions in the base genome and the y-axis representing percentage identity between the base and aligned genomes at that specified position. The quickest way to navigate the ECR browser is to type in the gene name or the chromosomal position into the ECR interface. However, for absolute accuracy it is advisable to submit a nucleotide sequence into the software to which the program will align. In this thesis the Human genome (freeze 18) was used as the base sequence which was aligned with the Dog and Mouse genomes. In certain regions Human-Mouse conservation is too high for alignments to usefully single-out specific conserved elements for further

study (Nobrega et al. 2003). Therefore, Human-Dog conservation was also analysed. The Dog genome is evolutionary more distant to the Human genome than Mouse, Rat and Chimpanzee genomes and therefore comparing Human, Mouse and Dog genomes should highlight only those regions that have been selectively conserved in Mammals. Only regions conserved between all three genomes were classed as ECRs. The sequence identity threshold for an alignment to be called an ECR was set at default (minimum sequence length 100 bp, minimum sequence identity 70%), thus detecting only very long or highly conserved ECRs. Through the conserved SNP tab the ECR browser also allows easy access to and data output of database SNPs residing within the reference sequence that lie within conserved regions in each species. This list of conserved SNPs can then be compared between species to highlight any SNPs that overlap species.

3. Psychosis Candidate Gene Association Study

3.1. Introduction

In this chapter, specific markers which have previously been directly or indirectly associated with psychosis, have been tested for association with LOAD+P. This experimental methodology is predominantly based on the hypothesis that psychosis susceptibly genes may act across disease divides, thus modifying psychotic symptoms in multiple distinct disorders. The hypothesis that psychosis susceptibility genes may affect psychosis in Alzheimer's disease is borne from the findings of recent studies which have shown overlap in the genetic susceptibility variants for distinct complex psychotic disorders such as bipolar disorder and schizophrenia (Craddock et al. 2009). The majority of the genes chosen for investigation in this study have long been considered to show association to either schizophrenia, bipolar disorder, or both disorders. The additional genes were chosen for investigation due to the recent (at the date of study commencement) identification of novel variants showing significant association to schizophrenia. The genes and where applicable, the specific variants studied were identified from literature searches, using the NCBI PubMed database using search terms; 'schizophrenia AND genetic AND association', 'bipolar AND genetic AND association' and 'psychosis AND genetic AND association'. The szgene website was used to identify and prioritise genes. Finally the list of genes identified was refined by a number of world renowned experts in the field of psychiatric genetics, including Professor's Michael Owen, Michael O'Donovan, Nick Craddock and Dr. Nigel Williams. Genes were also prioritised if they had previously shown a genetic association to a neurodegenerative disorder (Rubinsztein et al. 1997), or if they resided in a region of the genome identified as showing linkage to LOAD+P (Hollingworth et al. 2007). In this chapter the general experimental design of the study is discussed, before a literature review, the experimental design strategy, and the results of the studied genes are reported. Conclusions include a discussion of the study, its limitations and implications.

3.2. Genotyping Strategy

This study is based on the hypothesis that the genes investigated may modify psychotic symptoms across disease. Thus, in LOAD these genes are assumed to work independently of LOAD susceptibility genes. Therefore, the most appropriate and conservative study design is to compare allele and genotype frequencies between LOAD cases with psychosis (LOAD+P) with LOAD cases without psychosis (LOAD-P) in a case-control design. Using the diagnostic criteria described in chapter 2 2.3, there are 379 LOAD+P cases and 269 LOAD-P cases available for genotyping from the MRC genetic resource for LOAD sample. This dataset provides a limited power of 0.34 to detect an association with an effect size of 1.3 and a risk variant frequency of 30%. An alternative hypothesis is that these putative psychosis susceptibility genes may be involved in the aetiology of AD+P, as a sub-form of the disease. This hypothesis is tested by comparing allele and genotype frequencies of LOAD+P cases with control subjects (n=1,361) screened for both dementia and psychosis, and carefully matched with cases for age, sex, sex and ethnicity. This analysis provides a power of 0.57 to detect the association detailed above. As the latter analysis may identify variants associated with LOAD alone rather than the LOAD+P sub-form of disease, allele and genotype frequencies of LOAD cases (n=1,205) against control subjects have also been compared. This analysis has a power of 0.88 to detect the afore mentioned association. At loci where sex differences had previously been identified, data were re-analysed according to sex. For the association detailed above; male analyses had power of 0.11, 0.18, and 0.25 for LOAD+P (n=74) versus LOAD-P (n=67), LOAD+P versus control (n=423), and LOAD (n=282) versus control data respectively, female analyses had power of 0.23, 0.41, and 0.63 for LOAD+P (n=271) versus LOAD-P (n=164), LOAD+P versus control (n=695), and LOAD (n=693) versus control data respectively.

Markers were selected for each gene dependent on gene size. Where possible, variants were chosen to 'tag' the gene of interest ± 20 kb at an $r^2 \geq 0.8$ and a MAF ≥ 5% using Centre d'Etude du Polymorphisme Humain (CEPH) genotype data from the HapMap website or in house CEPH data from previous studies. However, where the gene size was large, requiring an excessive number

of markers to sufficiently 'tag' the region of interest, variants which have previously shown published association to psychosis were chosen for investigation. All SNPS were designed for genotyping using the Sequenom™ MassArray™ platform (Sequenom, San Diego, CA) extensively described in chapter 2. 7.3. The 4 bp *DISC1* variant was also genotyped using the Sequenom™ MassArray™ platform (Sequenom, San Diego, CA). The validity of this platform to genotype deletions has previously been confirmed (Green et al. 2006b). Where markers failed the assay design process or failed the genotyping quality control measures, they were instead genotyped using the Amplifluor™ UniPrimer Chemistry (chapter 2 .7.2). The two microsatellites and one TAA repeat studied were genotyped using the genescan fluorescent PCR methodology. The respective PCR and extension primers utilised are provided in Appendix 3. Example schematics for each genotyping method are provided in Appendix Figures 3.1, 3.2 and 3.3.

All assays were optimised, and deviation from Hardy–Weinberg equilibrium (HWE) was tested as described in chapter 2.7.4. The minimum genotyping success rate was set at 90% of the full sample set (n = 2,566). The percentage genotype for each variant in the full sample, and the HWE p-values for each SNP in each dataset (i.e. LOAD+P, LOAD-P, LOAD and control) are provided in Table 3.1. Ten SNPs show deviation from HWE in at least one dataset. The *DTNBP1* SNP rs2619539 shows deviation from HWE in both the LOAD-P and LOAD datasets (P = 0.001 and P = 0.04 respectively). The *GRM3* SNP rs2282966 shows a significant deviation from HWE in the control dataset (P = 0.001). The remaining 8 SNPs show nominally significant deviation from HWE (p-values 0.01 – 0.04) in 1 of the 4 datasets, with no over-representation in any 1 dataset.

For SNPs and haplotypes, statistically significant differences between genotype and allele frequencies were analysed as described in chapter 2.9. Microsatellite and TAA repeat genotype frequencies were estimated by direct counting. The significance of the distribution of alleles between comparison datasets were tested by the chi-square method, comparison between two groups was made with 95% confidence interval to estimate statistical significance. Power calculations are as described in chapter 2.9.6.

Table 3.1. All genotyped variants. Table provides chromosome, gene, marker name, base position, ancestral allele, marker function, CEU MAF, percentage genotyped in full sample, and HWE p-values in each comparison dataset.

								HWE			
Chr	**Gene**	**Marker**	**bp**	**Allele**	**Function**	**CEU MAF**	**% Geno**	**LOAD+P**	**LOAD-P**	**LOAD**	**Controls**
1	DISC1	rs3738401	228137030	G	Non-Syn	0.36	96.4	0.33	0.66	0.56	0.35
1	DISC1	rs6675281	228260836	C	Non-Syn	0.16	99.1	0.22	0.16	0.9	0.47
1	DISC1	rs821616	228451333	A	Non-Syn	0.31	97.2	0.89	1	0.28	0.57
1	DISC1	4bp deletion	n/a	n/a	Exonic	n/a	96.7	n/a	n/a	n/a	n/a
6	DTNBP1	rs12525702	15735750	C	Intronic	0.1	97.8	1	0.36	0.5	0.77
6	DTNBP1	rs17470454	15631427	G	Non-Syn	0.06	98.5	0.21	1	1	0.61
6	DTNBP1	rs2619538	15773188	T	5 prime	0.39	98.1	0.45	0.13	0.12	0.86
6	DTNBP1	rs2619539	15728834	C	Intronic	0.47	97	0.29	**0.001**	**0.04**	0.22
6	DTNBP1	rs3213207	15736081	A	Intronic	0.11	98.4	0.37	0.75	0.65	1
6	GRIK2	rs6922753	102354366	C	Intronic	0.1	92	0.65	0.46	0.8	0.9
6	GRIK2	TAA repeat	n/a	n/a	3'UTR	n/a	90.2	n/a	n/a	n/a	n/a
7	GRM3	rs13242038	86152356	C	Intronic	0.23	99.2	**0.03**	1	0.24	0.85
7	GRM3	rs1468412	86271387	A	Intronic	0.28	98.6	0.79	0.87	0.58	0.1
7	GRM3	rs187993	86101616	G	5 prime	0.34	99.9	0.3	0.06	0.5	0.7
7	GRM3	rs2228595	86253923	C	Synon	0.06	99.3	**0.01**	0.61	0.09	0.13
7	GRM3	rs2282966	86313603	G	Intronic	0.28	97.8	0.38	0.11	0.87	**0.001**
7	GRM3	rs6465084	86241411	A	Intronic	n/a	94	0.67	0.6	0.8	**0.04**
7	GRM3	rs917071	86191717	C	Intronic	0.28	95.5	0.51	0.88	0.53	1
8	NRG1	rs12681411	31582591	C	5 prime	0.35	97.8	0.5	0.5	0.05	0.15
8	NRG1	rs3924999	32572900	G	Non-Syn	0.43	96.2	0.82	1	0.39	1

8	NRG1	SNP8NRG221533	31593683	T	5 prime	n/a	95.6	0.76	0.54	0.49	0.96
8	NRG1	420M9-1395	n/a	n/a	5 prime	n/a	90.3	n/a	n/a	n/a	n/a
8	NRG1	478B14-848	n/a	n/a	5 prime	n/a	90.5	n/a	n/a	n/a	n/a
11	BDNF	rs6265	27636492	C	Non-Syn	0.2	99.8	1	0.17	0.03	0.24
13	DAOA	3' UTR	104927538	C	3' UTR	n/a	97.1	0.82	**0.02**	0.69	0.74
13	DAOA	rs1341402	104913510	T	5 prime	0.36	98.1	1	0.73	0.5	0.93
13	DAOA	rs1421292	104996236	T	3 prime	0.49	96.8	0.46	0.45	0.38	0.73
13	DAOA	rs2391191	104917447	C	Non-Syn	0.37	94.9	0.37	0.57	0.89	0.95
13	DAOA	rs3916965	104901361	G	5 prime	n/a	95.7	0.58	0.68	0.89	0.85
13	DAOA	rs3918342	104983750	C	3 prime	0.48	98.5	0.25	0.71	0.35	1
13	DAOA	rs778293	104967200	G	3 prime	0.39	97.7	0.59	0.11	0.4	0.33
13	DAOA	rs778294	104940236	G	Intronic	0.31	98.2	0.33	**0.04**	0.1	0.53
13	DAOA	rs954581	104950267	T	3 prime	0.12	97.7	0.38	**0.01**	0.22	0.27
17	CNP	rs55955100	37375664	A	Intronic	n/a	97.7	0.89	1	0.47	0.7
17	CNP	rs35967904	37379713	G	3' UTR	n/a	98.8	0.12	0.9	0.12	0.9
17	CNP	rs10540926	37371281	G	3 prime	n/a	98.1	0.71	0.36	0.44	0.75
17	CNP	rs11079028	37379659	G	3' UTR	0.23	97.9	0.87	0.18	0.51	0.18
17	CNP	rs11296	37380586	T	3' UTR	0.06	97.4	1	0.42	0.35	0.42
17	CNP	rs12602950	37377355	A	Intronic	0.25	97.7	0.08	0.88	0.64	0.88
17	CNP	rs12952915	37372078	G	5 prime	n/a	93.8	0.4	0.13	0.19	0.13
17	CNP	rs2070106	37379390	G	Synon	0.4	96.7	0.73	0.42	0.49	0.42
17	CNP	rs4796750	37368070	G	Intronic	0.25	96.6	**0.02**	0.82	0.2	0.82
17	CNP	rs4796751	37381063	C	3' UTR	0.12	98.8	0.66	1	0.45	1
21	OLIG2	rs62216115	33324327	G	Intronic	n/a	97.4	1	0.36	0.92	0.71
21	OLIG2	rs1005573	33320586	T	Intronic	0.3	96.6	0.45	0.54	0.66	0.53
21	OLIG2	rs1059004	33322333	A	3' UTR	n/a	95.4	0.33	1	0.9	0.51
21	OLIG2	rs11701698	33315815	T	Intronic	0.2	98.4	0.1	0.29	0.37	0.63

21	OLIG2	rs11701762	33325096	G	Intronic	n/a	96.6	0.4	0.15	0.87	0.11
21	OLIG2	rs13046814	33323247	T	3' UTR	n/a	96.2	0.26	0.77	0.58	0.41
21	OLIG2	rs2834070	33309322	G	Intronic	0.34	96.8	0.55	0.24	0.43	0.55
21	OLIG2	rs2834072	33332334	G	Intronic	0.48	96.8	1	0.16	0.9	0.6
21	OLIG2	rs762178	33321271	G	Synon	0.39	94.6	1	0.79	0.7	0.39
21	OLIG2	rs881666	33327136	G	Intronic	0.39	97.8	0.6	0.9	0.37	0.29
21	OLIG2	rs762237	33330047	C	Intronic	0.36	98.7	1	0.1	0.95	0.57
22	COMT	rs2020917	18303438	C	5 prime	0.33	97.7	1	0.05	0.4	0.19
22	COMT	rs737865	18304675	T	Intronic	0.33	99.1	0.52	0.08	0.29	0.1
22	COMT	rs4680	18325825	A	Non-Syn	0.48	95.2	0.16	0.7	0.05	0.48
22	COMT	rs165599	18331335	A	3' UTR	n/a	96.1	1	**0.01**	0.38	0.16

Note; SNP rs55955100 previously known as marker 3235AG, SNP rs35967904 previously known as marker 7284GA and SNP rs62216115 previously known as marker F16GA.

3.3. Disrupted in Schizophrenia 1

The disrupted in schizophrenia 1 (*DISC1*) locus was originally identified in a large Scottish pedigree, in which numerous members carried a balanced (1,11) (q42.1;q14.3) translocation that segregated with major psychiatric disorders, including schizophrenia, bipolar disorder and recurrent major depression (St Clair et al. 1990). The (1,11) (q42.1;q14.3) translocation lies in a gene desert on chromosome 11 and directly disrupts two genes, *DISC1* and Disrupted in schizophrenia 2 (*DISC2*) at 1q42 (Millar et al. 2000). Seven studies have reported linkage to the *DISC1* region (1q42) and neighbouring chromosomal bands in schizophrenia (Ekelund et al. 2001; Ekelund et al. 2004; Hovatta et al. 1999; Hwu et al. 2003) and bipolar disorder (Curtis et al. 2003; Detera-Wadleigh et al. 1999; Macgregor et al. 2004). However, these analyses have not consistently replicated, probably due to the employment of a more conservative phenotype (Macgregor et al. 2004; Segurado et al. 2003; Lewis et al. 2003). The strongest evidence for linkage in the region comes from analyses using a broad phenotype including schizophrenia, bipolar disorder, schizoaffective disorder and other non-affective psychotic disorders (Hamshere et al. 2005b; Hwu et al. 2003), suggesting the presence of one or more loci influencing susceptibility to a spectrum of psychotic disorders.

The first association study of *DISC1* produced negative results, with no association to schizophrenia or bipolar disorder (Devon et al. 2001). A positive association of multiple haplotypes across *DISC1* with a broad psychosis phenotype (schizophrenia, schizoaffective disorder, schizophrenia spectrum disorder and bipolar disorder or major depressive disorder) was reported in 2003 in a population of Finnish families (Hennah et al. 2003). The most significant association ($P = 3.1 \times 10^{-3}$) was seen with a common two SNP haplotype (rs751229 and rs3738401) (HEP3), spanning from intron 1 to exon 2, which also displayed sex differences ($P= 2.4 \times 10^{-4}$) (Hennah et al. 2003). Additional positive (Hodgkinson et al. 2004; Palo et al. 2007; Zhang et al. 2006) and negative (Thomson et al. 2005) associations of this haplotype have been documented in Caucasian samples, across a broad spectrum of diagnoses, with inconsistent findings of sex differences (Palo et al. 2007; Zhang et al. 2006). Independent association of *DISC1* to schizophrenia and

schizoaffective disorder has been provided by the non-synonymous SNP of the HEP3 haplotype in a UK sample (Zhang et al. 2006). However, this association has not replicated in multiple populations (Kim et al. 2007; Thomson et al. 2005) suggesting that variants in the HEP3 locality of *DISC1* influence risk for a broad range of psychotic disorders, specifically schizoaffective disorder and schizophrenia spectrum disorder. A two SNP haplotype (rs821616 and rs1411771) at the 3' end of *DISC1* has shown under-transmission to individuals with bipolar disorder ($P = 0.0002$) (Palo et al. 2007), with the non-synonymous variant of the haplotype rs821616 (Ser704Cys) showing reproducible independent association with schizophrenia in Caucasian and African American samples (Callicott et al. 2005; DeRosse et al. 2007; Qu et al. 2007). However, negative findings with rs821616 have also been reported (Hennah et al. 2008; Zhang et al. 2005), which are likely to be explained by ethnic and phenotypic differences between study groups. Among other positive associations of the *DISC1* locus to both schizophrenia (Cannon et al. 2005; Hwu et al. 2003; Qu et al. 2007) and bipolar disorder (Maeda et al. 2006; Thomson et al. 2005) is the *DISC1* mis-sense variant rs6675281 (allelic $P = 2.3 \times 10^{-6}$, Relative Risk = 2.42) (Hodgkinson et al. 2004). However, no study has replicated this association. A 4 bp deletion at the extreme 3' end of *DISC1* (exon 12) was identified in an American pedigree with schizophrenia but not found in 424 control individuals (Sachs et al. 2005). The mutation produces a truncated transcript that is detectable at a reduced level protein, and a mutant protein. A UK study failed to replicate this association with control subjects also carrying the mutation (Green et al. 2006b). Numerous negative associations of *DISC1* and psychosis have been documented, specifically in Asian samples (Chen et al. 2007; Kim et al. 2007; Zhang et al. 2005). Expression analysis of bipolar disorder patients, stratified by genomic risk variants, identified a significant decrease in DISC1 mRNA in bipolar disorder patients compared to unaffected subjects ($P = 0.006$) (Maeda et al. 2006), with the prevalence of manic symptoms correlating with lower levels of DISC1 expression ($P = 0.008$) (Maeda et al. 2006).

The *DISC1* gene is relatively large, spanning over 410 kb (Figure 3.3.). It is ubiquitously expressed with high expression in the central nervous system. The gene encodes an 854-amino acid wild-type protein which is functional in the nucleus, cytoplasm and mitochondria (James et al. 2004; Ozeki et al. 2003; Miyoshi et al. 2003; Sawamura et al. 2005). DISC1 appears to be an important

multi-functional protein active in intracellular transport, neuronal migration and architecture, neuronal cell signalling and gene expression (Hodgkinson et al. 2004), eliciting its roles through protein interactions, including proteins that transduce signals from membrane receptors (Morris et al. 2003a). Functional studies of DISC1 isoforms provide clear evidence that loss of normal DISC1 protein has detrimental effects on neurodevelopment, specifically neurite outgrowth (Kamiya et al. 2005) and positioning (Duan et al. 2007), which can result in schizophrenia-like phenotypes (Li et al. 2007; Pletnikov et al. 2007). DISC1 has shown interactions with other proteins implicated in susceptibility to schizophrenia, specifically phosphodiesterase 4B (PDE4B) (Pickard et al 2007; Millar et al. 2005). The reduced expression of *DISC1* seen in psychosis (Maeda et al. 2006) may minimise or eradicate these important protein interactions, resulting in a psychosis phenotype. Sub-cellular distribution of the wild-type DISC1 isoform is known to be significantly different in schizophrenia brains when compared with controls (Sawamura et al. 2005), suggesting the redistribution of this isoform by a mutant DISC1 isoform. This action further reduces the availability of wild-type DISC1 to perform its usual biological roles. In animal models mutant Disc1 fails to interact with an array of proteins and shows perturbed localisation (Morris et al. 2003a). Mutant Disc1 interacts with and redistributes wild-type Disc1 and disassociates the Disc1-dynein complex from the centrosome. Thus, impairing neurite outgrowth in vitro, and leading to impaired cerebral cortex development in embryonic mice (Kamiya et al. 2005).

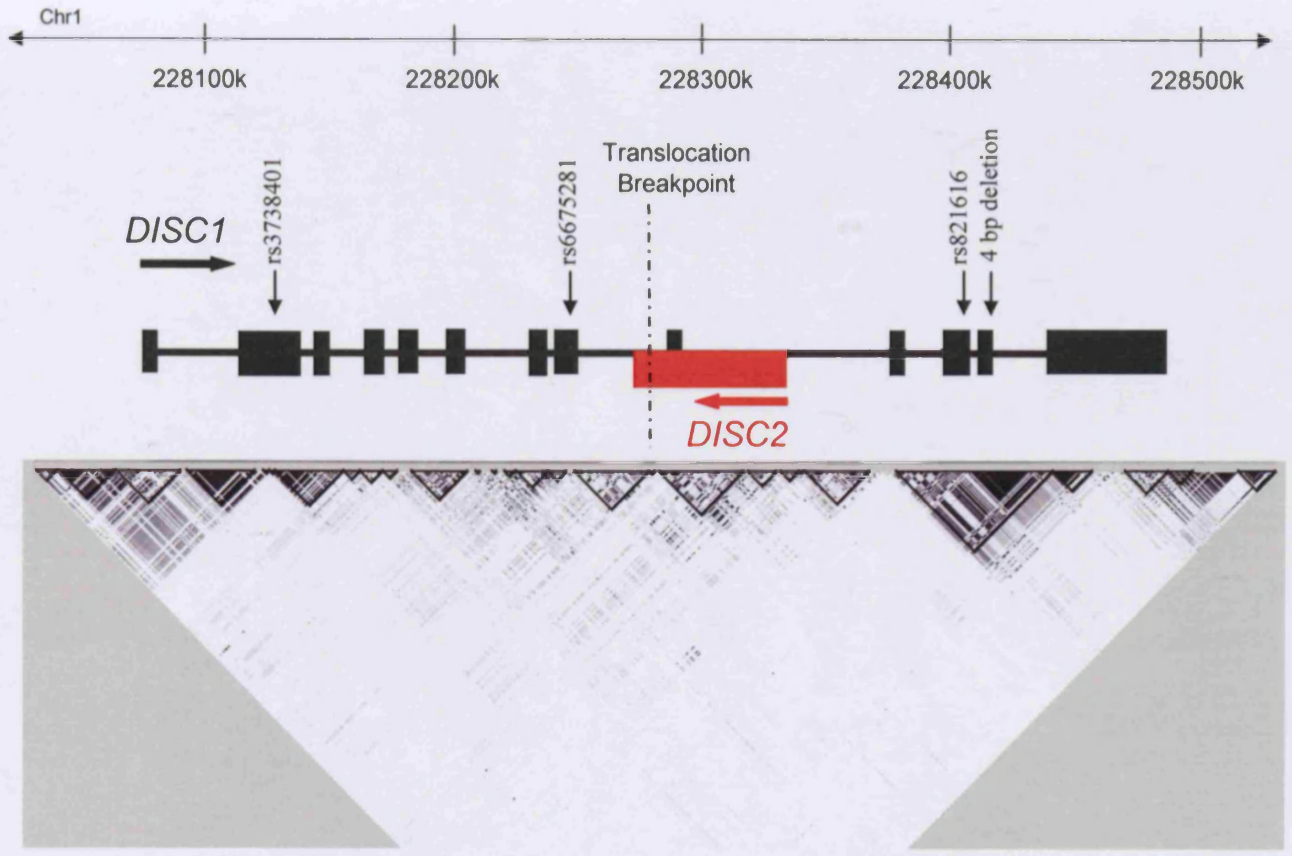


Figure 3.3. The *DISC1* locus. Schematic shows chromosomal position (bp), the SNPs and 4 bp deletion genotyped in this study, with their position within the gene indicated by an arrow, the direction of transcription indicated by black horizontal arrow, the *DISC1* gene with exons/UTR indicated by black bars. The balanced (1,11) (q42.1;q14.3) translocation breakpoint. The *DISC2* loci and its direction of transcription is indicated in red. The LD plot of the region (r^2) is provided at the bottom of the schematic.

3.3.1. Literature Summary

Convergent linkage data strongly suggest that variation in the 1q42 region influences susceptibility to mood-psychosis phenotypes that cut across the traditional Kraepelinian divide (Owen et al. 2007). Preliminary association studies within and around the *DISC1* gene have not produced compelling or consistent results, although the locus is large and no study has thoroughly covered the region. Numerous associations have been identified, generally with a broad spectrum of psychosis (Callicott et al. 2005; Hennah et al. 2003; Hodgkinson et al. 2004), some of which have replicated (Callicott et al. 2005). The 5' end of the gene shows most promise for association to psychosis (Hennah et al. 2003; Palo et al. 2007; Zhang et al. 2006), while the 3' end has displayed association to mood disorders (Palo et al. 2007). Other than the familial disruptions identified (St Clair et al. 1990; Sachs et al. 2005) there is no direct link between the risk haplotypes or genotypes identified and a specific biological or developmental dysfunction. However, *DISC1* remains an interesting functional and positional putative susceptibility gene for a wide spectrum of non-familial psychotic disorders. Functional studies provide clear evidence that loss of normal DISC1 protein has detrimental effects on neurodevelopment (Duan et al. 2007; Kamiya et al. 2005) which can result in schizophrenia-like phenotypes (Li et al. 2007; Pletnikov et al. 2007), which is consistent with the reduced expression of *DISC1* demonstrated in schizophrenia (Maeda et al. 2006).

3.3.2. Study Design

There are no compelling associations to specific *DISC1* markers or haplotypes documented in the literature. Therefore, three validated non-synonymous SNPs (rs3738401: Arg264Gln, rs6675281: Leu607Phe, and rs821616: Ser704Cys) within the *DISC1* gene were genotyped, in addition to the 4 bp deletion that has shown association to schizophrenia (Sachs et al. 2005). Variant rs821616 has shown the most consistent association to schizophrenia and bipolar disorder of any tested variant, and also shows association across numerous ethnic populations (Callicott et al. 2005; Thomson et al. 2005b; Kim et al. 2007; Qu

et al. 2007; Palo et al. 2007). Marker rs3738401 has shown independent association to schizophrenia (Zhang et al. 2006), while variant rs6675281 shows association to schizoaffective disorder (Hodgkinson et al. 2004). All markers were genotyped using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, California, USA) as described in chapter 2.7.3. The validity of this platform to genotype deletions has previously been confirmed (Green et al. 2006b). The LD structure across the *DISC1* locus and surrounding region according to the HapMap CEPH genotypes can be seen in Figure 3.3, along with the positions of the variants tested in this study. The relative marker PCR and extension primers are shown in Appendix Table 3.1.

3.3.3. Results

No LOAD individual with psychosis, but 1 LOAD individual without psychosis, 1 LOAD individual that did not meet criteria for LOAD+P or LOAD-P, and 1 control individual were identified to carry the 4 bp deletion in the heterozygous form. No difference in allele or genotype frequencies for any SNP was observed when comparing within cases, LOAD+P versus LOAD-P nor when comparing LOAD+P cases to controls. No allele or genotype differences were observed between the full sample of cases (n = 1,205) and controls (n = 1,361) denoting no association of the tested SNPs with LOAD (Tables 3.3a and 3.3b). Due to sex transmission differences previously seen at the locus (Chen et al. 2007; Hennah et al. 2003; Maeda et al. 2006), genotyped SNPs were analysed in females and males separately. No SNP showed a sex specific association with LOAD+P (Appendix Tables 3.2 - 3.5). The lack of association in these analyses is not surprising due to a further reduction in study power by sample size reduction. Analysis in female cases and controls produced no significant association with LOAD for any SNP. Conversely analysis in male LOAD cases and controls produced a significant association with marker rs3738401 ($P = 0.03$), however this association does not survive correction for multiple testing (permuted $P = 0.15$).

3.3.4. Discussion

The findings provide no evidence for the involvement of the *DISC1* locus in LOAD+P or LOAD. After allowing for sex effects a significant association with LOAD was seen in male subjects, but this association does not withstand correction for multiple testing. Only the non-synonymous variants within the gene and a familialy associated 4 bp deletion have been tested. Therefore, the locus can not be excluded as a potential susceptibility locus for psychosis in LOAD. The extensive size of the locus makes it difficult to investigate the region with a satisfactory number of markers at the present time. Future work at the *DISC1* locus will be to investigate variants within the gene region ± 20 kb in the LOAD+P genome-wide association study (GWAS) described in chapter 5.

Table 3.3a. MAF and genotype counts of tested *DISC1* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs3738401	A	0.32	0.31	0.31	0.33	40/148/172	22/108/112	96/448/474	133/501/534
rs6675281	T	0.15	0.13	0.15	0.14	5/102/260	7/53/197	23/262/767	20/294/882
rs821616	T	0.27	0.3	0.28	0.28	24/141/190	23/105/123	72/426/528	90/485/597

Table 3.3b. Individual genotyping of *DISC1* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs3738401	0.009	0.92	1.01 (0.79-1.3)	1.06	0.59	0.34	0.56	0.95 (0.79-1.13)	0.48	0.79	0.98	0.32	0.94 (0.83-1.07)	2.23	0.33
rs6675281	1.22	0.27	1.2 (0.87-1.67)	5.3	0.07	0.77	0.38	1.11 (0.88-1.4)	1.64	0.44	0.42	0.52	1.06 (0.89-1.25)	0.85	0.65
rs821616	1.74	0.19	0.84 (0.65-1.09)	1.84	0.4	0.83	0.36	0.92 (0.76-1.11)	0.85	0.66	0.19	0.66	0.97 (0.85-1.11)	0.36	0.84

3.4. Dystrobrevin Binding Protein 1

The gene encoding Dystrobrevin binding protein 1 (*DTNBP1*), also known as Dysbindin-1, is a 140 kb gene located on 6p22.3 (Figure 3.4), one of the best supported regions that have emerged from linkage studies of schizophrenia (Raybould et al. 2005). Consequently *DTNBP1* has been extensively studied in psychiatric disorders (Norton et al. 2006; Wray et al. 2008). Evidence of significant linkage to chromosomal region 6p24-22 in schizophrenia was identified in Irish pedigrees (LOD = 3.51, $P = 0.0002$) (Straub et al. 1995), with maximal linkage under an intermediate phenotype (Straub et al. 1995). There is support for schizophrenia linkage on chromosome 6p by independent groups, including 2 studies by Schwab and colleagues (Schwab et al. 1995; Schwab et al. 2000) and a collaborative study of 559-758 pedigrees (MLS = 2.68) (Schizophrenia collaborative group 1996). Several SNPs and 3 marker haplotypes between exon 5 and exon 1 of the *DTNBP1* gene have demonstrated a high degree of statistical significant association with schizophrenia ($P = 0.008–0.0001$) (Straub et al. 2002). Association at this gene, albeit with different haplotypes, has been identified in parent-offspring trio samples of German, Hungarian, Israeli and Bulgarian origin (Kirov et al. 2004; Schwab et al. 2003), in a case-control study of Swedish (Van Den Bogaert et al. 2003) and Hispanic (Funke et al. 2004) origin, but not in a case control study of Caucasian Irish Individuals (Morris et al. 2003b). Schwab and colleagues (Schwab et al. 2003) tested six of the most positively associated polymorphisms using their original linkage sample and a sib-pair sample. Evidence for association was observed for one SNP ($P = 0.0068$) and a multivariant haplotype ($P = 1x10^{-5}$). A study of dysbindin in a UK sample of 708 schizophrenia cases and 711 controls found strong evidence for association ($P < 0.0006$) with multiple novel 3-marker haplotypes, but these associations did not replicate (Williams et al. 2004). The evidence for *DTNBP1* as a susceptibility gene for schizophrenia extends to samples of non-European origin, with significant haplotypic association reported in a Chinese sample ($P = 0.0007$) (Tang et al. 2003) and a Japanese case-control sample ($P = 0.001$) (Numakawa et al. 2004). The absence of functional schizophrenia associated polymorphisms in *DTNBP1* strongly suggests that susceptibility is conferred by variation affecting mRNA expression. Such *cis*-acting variants have been shown to exist in *DTNBP1*, and to

operate in human brain (Bray et al. 2003b). Moreover, there is direct evidence linking schizophrenia risk haplotypes at the 3' end of the *DTNBP1* locus with reduced allelic expression of *DTNBP1* (Bray et al. 2005). This region of chromosome 6p22.3 also receives genome-wide significant support in bipolar disorder (Ewald et al. 2002). Some small studies show association of *DTNPB1* polymorphisms with bipolar disorder in Korean (Joo et al. 2007; Pae et al. 2007) and UK samples (Breen et al. 2006; Gaysina et al. 2008). Another UK study found modestly significant evidence for association of *DTNBP1* with a subset of cases with predominantly psychotic episodes of mood disturbance (Raybould et al. 2005). A trio based Ashkenazi Jew sample showed evidence that *DTNBP1* is associated with the development of both schizophrenia and bipolar disorder (Fallin et al. 2005).

The 352-amino acid 40 kDa DTNBP1 protein is ubiquitously expressed including a wide distribution in the brain. Dysbindin is part of the dystrophin-associated protein complex (DPC) (Benson et al. 2001), found in the sarcolemma of muscle but also in postsynaptic densities in a number of brain areas. In the brain dysbindin expression is thought to be confined to neurons, where it is pre- and postsynaptic (Benson et al. 2001). Of most relevance to this study, dysbindin is expressed in glutamatergic neurons and synapses of the hippocampus (Benson et al. 2001; Sillitoe et al. 2003). Dysbindin has emerged as a component of the biogenesis of lysosome-related organelles complex 1 (BLOC-1) (Li et al. 2003), which acts through a mechanism that may involve self-assembly and interaction with the actin cytoskeleton (Falcon-Perez et al. 2002). How dysbindin's role in the DPC or BLOC-1 relates to the pathogenesis of psychosis is unknown. Initially it was speculated dysbindin may influence schizophrenia risk through postsynaptic mechanisms (Straub et al. 2002). Compatible with the DPC's role in synaptic structure, maintenance and synaptic signalling, altered dysbindin/DPC function may lead to several of the structural and functional abnormalities that have been reported in schizophrenia, including altered function at glutamatergic and GABAergic synapses, and reduced synaptic density in frontal cortex and hippocampus (Straub et al. 2002). A presynaptic role has also been hypothesised following the documentation of a significant reduction in presynaptic dysbindin expression in several hippocampal regions and an inverse relationship between presynaptic dysbindin and the vesicular glutamate transporter suggests the

possibility of a mechanism related to glutamate release (Talbot et al. 2004). This hypothesis is supported by the demonstration that knockdown of endogenous dysbindin protein results in reduction of glutamate release (Numakawa et al. 2004). Reduced expression of dysbindin may result in disturbances of BLOC-1 function, and this among other things, may affect intracellular trafficking of glutamate vesicles and reduce glutamate release (Williams et al. 2005b). However, whether this mechanism has any relevance to the pathophysiology of schizophrenia is uncertain. Post-mortem evidence suggests *DTNBP1* is expressed in regions of the brain that are critical to cognitive function and that expression is reduced in hippocampus and prefrontal cortex in patients with schizophrenia (Weickert et al. 2004).

3.4.1. Literature Summary

There is a substantial body of support for association of the *DTNBP1* gene with schizophrenia which is far beyond what can be attributed simply to chance. However, there is considerable disagreement between studies with respect to the specific markers and haplotypes that associate with disease. No coding variant has yet been identified, strongly suggesting that susceptibility is conferred by variation affecting mRNA expression. There is direct evidence for this hypothesis, with reduced allelic expression of *DTNBP1* linked to schizophrenia (Bray et al. 2005). Some studies show association of *DTNPB1* with bipolar disorder (Breen et al. 2006; Fallin et al. 2005; Gaysina et al. 2008; Joo et al. 2007; Pae et al. 2007; Raybould et al. 2005). How dysbindin's function relates to the pathogenesis of psychosis is unknown, but both pre- (Numakawa et al. 2004; Talbot et al. 2004) and post-synaptic (Straub et al. 2002) hypotheses have been suggested and supported. The reduced expression of dysbindin seen in schizophrenia may affect intracellular trafficking of glutamate vesicles and reduce glutamate release (Williams et al. 2005b). However, whether this mechanism has any relevance to the pathophysiology of schizophrenia is uncertain.

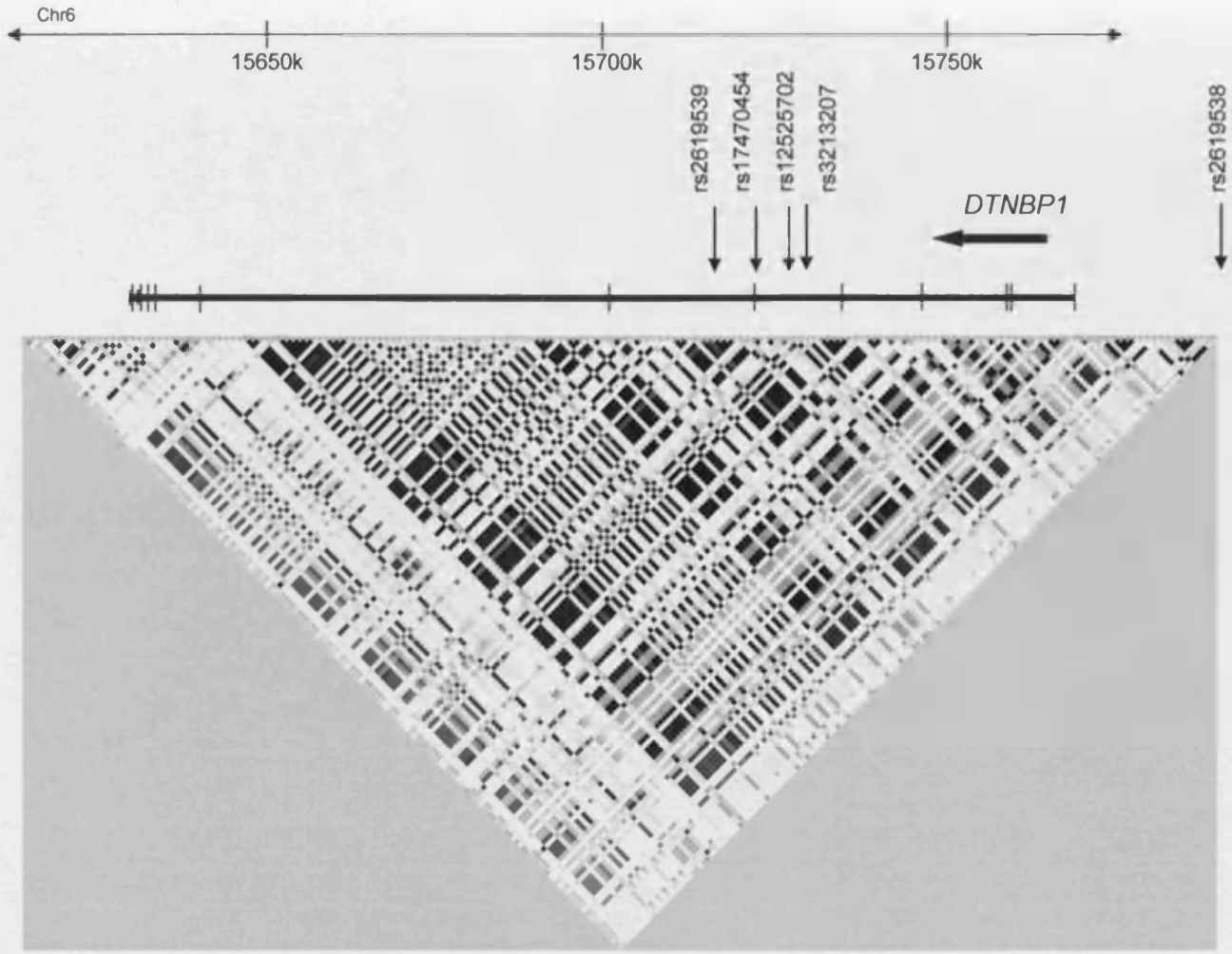


Figure 3.4. The *DTNBP1* locus. Schematic shows chromosomal position (bp), the SNPs genotyped in this study, with their position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *DTNBP1* gene with exons/UTR indicated by black bars (long/short bars respectively), and finally the LD plot of the region (r^2).

3.4.2. Study Design

Five markers which had previously shown evidence for association to schizophrenia (Carroll et al. 2009; Williams et al. 2005b) were tested for association with LOAD+P and LOAD. All markers were genotyped using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, California, USA) as described in chapter 2.7.3. The relative PCR and extension primers can be found in Appendix Table 3.6.

3.4.3. Results

One SNP in *DTNBP1* showed deviation from HWE. This variant, rs2619539, gave a HWE p-value of 0.001 in the without psychosis subset of LOAD and a HWE p-value of 0.04 in the full LOAD case sample. As this variant shows no association to LOAD+P or LOAD, and has a genotyping success rate of 97%, no explanation for the observed HWE deviation was sort. In the within case analysis (LOAD+P versus LOAD-P), one SNP rs12525702 showed a trend towards allelic association (P = 0.06; OR = 1.46), with the major C allele over-represented in cases with psychosis, 92% compared to 88% in cases without psychosis. The same SNP showed significant association to LOAD+P when this subset was compared to controls (allelic P = 0.03; OR = 1.39). The major C allele was again over-transmitted in cases with psychosis, 92% compared to 89% in controls. However, this association did not survive correction for multiple testing (Permuted P = 0.1). In the full analysis no individual variant showed an allelic or genotypic association to LOAD (Tables 3.4a and 3.4b). No significant haplotypic associations were seen, with global p-values of 0.77 for the within cases analysis (LOAD+P versus LOAD-P); 0.17 for the LOAD+P versus control analysis; and 0.28 for the LOAD versus control analysis, see Appendix Table 3.7.

Table 3.4a. MAF and genotype counts of tested *DTNBP1* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs17470454	A	0.05	0.04	0.06	0.06	2/31/310	0/21/219	3/109/881	5/124/996
rs2619539	C	0.47	0.43	0.46	0.47	80/162/101	56/96/88	223/464/306	234/579/312
rs12525702	T	0.08	0.12	0.1	0.11	2/53/288	5/46/189	12/176/805	15/221/889
rs3213207	G	0.1	0.1	0.12	0.12	5/60/278	2/46/192	16/201/776	15/242/868
rs2619538	A	0.41	0.43	0.43	0.45	64/156/123	50/104/86	194/458/341	228/548/349

Table 3.4b. Individual genotyping of *DTNBP1* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*P*	OR	χ^2	*P*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs17470454	0.33	0.57	1.18 (0.68-2.05)	1.42	0.49	0.71	0.4	1.18 (0.8-1.73)	1.19	0.55	0.05	0.82	1.03 (0.8-1.33)	0.29	0.87
rs2619539	1.48	0.22	1.16 (0.91-1.46)	3.94	0.14	0.03	0.85	1.02 (0.86-1.21)	1.99	0.37	0.22	0.64	1.03 (0.92-1.16)	4.8	0.09
rs12525702	3.64	0.06	1.46 (0.99-2.15)	4.26	0.12	**4.54**	**0.03**	**1.39 (1.03-1.87)**	4.55	0.1	1.31	0.25	1.12 (0.92-1.36)	1.38	0.5
rs3213207	0.01	0.91	1.02 (0.7-1.5)	0.7	0.71	1.82	0.18	1.21 (0.92-1.6)	2.6	0.27	0.13	0.72	1.03 (0.86-1.25)	0.75	0.69
rs2619538	0.14	0.71	1.05 (0.83-1.33)	0.49	0.78	2.22	0.14	1.14 (0.96-1.36)	2.83	0.24	1.76	0.19	1.09 (0.96-1.23)	2.67	0.26

3.4.4. Discussion

In a bid to decipher if *DTNBP1* is a susceptibility gene for Alzheimer's disease with psychosis, 5 polymorphisms were genotyped that have previously provided significant evidence for association to schizophrenia in Caucasian samples (Carroll et al. 2009; Williams et al. 2005b). This study provides moderate evidence that a variant in *DTNBP1* has some role in the aetiology of LOAD+P, with the major allele of rs12525702 over-transmitted in LOAD cases with psychosis, but this significant association does not withstand correction for multiple testing. If the within case analysis had greater power, through an increase in sample size, variant rs12525702 may have shown a significant association in this analysis. This is based on the observation that the effect size for rs12525702 is greater in the within case analysis (OR = 1.46) than the LOAD+P versus control analysis (OR = 1.39). The LOAD+P versus LOAD-P analysis has a power of 0.32, and the LOAD+P versus control analysis has power of 0.47 to detect an association with the MAF and OR seen with rs12525702. Future work will include analysis of the *DTNBP1* locus in the GWAS of LOAD with psychosis described in chapter 5, specifically variant rs12525702 or a proxy for this marker.

3.5. Glutamate Receptor Ionotropic Kainate 2

This gene is located on chromosome 6q16.3-q21 in a region identified by two psychosis covariate analyses of AD linkage screens (Bacanu et al. 2002; Hollingworth et al. 2007). Multiple genome-wide scanning studies mapped a schizophrenia (Cao et al. 1997; Levinson et al. 2000; Martinez et al. 1999) and/or bipolar disorder (Dick et al. 2003; Lambert et al. 2005; Pato et al. 2004) susceptibility locus in the vicinity of *GRIK2* (6q21–q22.3). There are relatively few genetic association studies of *GRIK2* with psychosis. Previous studies have shown significant statistical association of the gene with obsessive compulsive disorder (OCD) (Delorme et al. 2004) and autism (Jamain et al. 2002), which has been linked to schizophrenia (Konstantareas and Hewitt 2001). Significant association between *GRIK2* and schizophrenia has been reported (Bah et al. 2004), with the

results similar to those previously reported for autism. Several studies report decreased kainate receptor expression (including *GRIK2*) in the brain of schizophrenic patients (Meador-Woodruff et al. 2001a; Meador-Woodruff et al. 2001b; Porter et al. 1997). However, a Japanese case control study failed to find any association between *GRIK2* and schizophrenia (Shibata et al. 2002). Of relevance to this study, genetic variation at the *GRIK2* locus has shown significant association, in multiple studies, to the neurodegenerative disorder Huntington's disease (Metzger et al. 2006; Zeng et al. 2006). A TAA repeat in the 3' untranslated region has been shown to associate with age at onset of Huntington's disease (AAO) (Rubinsztein et al. 1997).

The glutamate receptor *GRIK2* is an excellent functional candidate for susceptibility to schizophrenia. Glutamate is an excitatory neurotransmitter in the central nervous system (CNS), which acts on two different groups of receptors: metabotropic and ionotropic receptors (Hollmann and Heinemann 1994). The ionotropic receptors are ionic channels separated into three different families: NMDA, AMPA and kainate receptors. GRIK2 is a member of the ionotropic kainate receptor family. It is expressed during brain development and post-transcriptionally modified by editing. The gene consists of 18 exons spanning over 700 kb (Figure 3.5). The encoded protein is a 908 amino acid, 103 kDa subunit (GluR6) of a kainate glutamate receptor which assembles into a kainate-gated homomeric channel. Genetic variation at the *GRIK2* locus alters the calcium permeability of this channel. The GRIK2 receptor plays an important role in synaptic plasticity and neural development (Nakanishi et al.1998), may be important for learning and memory (Shimizu et al. 2000}, and may have a role in neurodegeneration (Nakanishi et al.1998). Studies have reported abnormal kainate receptor expression and editing of *GRIK2* in brains from schizophrenic patients (Meador-Woodruff et al. 2001b). The glutamatergic dysfunction hypothesis of schizophrenia suggests genes involved in glutamatergic transmission as strong candidates for schizophrenia-susceptibility genes (Shibata et al. 2002), based on the fact that an antagonist of a glutamate receptor induces phenotypes similar to schizophrenia in human and in animal models.

3.5.1. Literature Summary

There is strong evidence for linkage to schizophrenia (Cao et al. 1997; Levinson et al. 2000; Martinez et al. 1999), bipolar disorder (Dick et al. 2003; Lambert et al. 2005; Pato et al. 2004) and AD+P (Bacanu et al. 2002; Hollingworth et al. 2007) on chromosome 6q. However there is little evidence from association studies to support these findings. Association with alternative psychotic disorders (autism and OCD) has been documented (Delorme et al. 2004; Jamain et al. 2002), but there is no convincing data for the role of *GRIK2* in the aetiology of schizophrenia from the 2 association studies (Bah et al. 2004; Shibata et al. 2002). The functional candidacy of *GRIK2* as a susceptibility gene for psychosis is strong, due to the glutamatergic dysfunction hypothesis of schizophrenia and the report of abnormal kainate receptor expression and editing in the brains of schizophrenic patients (Meador-Woodruff et al. 2001b).

3.5.2. Study Design

GRIK2 has not been extensively studied in psychosis and the rationale for including it in this study is 1) It's functional candidacy as a gene involved in glutamatergic transmission, 2) The gene's chromosomal position in an overlapping linkage region for schizophrenia, bipolar disorder and AD+P, and 3) Its previous association with a neurodegenerative disease. The *GRIK2* TAA repeat which has shown association to HD (Rubinsztein et al. 1997) was chosen for investigation in this study along with SNP rs6922753 which showed significant evidence for association with schizophrenia in the study of Bah and colleagues (Bah et al. 2004). The genomic position of the TAA repeat was identified from the paper of Rubinsztein and colleagues (Rubinsztein et al. 1997). This variant was genotyped using the genescan fluorescent PCR technology using a standard 57°C PCR program, see chapter 2.7.1. Polymorphism rs6922753 was genotyped using the Amplifluor™ Uniprimer Chemistry technique using a 58°C program with 22 cycles see chapter 2.7.2. The utilised PCR primer sequences are provided in Appendix Table 3.8.

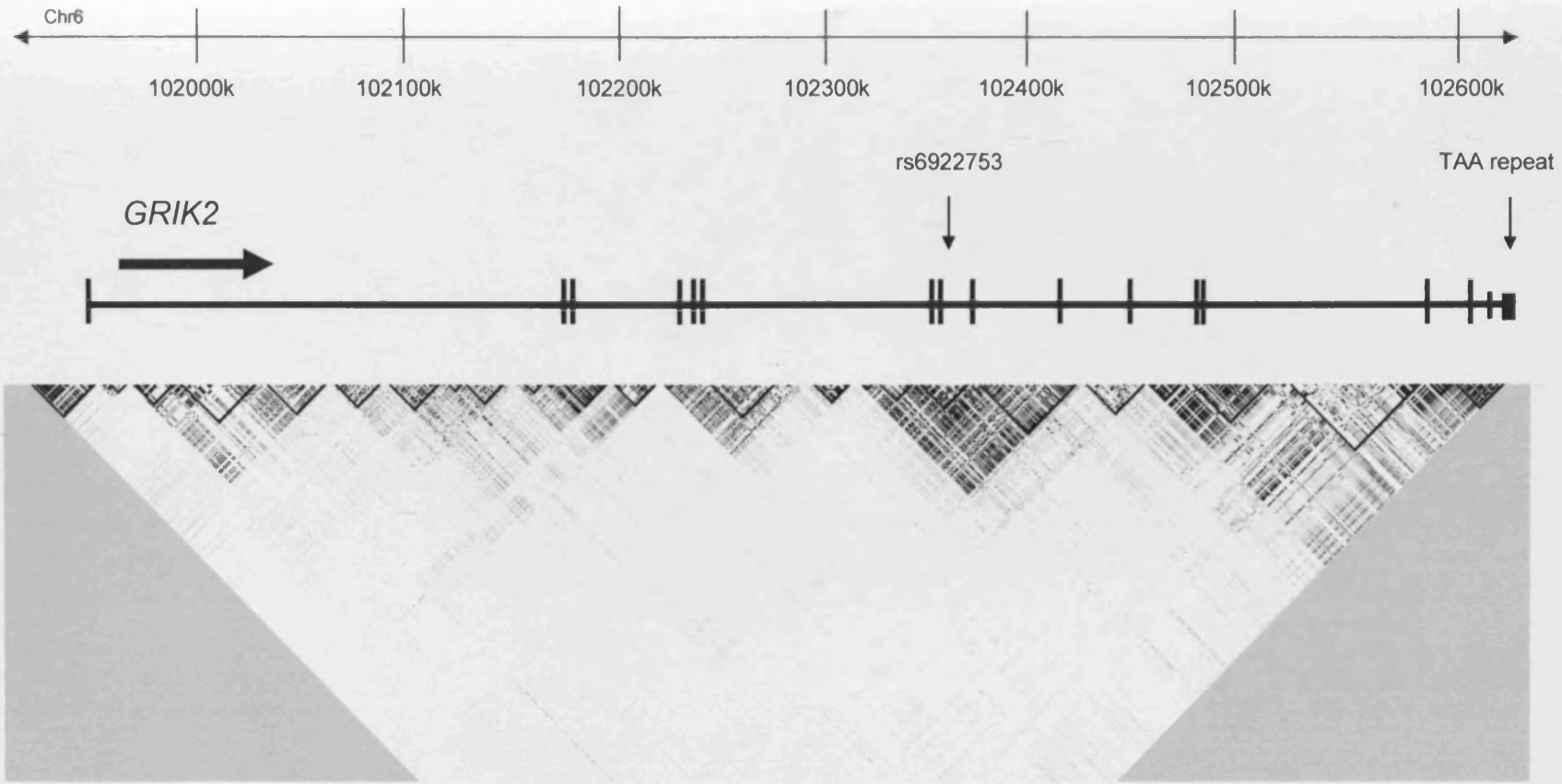


Figure 3.5. The *GRIK2* locus. Schematic shows chromosomal position (bp), the SNP and TAA repeat genotyped in this study, with their position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *GRIK2* gene with exons/UTR indicated by black bars (long/short bars respectively), and finally the LD plot of the region (r^2).

3.5.3. Results

The *GRIK2* SNP (rs6922753) shows no association to LOAD+P in the within case analysis (allelic $P = 0.65$, OR = 1.11, genotypic $P = 0.73$) nor in the LOAD+P versus control analysis (allelic $P = 0.97$, OR = 1, genotypic $P = 0.91$). The variant also shows no association to LOAD alone (allelic $P = 0.31$, OR = 1.09, genotypic $P = 0.55$) (Tables 3.5a and 3.5b). The TAA repeat had 15 observed genotypes, chi square statistics were used to determine if the number of genotypes differed significantly between 'cases' and 'controls' of each dataset analysed (Table 3.5c). There were no significant differences between the TAA genotypes in any analysis. The chi square statistic and p-value for each comparison dataset are shown in Table 3.5d.

3.5.4. Discussion

In this study the 3'UTR *GRIK2* TAA repeat which has shown association to HD (Rubinsztein et al. 1997) and SNP rs6922753 which has shown significant evidence for association with schizophrenia were investigated (Bah et al. 2004). Neither of these variants show an association to LOAD+P or LOAD. However, due to the strategy of this study coverage of the *GRIK2* locus is low meaning that variation at *GRIK2* may prove to be a risk factor for psychosis in LOAD in future studies.

Table 3.5a. MAF and genotype counts of tested *GRIK2* SNP. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs6922753	T	0.14	0.15	0.15	0.14	252/83/5	74/29/1	706/241/22	851/271/20

Table 3.5b. Individual genotyping of *GRIK2* SNP through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*P*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs17470454	0.2	0.65	1.11 (0.71-1.72)	0.63	0.73	0.002	0.97	1 (0.78-1.29)	0.18	0.91	1.03	0.31	1.09 (0.92-1.3)	1.19	0.55

Table 3.5c. Observed genotypes and genotype counts for the *GRIK2* TAA repeat.

Genotype	LOAD+P	LOAD-P	LOAD	Controls
143:143	12	9	34	37
146:134	13	11	39	35
146:143	52	39	156	188
146:146	61	47	183	199
149:140	31	23	89	85
149:143	20	15	59	67
152:146	40	30	120	136
152:149	33	26	98	119
152:152	31	23	89	94
155:143	12	9	36	45
155:149	23	17	69	76
155:152	7	7	21	18
155:155	15	12	45	56
158:146	9	6	25	36
158:149	11	8	31	29

Table 3.5d. Chi-square statistic and p-value for each analysis of the *GRIK2* TAA repeat.

	χ^2	*P*
LOAD+P v LOAD-P	0.4	0.98
LOAD+P v Control	2.5	0.64
LOAD v Control	2.49	0.63

3.6. Glutamate Receptor Metabotropic 3

The glutamate receptor metabotropic 3 gene (*GRM3*) is one of the most hypothesised putative functional candidate genes for neurological disorders. Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system and is involved in most aspects of normal brain function. The 220 kb *GRM3* gene (Figure 3.6) maps to locus 7q21.1-q21.2. This locus is on the edge of region 7q11.23-7q21.12 which provides significant evidence of linkage to psychosis in AD, making *GRM3* a putative functional and positional candidate for AD with psychosis. Multiple groups have examined polymorphisms in the *GRM3* gene for association with schizophrenia and/or bipolar disorder. The results are conflicting with no study providing compelling evidence for genetic association with either disorder. Significant association of the synonymous polymorphism rs2228595, which is thought to modulate *GRM3* splicing (Sartorius et al. 2008), was reported in German (P = 0.002) (Marti et al. 2002), European American (P = 0.08) (Egan et al. 2004), and Han Chinese (P = 0.03) (Chen et al. 2005) schizophrenia samples. However, these associations were not all in the same direction and do not replicate in large case-control (Marti et al. 2002) or familial samples (Egan et al. 2004). The intron 2 SNP rs6465084 is thought to modulate synaptic glutamate, with AA homozygotes having lower mRNA levels of a glial glutamate transporter. This variant has shown significant evidence for association to schizophrenia in a Caucasian sample (Egan et al. 2004) which replicates in a sample of German Caucasian origin (Mössner et al. 2008). Negative associations of this marker with schizophrenia have also been reported (Egan et al. 2004). Nominally significant evidence for association with intronic marker rs1468412 has been reported in Japanese (P = 0.01) (Fujii et al. 2003) and African America (P = 0.03) (Egan et al. 2004) schizophrenia samples, in addition to a number of 2 and 3 marker haplotype combinations in the same sample sets (Egan et al. 2004; Fujii et al. 2003). Entirely negative results have been reported in population samples of Caucasian (Bishop et al. 2007; Fallin et al. 2005; Norton et al. 2005; Schwab et al. 2008) and Asian ethnicity (Albalushi et al. 2008; Tochigi et al. 2006). There is no consistent pattern of diagnosis-related changes for GRM3 mRNA from studies of GRM3 expression in schizophrenia (Corti et al. 2007; Crook et al. 2002; Gupta et

al. 2005; Ohnuma et al. 1998; Richardson-Burns et al. 2000; Sartorius et al. 2008). However, the study of Corti and colleagues (Corti et al. 2007) is of specific interest, as they found a marked decrease of the dimeric form of GRM3 in schizophrenia, which is though to denote a decrease in the functional activity of GRM3 in that disease (Harrison et al. 2008). Two of three studies have reported association of *GRM3* with bipolar disorder (Fallin et al. 2005; Green et al. 2006a), suggesting that there may be a genetic association with a broader psychosis phenotype. Fallin and colleagues (Fallin et al. 2005) identified a four-marker haplotype that was associated with bipolar disorder in Ashkenazi Jew case-parent trios, but this association would not survive correction for multiple testing. Green and colleagues (Green et al. 2006a) found a significant association of the intron 2 SNP rs6465084 in a case–control study from a UK-Irish population. While, Marti and colleagues provide the only negative study in a German case-control sample (Marti et al. 2002).

The postsynaptic actions of glutamate are mediated by two types of receptors. The ionotropic receptors are glutamate-gated ion channels, which cause neuronal depolarization. While the metabotropic glutamate receptors are a family of G protein-coupled receptors, that have been divided into 3 groups on the basis of sequence homology, putative signal transduction mechanisms and pharmacologic properties (Kuramoto et al. 1994). Group II includes GRM2 and GRM3 which have key roles in synaptic plasticity via inhibition of glutamate release (Johnson et al. 2005). Abnormal glutamatergic transmission has been proposed in schizophrenia, initially from the observation that exposure to ionotropic receptor antagonists can cause symptoms of schizophrenia and also relapse in patients with schizophrenia (Javitt and Zukin 1991; Kim et al. 1980). Interest in group II metabotropic glutamate receptors was stimulated when agonists of metabotropic glutamate receptors 2 and 3 (GRM2 and GRM3) were shown to ameliorate the adverse behavioural effects induced by ionotropic receptor antagonists in the mouse (Moghaddam and Adams 1998). This finding has been confirmed in subsequent rodent studies (Cartmell et al. 1999; Cartmell et al. 2000a; Cartmell et al. 2000b; Carter et al. 2004; Greco et al. 2005; Homayoun et al. 2005; Lorrain et al. 2003). The *GRM3* gene has been specifically highlighted as a putative psychosis candidate gene for schizophrenia by three relatively recent developments. 1) Several studies have shown genetic association of *GRM3* with schizophrenia (Chen et al. 2005; Egan et al. 2004; Fujii

et al. 2003; Marti et al. 2002; Mössner et al. 2008; Schwab et al. 2006). 2) A clinical trial of a group II metabotropic glutamate receptor agonist has shown unequivocal antipsychotic efficacy (Patil et al. 2007). 3) A splice variant of GRM3 (GRM3Δ4) has been identified which encodes a truncated form of the receptor that is expressed in brain (Sartorius et al. 2008). Increased expression of GRM3Δ4 has been found to correlate with genetic risk factor for schizophrenia (Sartorius et al. 2008).

3.6.1 Literature Summary

The existing studies do not provide compelling evidence for association of *GRM3* with schizophrenia or bipolar disorder. Although, the majority of studies have been inadequately powered. For example, Fallin and colleagues noted that lack of power was a serious limitation of their study; with only 7% power to detect a susceptibility variant with an OR of 1.5, assuming a minimum MAF of 10% and that the tested variant is in a r^2 of 1 with the functional variant (Fallin et al. 2005). Two SNPs (rs1468412 and rs6465084) within *GRM3* have shown association to psychosis that replicates in independent samples (Egan et al. 2004; Fujii et al. 2003; Green et al. 2006a; Mössner et al. 2008). The positive associations of *GRM3* and psychosis are centred around exon 3 and adjoining introns, providing a degree of intragenic convergence that likely increases the chances that a true causal variant (or variants) is located in this region of the gene (Harrison 2008). Moreover, this localization leads to a prediction: if there is a pathophysiological consequence of allelic variation in the vicinity, it is likely mediated by effects on expression, and more specifically splicing, of the gene (Harrison and Weinberger 2005). The biological plausibility of *GRM3* as a susceptibility gene for psychosis is more convincing, with a clinical trial of a group II metabotropic glutamate receptor agonist showing antipsychotic efficacy (Patil et al. 2007), and increased expression of a splice variant of GRM3 correlating with risk for schizophrenia (Sartorius et al. 2008).

3.6.2. Study Design

Seven variants were tested for association with LOAD+P and LOAD. These were *GRM3* markers which have previously shown significant evidence for association to schizophrenia in Caucasian samples. One SNP (rs2228595) showed significance in an early German sample (Marti et al. 2002), while the remaining SNPs were reported by Egan and colleagues in a large UK based case control sample (Egan et al. 2004). These SNPs and haplotypes have since been studied in an additional UK case control sample of comparable size to that of Egan and colleagues where they show no evidence of association to schizophrenia (Norton et al. 2005). All markers except rs187993 were genotyped using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, CA) (see chapter 2.7.3). Polymorphism rs187993 was genotyped using the Amplifluor™ UniPrimer chemistry, comprehensively described in chapter 2.7.2. This SNP was genotyped on a 58°C program with 22 cycles. Marker PCR and extension primers are provided in Appendix Table 3.9.

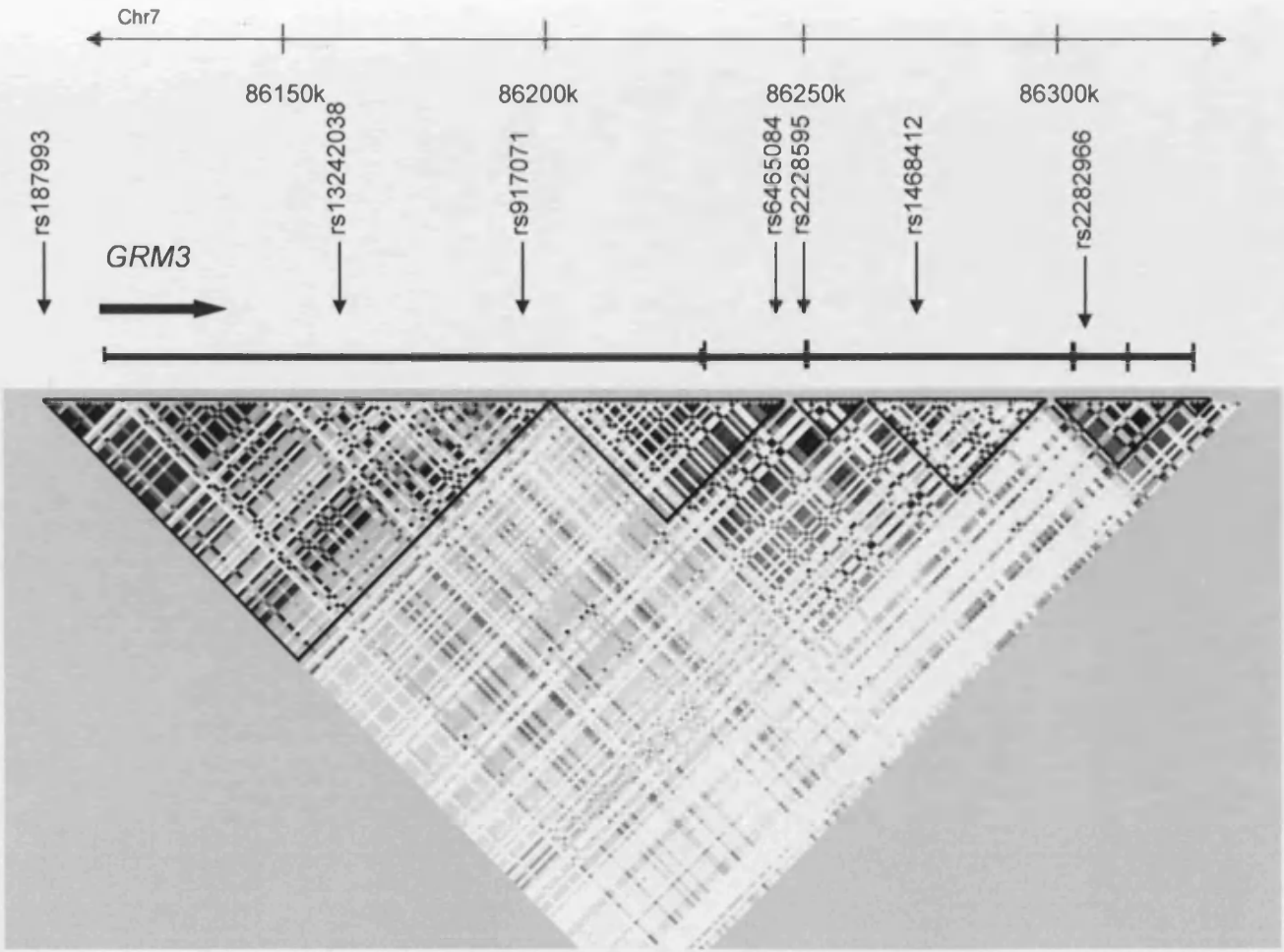


Figure 3.6. The *GRM3* locus. Schematic shows chromosomal position (bp), the SNPs genotyped in this study, with their position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *GRM3* gene with exons/UTR indicated by black bars (long/short bars respectively, and finally the LD plot of the region (r^2).

3.6.3. Results

Four SNPs showed deviation from HWE in one of the sample groups. Polymorphism rs13242038 shows deviation from HWE ($P = 0.03$) in the LOAD+P group as does variant rs2228595 (HWE $P = 0.01$). Marker rs6465084 has a nominally significant deviation from HWE in the control group ($P = 0.04$), while rs2282966 shows a greater deviation from HWE in this sample set ($P = 0.001$). No genotyping error was found, genotypes of HapMap samples included on the sample plates were checked and confirmed against HapMap data, and none of the SNPs were found to have a non-specific assay (where a primer shows high homology to multiple genomic regions) or a primer covering a known variant. Therefore, no reasons for the observed deviations from HWE were identified; hence the raw genotype data and the statistics calculated from these were received with caution (Martin et al. 2000; Wang et al. 2003). One of the *GRM3* SNPs (rs2228595) showed a nominal genotypic ($P = 0.04$) but no allelic difference between LOAD+P and LOAD-P cases. This genotypic association does not survive correction for multiple testing (permuted $P = 0.28$). The same SNP showed a significant association with both an allelic ($P = 0.003$, OR = 1.71, permutated $P = 0.01$) and genotypic ($P = 0.003$, permutated $P = 0.02$) association to LOAD+P as a subtype of disease which survives correction for multiple testing. However, as discussed this variant shows deviation from HWE in the LOAD+P subgroup. Four SNPs (rs917071, rs6465084, rs2228595, rs1468412) showed a significant allelic association with LOAD. Two of these associations survived correction for multiple testing. Marker rs6465084 showed the most significant association with LOAD ($P = 0.002$, OR = 1.27; permuted $P = 0.009$), but this variant shows slight deviation from HWE in the control dataset ($P = 0.04$). Polymorphism rs2228595 also showed a significant association with LOAD that survived correction for multiple testing ($P = 0.005$, OR = 1.47, permuted $P = 0.021$). The minor T allele of this variant is over-represented in cases with a MAF of 7% compared to a MAF of 5% in controls (see Tables 3.6a and 3.6b). Haplotype analyses revealed no stronger association than the allelic associations seen with LOAD+P, with global p-values of 0.93 and 0.2 respectively, see Appendix Table 3.10. In the LOAD versus control dataset a global haplotypic p-value of 0.029 was detected, see Appendix Table 3.10. The most significant association was seen with a four marker haplotype ($P = 0.0005$)

including variants rs917071, rs2282966, rs1468412 and rs6465084. Two of these variants show deviation from HWE in the control dataset (rs282966, $P = 0.001$; rs6465084, $P = 0.04$)

3.6.4. Discussion

Association to LOAD+P was seen with the synonymous SNP rs2228595, this SNP shows deviation from HWE in the LOAD+P dataset and therefore the genotype data can not be relied upon. However, the association remains interesting and would be worthy of investigation in an independent dataset. Given the modest number of variants genotyped in this study it is possible that an alternate variant at the *GRM3* locus is associated with LOAD+P. Four variants showed association to LOAD, two of which survived correction for multiple testing. The greatest association of *GRM3* to LOAD was seen with a 4 SNP haplotype of these associated variants, however, variants rs2282966 and rs6465084 which are included in this haplotype show deviation from HWE. The association of *GRM3* with LOAD is interesting, but modest considering the ample size of our case-control sample. It warrants replication in an independent dataset and examination in a LOAD GWAS. Future work will include analysis of the *GRM3* locus in a LOAD+P GWAS, specifically the rs2228595 variant or a proxy for this marker. This will be discussed further in chapter 5.

Table 3.6a. MAF and genotype counts of tested *GRM3* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs187993	T	0.33	0.32	0.32	0.34	38/152/155	25/106/113	116/526/546	140/526/521
rs13242038	T	0.18	0.22	0.2	0.19	17/80/226	11/75/135	45/276/581	36/312/658
rs917071	T	0.28	0.3	0.28	0.24	29/122/172	20/91/110	77/345/480	62/ 66/578
rs6465084	G	0.25	0.25	0.25	0.21	19/126/178	15/79/127	62/332/508	53/317/636
rs2228595	T	0.08	0.06	0.07	0.05	6/39/278	0/26/195	8/108/786	3/90/913
rs1468412	T	0.27	0.27	0.27	0.24	25/122/176	17/85/119	72/340/490	68/346/592
rs2282966	A	0.23	0.27	0.25	0.25	20/106/197	12/94/115	59/337/506	82/332/592

Table 3.6b. Individual genotyping of *GRM3* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*P*	OR	χ^2	*P*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs187993	0.15	0.7	1.05 (0.82-1.35)	0.15	0.93	0.2	0.66	1.04 (0.87-1.25)	0.21	0.9	2.26	0.13	1.1 (0.97-1.24)	2.84	0.24
rs13242038	3.1	0.1	1.31 (0.97-1.78)	5.45	0.07	0.66	0.42	1.1 (0.87-1.39)	5.74	0.06	0.87	0.35	1.08 (0.92-1.27)	2.33	0.31
rs917071	0.4	0.5	1.09 (0.84-1.42)	0.7	0.71	3.2	0.07	1.2 (0.98-1.47)	3.73	0.16	**5.42**	**0.02**	**1.19 (1.03-1.37)**	5.67	0.06
rs6465084	0.07	0.8	1.04 (0.79-1.37)	0.67	0.71	**5.41**	**0.02**	**1.28 (1.04-1.57)**	**6.92**	**0.03**	**9.71**	**0.002**	**1.27 (1.09-1.48)**	**9.73**	**0.01**
rs2228595	1.14	0.2	1.37 (0.84-2.24)	**6.62**	**0.04**	**9.13**	**0.003**	**1.71 (1.2-2.43)**	**11.8**	**0.003**	**7.74**	**0.005**	**1.47 (1.12-1.94)**	**7.76**	**0.02**
rs1468412	0.01	0.9	1.02 (0.77-1.33)	0.03	0.99	1.88	0.17	1.15 (0.94-1.41)	1.93	0.38	**4.15**	**0.042**	**1.16 (1.01-1.35)**	4.13	0.13
rs2282966	2.4	0.1	1.25 (0.94-1.65)	5.33	0.07	1.12	0.29	1.12 (0.91-1.38)	1.42	0.49	0.17	0.68	1.03 (0.89-1.19)	4.87	0.09

3.7. Neuregulin 1

Neuregulin 1 (*NRG1*) (Figure 3.7) is genetically one of the most well supported susceptibility genes for schizophrenia (Craddock et al. 2005). Schizophrenia mapped to chromosome 8p21-22 in a genome-wide scan of Icelandic families (Stefansson et al. 2002), with the highest multipoint LOD score of 3.06 at marker D8S532 (Stefansson et al. 2002). This region is 10-15 cM centromeric to the previously reported linkage to 8p (Brzustowicz et al. 1999; Blouin et al. 1998; Gurling et al. 2001; Kaufmann et al. 1998; Kendler et al. 1996; Levinson et al. 1996; Pulver et al. 1995; Shaw et al. 1998). The localisation discrepancy is thought to be accounted for by low marker density, low resolution and uncertainty with maps in older studies (Stefansson et al. 2002). Extensive fine mapping of the locus revealed association between schizophrenia and a multi-marker haplotype at the 5′ end of *NRG1* (Stefansson et al. 2002). Strong evidence for association with the same haplotype was subsequently found in a large sample from Scotland ($P = 0.00031$, RR = 1.8) (Stefansson et al. 2003) and a UK and Ireland sample ($P < 0.04$; RR = 1.25) (Williams et al. 2003a). The multi- marker haplotype can be identified using only 3 markers (SNP8NRG221533 and microsatellites 478B14-848 and 420M9-1395). Negative findings with this "Icelandic" haplotype have been reported in Caucasian populations (Bakker et al. 2004; Corvin et al. 2004; Hall et al. 2004; Petryshen et al. 2005; Thiselton et al. 2004). However, association with individual variants of the Icelandic haplotype (Bakker et al. 2004) or alternative haplotypes (Corvin et al. 2004; Hall et al. 2004; Petryshen et al. 2005) have been reported in many of these studies. Summary analysis of the 'Icelandic' haplotype data from all Caucasian studies (n ~ 4,500) indicates a significant association with an odds ratio of approximately 1.5 (Tosato et al. 2005). In Asian populations, where the 'Icelandic' haplotype is not found, there is good evidence from several studies of association with other markers in the same region (Hong et al. 2004; Li et al. 2004b; Tang et al. 2004; Yang et al. 2003), although one negative study has been reported (Iwata et al. 2004). Two studies have investigated *NRG1* in bipolar disorder, one of which found significant evidence for association of the 'Icelandic' risk haplotype with a similar effect size to

that seen by the same group in schizophrenia (Green et al. 2005), the other found association with an alternative *NRG1* variant (Georgieva et al. 2008). These findings suggest that *NRG1* plays a role in influencing susceptibility to both bipolar disorder and schizophrenia, perhaps via alterations to gene expression or regulation due to the lack of genetic association with a coding variant. Go and colleagues (Go et al. 2005) performed linkage analysis on chromosome 8p in a NIMH Alzheimer disease sample. A specific linkage peak for Alzheimer disease with psychosis was demonstrated on 8p12, a region which encompasses the *NRG1* gene. The authors subsequently demonstrated a significant association between an *NRG1* SNP (rs3924999) and AD with psychosis ($P = 0.008$). This SNP is part of a 3-SNP haplotype preferentially transmitted to individuals with the phenotype suggesting that *NRG1* plays a role in increasing the genetic risk for psychosis in a proportion of Alzheimer disease families (Go et al. 2005).

An extraordinary variety of different isoforms are produced from the *NRG1* gene by alternative splicing. These variants, which include glial growth factors and sensory and motor neuron-derived factors, show tissue-specific expression and differ significantly in their structure. In the brain, *NRG1* is thought to encode around 15 proteins with a diverse range of functions, including cell-cell signalling, receptor interactions, axon guidance, synaptogenesis, glial differentiation, myelination, and neurotransmission (Stefansson et al. 2004). A number of the proteins have a clear role in the expression and activation of neurotransmitter receptors, including the NMDA glutamate receptor (Stefansson et al. 2002) and are essential for neuronal development. NRG1 is a plausible susceptibility gene for schizophrenia because of its involvement in neurodevelopment, regulation of glutamate and other neurotransmitter receptor expression, and synaptic plasticity, any of which could potentially influence susceptibility to schizophrenia (Craddock et al. 2005). However, the pathophysiological mechanisms by which altered NRG1 function might lead to schizophrenia are unclear. The view that the association is related to altered NRG1 function or expression is supported by the observation of alteration in the ratios of NRG1 mRNA species in schizophrenic brain (Hashimoto et al. 2004). In animals, mutant NRG1 hypomorphic mice show a behavioural phenotype that overlaps with mouse models for schizophrenia, which can be partially reversed with clozapine, an atypical antipsychotic drug used to treat schizophrenia

(Stefansson et al. 2002). Furthermore, NRG1 hypomorphic mice have fewer functional NMDA receptors than wildtype mice (Stefansson et al. 2002).

3.7.1. Literature Summary

There is strong evidence from several studies that genetic variation in *NRG1* confers risk to schizophrenia, but as yet, and in spite of extensive re-sequencing, specific susceptibility and protective variants have not been identified (Craddock et al. 2005). The most consistent association in Caucasian populations is with a multi-marker haplotype, located at the 5 prime end of the gene. This "core" haplotype was originally identified in an Icelandic sample (Stefannson et al. 2002) and has replicated in Scottish (Stefannson et al. 2003) and UK (Williams et al. 2003a) samples. This Icelandic haplotype has also shown significant association with risk for bipolar disorder. The lack of identification of a coding *NRG1* variant leads to the hypothesis that *NRG1* contributes to schizophrenia aetiology via a reduction in protein function or expression (Hashimoto et al. 2004; Stefansson et al. 2002). *NRG1* is thought to contribute to the aetiology of psychosis through one of its many roles in neurodevelopment, regulation of glutamate and other neurotransmitter receptor expression, and synaptic plasticity. Of particular interest to this study is that *NRG1* SNP (rs3924999) has shown association to AD with psychosis (Go et al. 2005).

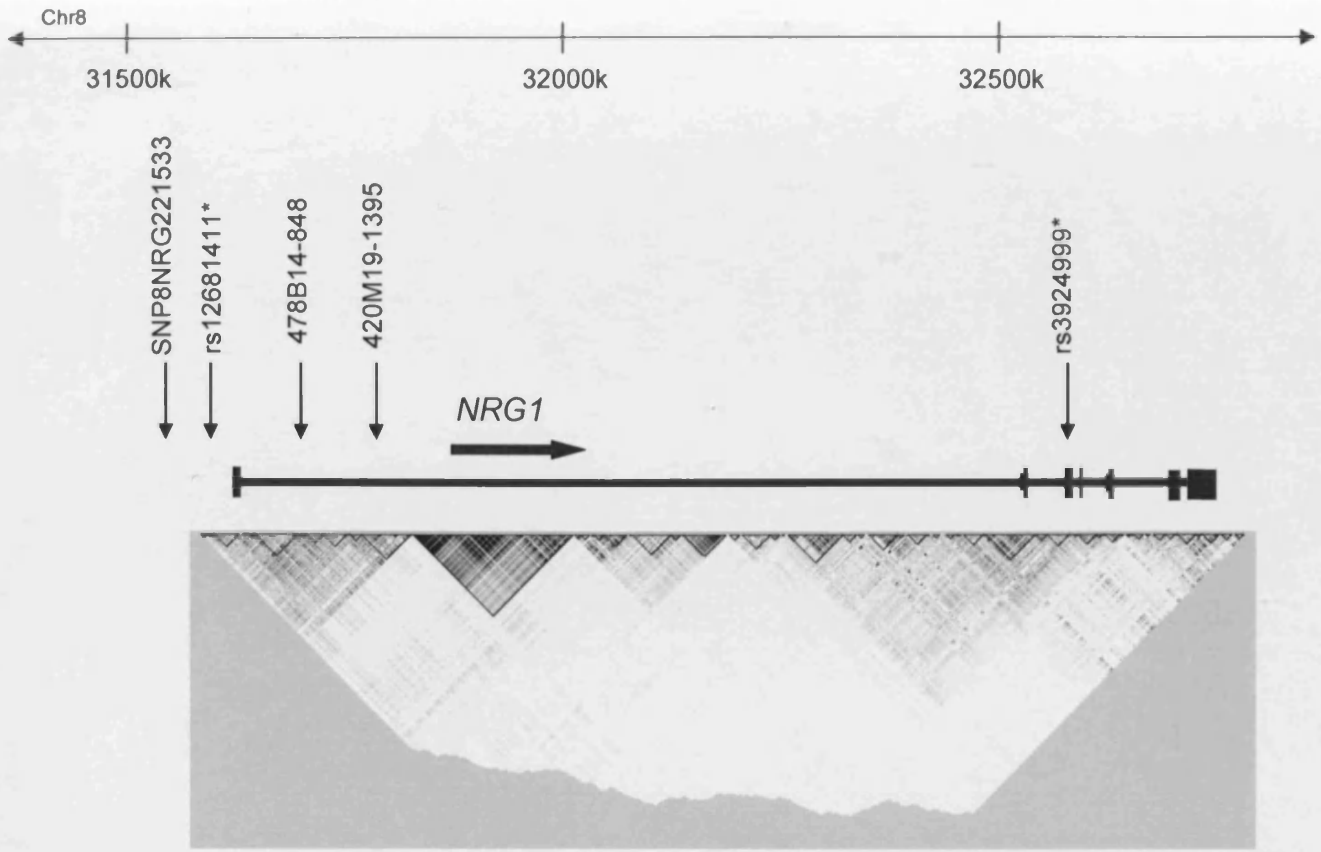


Figure 3.7. The *NRG1* locus. Schematic shows chromosomal position (bp), the markers genotyped in this study (* indicates database SNP), with their position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *NRG1* gene with exons/UTR indicated by black bars (long/short bars respectively), and finally the LD plot of the region (r^2).

3.7.2. Study Design

Three of the *NRG1* variants investigated in this thesis were chosen due to their previous haplotypic association with schizophrenia (Stefansson et al. 2003). The relevant oligomeric primers given by the Stefansson et al. (2003) study were used to genotype the markers. The remaining variants (rs3924999 and rs12681411) were identified from two separate studies. Marker rs3924999 is associated with schizotypical personality (Lin et al. 2005a) and has shown association to AD+P (Go et al. 2005). Marker rs12681411 has shown evidence for correlation with gene expression (Davelos-Baines et al. 2008). All SNPs were genotyped using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, CA) as described in chapter 2.7.3. Microsatellites 420M9-1395 and 478B14-848 were genotyped by genescan fluorescent PCR (chapter 2.7.1) on 50°C 34 cycle touchdown, and 57°C standard PCR programs respectively. PCR and extension primer sequences are provided in Appendix Table 3.11.

3.7.3. Results

No SNP showed association to LOAD+P or LOAD (Tables 3.7a and 3.7b). Microsatellite 420M9-1395 had 21 observed genotypes, and microsatellite 478B14-848 had 22 observed genotypes (Tables 3.7c and 3.7e). Chi square statistics were used to determine if the number of microsatellite genotypes differed significantly between 'cases' and 'controls' (Tables 3.7d and 3.7f). There were no significant differences between the genotypes in any analysis. Haplotypic analysis of the Icelandic haplotype produced no significant results in any analysis (LOAD+P vs. LOAD-P global $P = 0.59$; LOAD+P vs. control global $P = 0.49$; LOAD vs. control global $P = 0.41$ see Table 3.7g).

Table 3.7a. MAF and genotype counts of tested *NRG1* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs12681411	G	0.38	0.35	0.37	0.37	52/156/135	27/119/98	146/437/411	146/549/448
SNP8NRG221533	C	0.31	0.29	0.32	0.3	49/165/138	31/121/93	132/448/445	139/560/541
rs3924999	A	0.4	0.42	0.4	0.39	56/163/124	42/119/83	166/463/365	173/546/424

Table 3.7b. Individual genotyping of *NRG1* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*P*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs12681411	0.74	0.39	1.11 (0.87-1.41)	2.13	0.34	0.28	0.6	1.05 (0.88-1.25)	1.49	0.47	0.01	0.94	1.01 (0.89-1.14)	3.95	0.14
SNP8NRG221533	0.69	0.47	1.24 (0.83-1.52)	2.23	0.29	0.57	0.23	1.12 (0.86-1.22)	1.52	0.53	0.36	0.41	1.08 (0.91-1.21)	2.6	0.34
rs3924999	0.27	0.6	1.06 (0.84-1.35)	0.3	0.86	0.25	0.62	1.05 (0.88-1.24)	0.31	0.86	0.42	0.52	1.04 (0.92-1.18)	1	0.61

Table 3.7c. Observed genotypes and genotype counts for microsatellite 420M19-1395.

Genotype	LOAD+P	LOAD-P	LOAD	Controls
181:181	1	0	3	4
181:183	2	1	6	8
181:185	7	6	3	4
183:183	9	6	27	36
183:185	11	8	33	44
183:187	17	14	51	62
183:189	25	18	75	95
185:185	20	15	60	77
185:187	35	26	105	113
185:189	28	23	84	102
185:193	12	9	3	4
187:187	44	33	132	155
187:189	54	47	162	189
187:193	27	15	81	91
189:189	14	12	42	51
189:193	8	4	24	32
193:193	7	3	21	24
196:187	5	1	15	18
196:189	2	0	6	8
196:193	0	0	2	0
196:196	0	0	2	0

Table. 3.7d. Chi-square statistic and p-value for each analysis of microsatellite 420M19-1395.

	χ^2	*P*
LOAD+P v LOAD-P	1.34	0.86
LOAD+P v Control	0.16	0.99
LOAD v Control	0.33	0.98

Table 3.7e. Observed genotypes and genotype counts for microsatellite 478B14-848.

Genotype	LOAD+P	LOAD-P	LOAD	Controls
215:215	0	0	0	2
215:217	1	0	1	0
215:219	6	7	17	19
217:217	9	6	28	34
217:219	8	6	24	25
217;221	15	14	46	53
217:223	27	19	85	95
219:219	61	43	177	198
219:221	45	38	143	162
219:223	27	16	72	81
219:225	12	11	41	46
219:227	33	23	105	118
221:221	34	22	97	107
221:223	18	13	54	65
221:225	25	19	84	100
221:227	12	7	31	38
223:223	1	0	2	7
223:225	1	1	3	0
223:227	3	2	9	8
225:225	1	0	1	0
225:227	0	0	1	0
227:227	0	0	0	1

Table 3.7f. Chi-square statistic and p-value for each analysis of microsatellite 478B14-848.

	χ^2	*P*
LOAD+P v LOAD-P	1.97	0.74
LOAD+P v Control	4.31	0.37
LOAD v Control	1.83	0.77

Table 3.7g. Results of haplotype analysis at the *NRG1* locus. Table shows analysis dataset, number of markers analysed, chi square statistic and p-value.

Dataset	Number of Markers Analysed	χ2	Global *P*
LOAD+P v LOAD-P	3	0.3	0.59
LOAD+P v Control	3	0.54	0.49
LOAD v Control	3	0.67	0.41

3.7.4. Discussion

This study investigated the 'Icelandic/Stefansson' haplotype, in addition to the functional variant rs3924999, which has previously shown association with schizotypical personality disorder (Lin et al. 2005a), and AD+P (Go et al. 2005), plus SNP rs12681411 which has been shown to correlate with gene expression. No individual variant showed an association to LOAD+P or LOAD in this study, meaning the association of rs3924999 to AD+P has not replicated. The original association was discovered in a familial sample of 65 pedigrees, suggesting the *NRG1* variant rs3924999 is familialy associated with AD+P and may not be a risk variant in the general population. Haplotype analysis of the 'Icelandic' haplotype also revealed no significant associations with LOAD+P or LOAD. Despite the negative findings of this study *NRG1* remains an interesting candidate gene for AD+P due to its previous associations with psychosis and its strong functional candidacy. This study only investigates 5 variants. Further investigation of this locus is warranted, and will be discussed in chapter 5 of this thesis where the *NRG1* gene will be explored in a LOAD+P GWAS.

3.8. Brain-derived Neurotrophic Factor

The Brain Derived Neurotrophic Factor (*BDNF*) is a functional candidate gene that has attracted a great deal of interest in schizophrenia (Neves-Pereira et al. 2005) and bipolar disorder (Green et al. 2003). Only one frequent, non-conservative polymorphism in the human *BDNF* gene has been identified, a SNP that causes a valine to methionine substitution at codon 66 (val66met) and may have a functionally relevant effect by modifying the processing and trafficking of *BDNF* (Egan et al. 2003).Su bjects with the met allele show abnormal hippocampal activity and poorer performance during episodic memory tasks, consistent with a reduced functional effect of BDNF (Egan et al. 2003). The variant has shown positive association to bipolar disorder in at least 3 family-based Caucasian samples (Geller et al. 2004; Neves-Pereira et al. 2002; Sklar et al. 2002), all showing over-representation of the common valine allele. There have been multiple case-control association studies of varying ethnicities which show no evidence for

an allelic or genotypic association to bipolar disorder (Green et al. 2003; Hong et al. 2003; Nakata et al. 2003; Neves-Pereira et al. 2005; Oswald et al. 2004; Skibinska et al. 2004). However, one bipolar disorder case-control analysis in a European population showed a significant increase of the val allele in cases (P = 0.028; OR of 1.22) (Lohoff et al. 2005). *BDNF* has attracted less interest in schizophrenia, but there are reports of positive association at val66met and a 2 locus haplotype including this polymorphism in schizophrenia (Neves-Pereira et al. 2005) and schizophrenia-spectrum disorder (Rosa et al. 2006). This variant has shown association to AD in one Italian case-control analysis (Ventriglia et al. 2002) but not in multiple other studies (Bodner et al. 2005; He et al. 2007; Tsai et al. 2004; Tsai et al. 2006), and has also shown association with age at disease onset in Parkinson disease (Karamohamed et al. 2005). Expression of the *BDNF* gene is reduced in both Alzheimer's and Huntington disease patients (Pomponi et al. 2008; Zala et al. 2008).

The *BDNF* gene contains 11 exons and spans about 70 kb (Figure 3.8) (Pruunsild et al. 2007) of chromosome 11p13 (Maisonpierre et al. 1991). It encodes a precursor peptide, which is cleaved proteolytically to form the mature protein (Seidah et al. 1996). The mature protein is a member of the nerve growth factor family. It is a pro-survival factor induced by cortical neurons, and is necessary for survival of striatal neurons in the brain (Zuccato et al. 2001). BDNF elicits long-term neuronal adaptations by controlling the responsiveness of its target neurons to the important neurotransmitter dopamine (Guillin et al. 2001). The biologic action is regulated by proteolytic cleavage, with proforms preferentially activating apoptosis and mature forms activating receptors to promote survival (Lee et al. 2001). The mature form of BDNF is identical in all mammals examined, with conserved tissue distributions and neuronal specificities (Maisonpierre et al. 1991). Multiple transcript variants encoding distinct isoforms have been described for this gene but the full-length nature of only some have been determined. The transcripts differ in their use of alternative promoters, alternative splice donor and acceptor sites, and alternative polyadenlylation sites (Liu et al 2005; Pruunsild et al. 2007). In summary, the gene plays an important role in promoting and modifying growth, development, and survival of neuronal populations and, in the mature nervous system, is involved in activity-dependent neuronal plasticity (Duman et al. 1999).

Of relevance to psychosis, mature BDNF has been shown to mediate long-term potentiation in denate cells of mouse hippocampus (Kovalchuk et al. 2002; Pang et al. 2004), with induction of BDNF expression by selective activation of calcium influx (Tao et al. 2002). Indeed, calcium-responsive enhancer elements within the BDNF promoter have been identified (Tao et al. 2002). Also, BDNF has been shown to rapidly shift the neurotransmitter release properties of rat sympathetic neurons from excitatory to inhibitory cholinergic transmission in response to neural stimulation (Yang et al. 2002). Of interest to this study is that the *BDNF* gene has been implicated in Huntingdon's disease (HD), with the Huntingtin protein upregulating transcription of BDNF (Zuccato et al. 2001), and enhancing vesicular transport (Gauthier et al. 2004). These events result in insufficient neurotrophic support and neuronal toxicity (Gauthier et al. 2004; Zuccato et al. 2001) meaning that increasing BDNF production may be a therapeutic approach for treating HD. (Zuccato et al. 2001). Reduced expression of BDNF has also been noted in AD (Egan et al. 2003), which may have a result similar to that seen in HD. The oxidative stress hypothesis for the aetiology of AD lends support to this theory, as BDNF has been shown to attenuate neuronal damage induced by chemical hypoxia in vivo by a mechanism which may involve attenuation of oxidative stress (Kirschner et al. 1996).

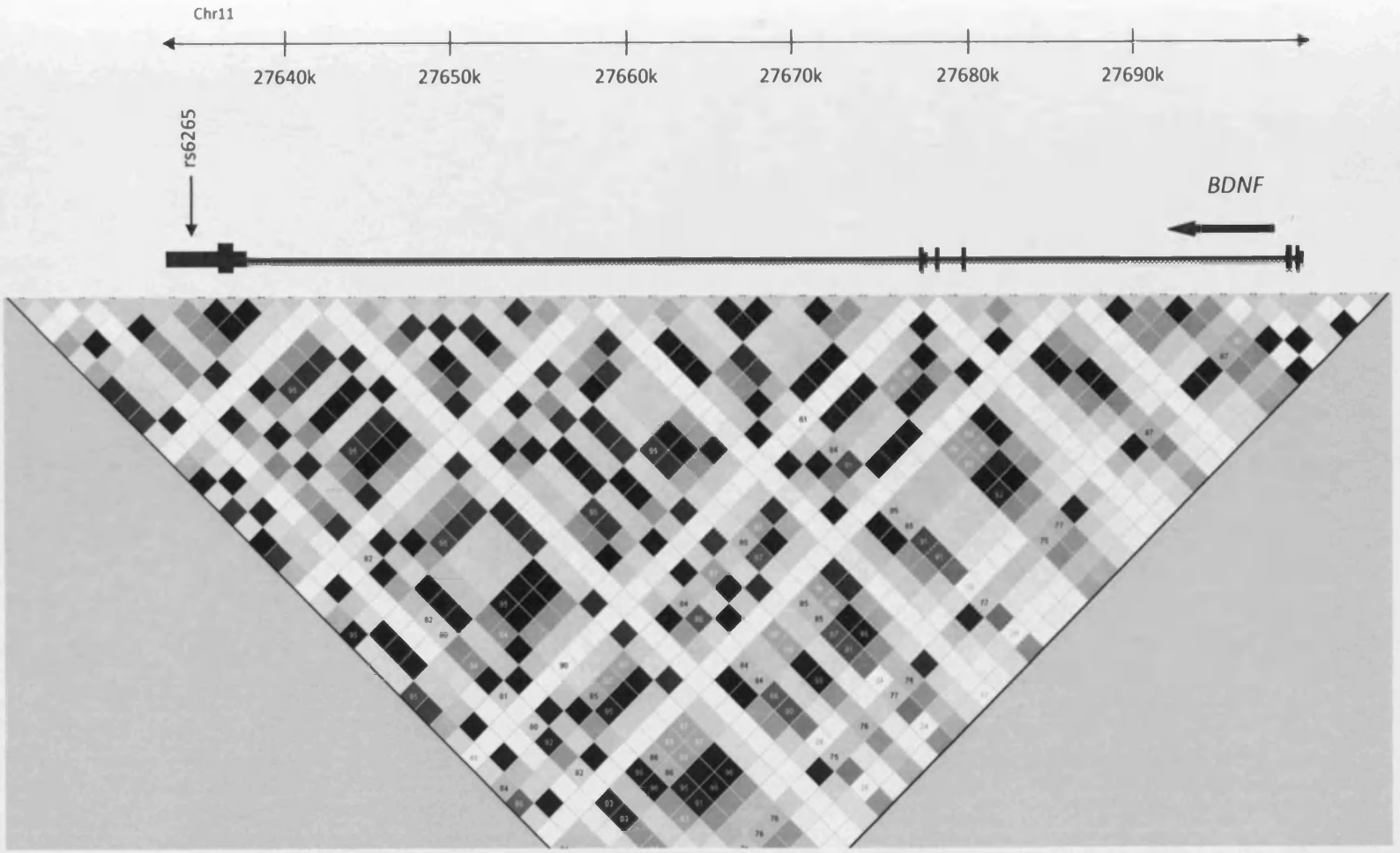


Figure 3.8. The *BDNF* locus. Schematic shows chromosomal position (bp), the SNP genotyped in this study, with its position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *BDNF* gene with exons/UTR indicated by black bars (long/short bars respectively), and finally the LD plot of the region (r^2).

3.8.1. Literature Summary

The *BDNF* locus has not been extensively studied in psychotic illness or neurodegenerative disease. The val66met variant has shown positive association to bipolar disorder (Lohoff et al. 2005) and schizophrenia (Neves-Pereira et al. 2005), but with no consistency or certainty. The marker has also shown association to AD (Ventriglia et al. 2002) but only in one study which must be set against the numerous negative findings (Bodner et al. 2005; He et al. 2007; Tsai et al. 2004; Tsai et al. 2006). The protein is a good putative functional candidate for both psychosis and neurodegeneration with its involvement in the action of the neurotransmitter dopamine, and its actions as a pro-survival factor (Zuccato et al. 2001). BDNF has been shown to be induced by calcium influx (Tao et al. 2002), a pathway which has recently seen a wealth of interest from the field of psychiatric genetics (O'Donovan et al. 2009). The protein has also shown reduced expression in two neurodegenerative disorders, including AD (Egan et al. 2003).

3.8.2. Study Design

Brain derived neurotrophic factor (*BDNF*) is a good putative functional candidate gene for schizophrenia and neurodegeneration. The val66met polymorphism (rs6265) of BDNF is the only gene variant to show consistent association with schizophrenia (Neves-Pereira et al. 2005) and more broadly with psychosis (Rosa et al. 2006), as well as neurodegenerative disorders including AD (Ventriglia et al. 2002). This study investigates the val66met polymorphism (rs6265) with respect to psychosis in LOAD. Polymorphism rs6265 was genotyped using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, CA) as described in chapter 2.7.3. The relative marker PCR and extension primers are shown in Appendix Table 3.12.

3.8.3. Results

Marker rs6265 showed no evidence for association with LOAD+P under either analysis model (Table 3.8a and 3.8b). The minor T allele of the marker showed a frequency of 19% in the LOAD+P dataset and 17% in both LOAD-P and control datasets. Marker rs6265 did show a significant allelic association with LOAD (P = 0.01, OR = 1.2). The minor T allele of the marker is over-represented in cases, with a frequency of 19% compared to a frequency of 17% in controls. This difference in allele frequency is equal to that seen in both analyses of LOAD+P, but the LOAD sample has greater power to detect the association. Sex effects have previously been documented at the *BDNF* locus (Verhagen et al. 2008) Therefore, the datasets were analysed by sex. This analysis reduces the sample size and therefore the power of the analysis. Differences in allele and genotype frequencies did not significantly differ in male or female samples for the disease modifier analysis. Analysis of the disease subtype model found a significant association of LOAD+P with males (P = 0.04, OR = 1.53), although this association did not withstand correction for multiple comparisons (permuted P = 0.12). Sex analysis of the *BDNF* marker in LOAD showed a significant association with males (P = 0.04, OR = 1.3), which did not survive correction for multiple comparisons (permuted P = 0.09) and did not surpass the association seen with LOAD irrelevant of sex (see Appendix Tables 3.13 - 3.16).

Table 3.8a. MAF and genotype counts of tested *BDNF* SNP. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

SNP ID	MA	MAF				Genotype Counts			
		LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs6265	T	0.19	0.17	0.19	0.17	12/111/242	14/74/167	54/347/768	44/360/931

Table 3.8b. Individual genotyping of *BDNF* SNP through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

SNP ID	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
	χ^2	*p*	OR	χ^2	*p*	χ^2	*P*	OR	χ^2	*P*	χ^2	*p*	OR	χ^2	*p*
rs6265	0.44	0.51	1.1 (0.83-1.47)	1.85	0.4	1.19	0.28	1.13 (0.91-1.39)	1.72	0.42	**6.07**	**0.01**	**1.2 (1.04-1.38)**	5.92	0.05

3.8.4. Discussion

The *BDNF* variant val66met (rs6265), previously associated with schizophrenia (Neves-Pereira et al. 2005) and bipolar disorder (Lohoff et al. 2005), does not associate with psychosis in LOAD in this study. Marker rs6265 does show significant association with LOAD with the met allele overrepresented in cases. The association appears to be stronger in males ($P = 0.04$) than females ($P = 0.11$), but does not exceed the association seen with full sample set irrespective of sex ($P = 0.01$, OR = 1.2). Polymorphism rs6265 is a non-synonymous variant that causes a substitution of valine to methionine in a portion of the protein thought to play a role in cytoplasmic trafficking and activity-dependent secretion of BDNF (Egan et al. 2003). Variation at rs6265 may reduce protein function, which fits well with the oxidative stress hypothesis of AD aetiology. The variant has previously shown association to AD (Ventriglia et al. 2002) but this is set against numerous negative reports (Bodner et al. 2005; He et al. 2007; Tsai et al. 2004; Tsai et al. 2006). Investigation of rs6265 in independent LOAD datasets is warranted.

3.9. D-amino Acid Oxidase Activator

The D-amino acid oxidase activator (*DAOA*) encodes a protein that activates the enzyme D-amino acid oxidase (DAO). DAO degrades the gliotransmitter D-serine, a potent activator of NMDA type glutamate receptors, resulting in decreased NMDA receptor functioning.Hypof unction of the NMDA receptor is a well regarded model in the aetiology of psychosis (Farber 2003). A broad length of chromosome 13q (~50 cM), which harbours the *DAOA* gene, is implicated as a linkage region for both schizophrenia and bipolar disorder (Badner and Gershon 2002; Berrettini 2003; Gershon and Badner 2001). Linkage of schizophrenia to chromosome 13q14-q22 was first reported by Lin and colleagues (Lin et al. 1995) in large pedigrees from the UK and Japan (MLS = 1.61). A meta-analysis of 44 independent pedigrees found suggestive linkage with the maximum LOD score (MLS = 2.58) mapping to band 13q32 in a Caucasian but not an Oriental population (Lin et al. 1997). Significant (Blouin et al. 1998; Brzustowicz et al. 1999) and suggestive (Abecasis et al. 2004; Faraone et al. 2002; Shaw et al. 1998)

evidence for linkage to 13q32 have been reported in multiple ethnic and geographical populations, using varying diagnostic phenotypes. Negative findings of linkage to this region in large schizophrenia samples are also apparent (Coon et al. 1994; DeLisi et al. 2000; Faraone et al. 1998; Gurling et al. 2001; Kaufmann et al. 1998; Kendler et al. 1996; Levinson et al. 2000; Mowry et al. 2000; Riley et al. 1998; Schwab et al. 2000). Meta analyses of the region provide inconsistent results, with one positive (Badner and Gershon 2002) and one negative study (Lewis et al. 2003). The study of Lewis and colleagues (Lewis et al. 2003) is the more systematic of the two, being based on data collected using identical methods across many studies both published and unpublished, whereas Badner and Gershon (Badner and Gershon 2002) relied on published data that had been analysed using different methods (Owen 2005). Linkage evidence for chromosome 13q32 in bipolar disorder is equally as inconsistent. Significant evidence for linkage was found in a small study under a broad phenotypic (Detera-Wadleigh et al. 1999) and suggestive linkage using a narrow diagnostic phenotype (bipolar I and schizoaffective disorder bipolar type) (Stine et al. 1997). Numerous bipolar disorder genome scans of substantial size and across multiple nationalities have not found evidence for linkage to the region (Bennett et al. 2002; Cichon et al. 2001; Coon et al. 1993; Foroud et al. 2000; Friddle et al. 2000). One meta-analysis has shown significant linkage with this region and bipolar disorder (Badner and Gershon 2002), but two more extensive and detailed meta-analyses produced negative findings (McQueen et al. 2005; Segurado et al. 2003).

Chumakov and colleagues (Chumakov et al. 2002) used linkage disequilibrium-based positional cloning to identify genes within the linkage region on chromosome 13q22-34. Two regions contained markers associated with schizophrenia in a French-Canadian sample, and two markers from the distal region replicated the association in a Russian sample. *DAOA* was identified as a gene residing within this region. Replication studies have subsequently reported positive associations of *DAOA* and schizophrenia in samples from Germany (Schumacher et al. 2004), China (Hong et al. 2006; Ma et al. 2006; Wang et al. 2004; Yue et al. 2007; Zou et al. 2005), Ashkenazi Jew (Korostishevsky et al. 2004), Ireland (Corvin et al. 2007), Scotland (Ma et al. 2006), Korea (Shin et al. 2007), the United States (Hall et al. 2004; Nicodemus et al. 2006) and South Africa (Hall et al. 2004), among others (Addington et al. 2004; Korostishevsky et al. 2005;

Korostishevsky et al. 2006a; Shinkai et al. 2007; Zou et al. 2005). However, the associated alleles and haplotypes are not consistent across studies with some variants located outside the gene (Detera-Wadleigh and McMahon, 2006), and no pathologically relevant variant has yet been identified. Several studies show negative association of *DAOA* and schizophrenia (Liu et al. 2006), predominantly in samples from the United States (Fallin et al. 2005; Sanders et al. 2008; Wood et al. 2007), including those of mixed race (Mulle et al. 2005), and from Europe (Bakker et al. 2007; Vilella et al. 2008; Williams et al. 2006). There are three independent meta-analysis of *DAOA* in schizophrenia, all show association of several *DAOA* markers with disease, in both Asian and European samples (Detera-Wadleigh and McMahon 2006; Li and He 2007; Shi et al. 2008). However, there is no inter-study consensus as to the associated markers. Gene expression analysis of *DAOA* in a schizophrenia case-control sample has identified a tendency toward over-expression of *DAOA* in prefrontal cortex samples of cases (Korostishevsky et al. 2006b). Thus, supporting the biologic plausibility of *DAOA* as a candidate gene, based on NMDA receptor hypofunction. Association at the *DAOA* locus has been reported with bipolar disorder in two US family samples (Hattori et al. 2003), which replicated in a further US family sample (Chen et al. 2004c), German case–control samples (Schulze et al. 2005; Schumacher et al. 2004), a Polish case-control sample (Schulze et al. 2005) and UK case-control samples (Prata et al. 2008; Williams et al. 2006). In all studies, evidence for association came from individual SNPs as well as multivariant haplotypes, although there is variation between studies as to the specific SNPs and haplotypes showing association. One of the two bipolar disorder meta-analyses of *DAOA* shows positive association of several markers, albeit to a lesser extent than seen in schizophrenia (Detera-Wadleigh and McMahon 2006). The other meta-analysis found no overall association with bipolar disorder (Shi et al. 2008). Recently *DAOA* has shown association to AD with delusions in a cohort of 185 Alzheimer's disease patients (Di Maria et al. 2009). The analysis demonstrated a nominally significant association ($P < 0.05$) with one SNP (rs2153674), with multivariate regression showing that the genotype accounts for up to 15% of the variance in delusions severity (Di Maria et al. 2009).

3.9.1. Literature Summary

The data suggests *DAOA* as a strong functional and positional candidate gene for both schizophrenia and bipolar disorder. Association analyses in schizophrenia are inconsistent with both positive and negative associations seen across numerous populations. Studies in bipolar disorder are more consistent, with positive associations seen in all the published studies except the most recent meta-analysis (Shi et al. 2008), although, the identified associations are generally weaker than those seen in schizophrenia. There is no consensus between studies in either disorder as to which variants and/or haplotypes are associated with disease status. Of recent interest is the study of Williams and colleagues (Williams et al. 2006) who analysed *DAOA* polymorphisms in both schizophrenia and bipolar disorder patients across traditional diagnostic categories and revealed significant evidence for association in a subset of cases in which episodes of major mood disorder had occurred (Williams et al. 2006). By contrast, no evidence for association was observed in the subset of cases in which psychotic features occurred (Williams et al. 2006). Thus, suggesting that variation at the *DAOA* locus does not primarily increase susceptibility to psychosis but instead influences susceptibility to episodes of mood disorder across the traditional categories of bipolar disorder and schizophrenia. However, this finding requires replication particularly as it conflicts a number of studies which find stronger *DAOA* associations in schizophrenia and not bipolar disorder (Detera-Wadleigh and McMahon 2006; Schumacher et al. 2004). One *DAOA* variant has recently shown evidence of association to AD with delusions (Di Maria et al. 2009). The identified polymorphisms are almost certainly not the true pathogenic variants, as even in the most convincing cases, the risk haplotypes appear to be associated with small effect sizes (Owen 2005), and do not account for the linkage observed. Alternatively the linkage could in fact reflect variation at more than one susceptibility site within *DAOA*, or within a neighbouring gene(s).

3.9.2. Study Design

Nine variants were tested for association with LOAD+P and LOAD. Five markers were chosen from the study of Williams and colleagues (Williams et al.

2006) to tag the *DAOA* gene and approximately 6 kb of flanking sequence. Variants were identified through sequence analysis of schizophrenia cases, marker-marker linkage disequilibrium was calculated, and the Haploview software (Barrett et al. 2005) used to examine the haplotype block structure and to select a set of maximally informative single-nucleotide polymorphisms (SNPs), with a minor allele frequency (MAF) greater than 10%, that tagged all of the haplotypes with a frequency greater than 5%. The remaining four polymorphisms (rs3918342, rs3916965, rs778293, and rs1421292) were selected from the Single Nucleotide Polymorphism Database (dbSNP) and genotyped to supplement the tag SNPs so that the markers spanned the *DAOA* locus at an average of 7.9 kb, these supplementary SNPs had also been shown to associate with schizophrenia (Chumakov et al. 2002). The LD structure across the *DAOA* locus and surrounding region, inclusive of the studied variants, according to the HapMap CEPH genotypes can be seen in Figure 3.9. All markers were genotyped using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, California, USA) as described in chapter 2.7.3. The relative marker PCR and extension primers are shown in Appendix Table 3.17.

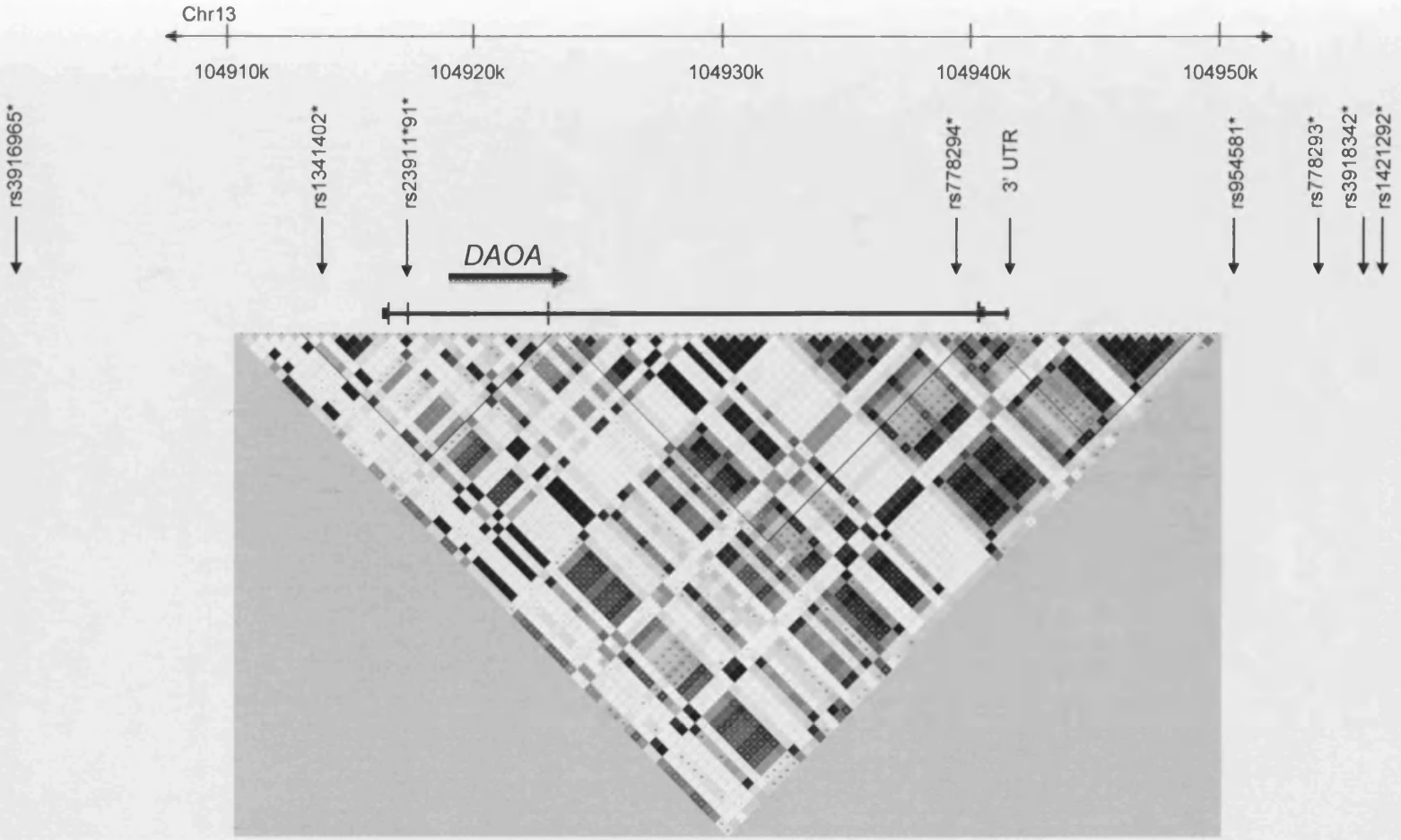


Figure 3.9. The *DAOA* locus. Schematic shows chromosomal position (bp), the SNPs genotyped in this study (* indicates database SNP), with their position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *DAOA* gene with exons/UTR indicated by black bars (long/short bars respectively), and finally the LD plot of the region (r^2).

3.9.3. Results

Three variants within *DAOA* showed deviation from HWE in the LOAD-P dataset. These variants are the 3'UTR marker, rs778294 and rs954581, with p-values of 0.02, 0.04 and 0.01 respectively. None of these markers showed association with LOAD+P or LOAD. No variant showed a difference in allele or genotype frequency in the disease modifier analysis. A nominal allelic association was observed with marker rs778293 ($P = 0.04$) when LOAD+P cases were compared to controls, with the major A allele over-represented in cases by 4%. This association does not survive correction for multiple testing (permuted $P = 0.3$). No difference in allele or genotype frequency was observed in the LOAD versus control analysis. The greatest trend towards association was with the novel UTR variant (allelic $P = 0.07$), see Tables 3.8a and 3.8b. Haplotype analysis revealed no further evidence for association (global $P = 0.42$, 0.74 and 0.48, for the disease modifier, disease subtype and LOAD analysis, respectively), see Appendix Table 3.18.

3.9.4. Discussion

Our results provide no evidence for the involvement of the *DAOA* locus in LOAD+P, under either disease model. The *DAOA* locus has been comprehensively examined in this study, with coverage of all LD blocks within the region by both a "tagging" and "SNP per kb approach". However variants with a MAF < 10% and rare haplotypes with frequency < 5% have not been analysed. Also, variants were selected by LD in the HapMap CEPH trios and in a schizophrenia and bipolar disorder case-control sample using phase 1 of the HapMap project. More recent HapMap phases include additional genotype data which may increase the number of markers required to tag the locus. Also, the LOAD sample used in this study may show a differing pattern of LD. However, the pattern of LD in the CEPH trios is highly concordant with that of the schizophrenia and bipolar disorder case-control samples, making it unlikely that the pattern of LD in our LOAD sample would be significantly different. Also the previously associated AD with delusion variant rs2153674 is in LD (D' = 1, r2 = 0.6) with marker rs778294, suggesting that the previously identified association would not replicate in our sample set.

Table 3.9a. MAF and genotype counts of tested *DAOA* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs3916965	A	0.39	0.37	0.38	0.38	56/163/134	35/112/101	149/485/385	163/546/443
rs1341402	C	0.23	0.24	0.24	0.22	19/128/216	16/91/146	64/372/606	60/409/712
rs2391191	A	0.38	0.36	0.38	0.38	55/159/140	32/104/99	143/475/386	165/540/447
3' UTR	A	0.37	0.39	0.36	0.34	144/163/49	102/100/47	419/472/141	522/522/136
rs778294	A	0.31	0.32	0.31	0.29	175/148/40	125/97/33	506/425/113	605/488/107
rs954581	C	0.18	0.15	0.17	0.17	244/103/15	187/52/11	723/276/34	816/333/42
rs778293	G	0.41	0.38	0.39	0.38	58/181/124	42/107/106	168/487/393	182/547/464
rs3918342	A	0.51	0.49	0.51	0.49	99/171/94	64/125/67	264/508/275	311/592/283
rs1421292	A	0.47	0.49	0.48	0.47	81/171/107	62/115/69	244/498/283	264/582/334

Table 3.9b. Individual genotyping of *DAOA* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs3916965	0.63	0.43	1.1 (0.87-1.4)	0.61	0.74	1.41	0.24	1.12 (0.93-1.34)	0.65	0.72	0.12	0.73	1.02 (0.9-1.16)	0.15	0.93
rs1341402	0.35	0.56	1.08 (0.83-1.41)	0.42	0.81	0.03	0.87	1.02 (0.83-1.25)	0.07	0.96	1.51	0.22	1.09 (0.95-1.25)	1.72	0.42
rs2391191	0.59	0.44	1.1 (0.86-1.4)	0.6	0.74	0.45	0.5	1.06 (0.89-1.28)	0.54	0.76	0.01	0.94	1 (0.89-1.14)	0.04	0.98
3' UTR	0.47	0.49	1.09 (0.86-1.38)	3.49	0.17	0.99	0.32	1.1 (0.91-1.32)	2.19	0.33	3.25	0.07	1.12 (0.99-1.27)	4	0.14
rs778294	0.07	0.79	1.03 (0.81-1.32)	0.77	0.68	0.6	0.44	1.08 (0.89-1.3)	1.58	0.45	1.9	0.17	1.09 (0.96-1.24)	2.5	0.29
rs954581	2.56	0.11	1.29 (0.94-1.76)	4.59	0.1	0.64	0.43	1.1 (0.87-1.37)	0.36	0.84	0.57	0.45	1.06 (0.91-1.24)	0.58	0.75
rs778293	1.54	0.22	1.16 (0.92-1.46)	4.24	0.12	**4.33**	**0.04**	**1.21 (1.01-1.44)**	2.7	0.26	0.48	0.49	1.04 (0.92-1.18)	0.54	0.76
rs3918342	0.2	0.66	1.05 (0.84-1.32)	0.39	0.82	0.03	0.85	1.02 (0.86-1.21)	1.03	0.6	1.3	0.26	1.07 (0.95-1.2)	1.73	0.42
rs1421292	0.6	0.44	1.1 (0.87-1.38)	0.61	0.74	0.07	0.79	1.02 (0.86-1.22)	0.38	0.83	0.58	0.45	1.05 (0.93-1.18)	0.64	0.72

3.10. 2', 3'-Cyclic Nucleotide 3' Phosphodiesterase

The 9 kb 2', 3'-cyclic nucleotide 3' phosphodiesterase (*CNP*) gene is located at chromosome 17q21 (Douglas et al. 1992; Monoh et al. 1993; Sprinkle et al. 1992) (Figure 3.10). There are two transcription start points and, in human brain, two forms of CNP mRNA are produced by alternative splicing (Monoh et al. 1993; Scherer et al. 1994). Region 17p11.2-q25.1 (44–97 cM) has shown highly suggestive evidence for linkage with schizophrenia (MLS = 3.35) in a UK sample (Williams et al. 2003b), although much of the linkage signal could be attributed to a single pedigree (MLS = 8.68) (Williams et al. 2003b). This region of chromosome 17 overlaps a previously identified region showing significant evidence for linkage to schizophrenia following meta-analysis (Lewis et al. 2003). Peirce and colleagues (Peirce et al. 2006) identified a marker within *CNP* (rs2070106) that associates with schizophrenia in a Caucasian case-control sample ($P = 0.04$) (Peirce et al. 2006). The A allele of the marker is more common in affected individuals and is associated with lower *CNP* expression ($P < 0.001$) (Peirce et al. 2006). All affected siblings of the pedigree previously showing linkage to chromosome 17 p11.2-q25.1 (Williams et al. 2003b) were homozygous for the lower-expression allele (Williams et al. 2003b). A significant effect of rs2070106 ($P = 0.03$) on *CNP* gene expression in the dorsolateral prefrontal cortex (DLPFC) grey matter, with the A allele correlating with reduced expression, provides independent support for this variation as a risk factor for schizophrenia (Mitkus et al. 2007). No significant association with schizophrenia was observed by single-marker or haplotype analysis of *CNP* in Japanese (Usui et al. 2006) or Han Chinese (Tang et al. 2007) populations. This failure to replicate the previous finding may be due to ethnic differences (Marchini et al. 2005), a theory supported by differences in the LD block structure of *CNP* between the studied populations in the HapMap database. Multiple microarray studies have reported the down regulation of oligodendrocyte and myelination related genes, including *CNP*, in the schizophrenic brain (Aston et al. 2004; Hakak et al. 2001; Katsel et al. 2005; Tkachev et al. 2003). Assessment of oligodendrocyte-related gene expression in white matter finds independent evidence of reduced *CNP* expression in the tissue of schizophrenic patients (Prabakaran et al. 2004; McCullumsmith et al. 2007). Down-regulation of *CNP*

mRNA has been confirmed at the protein level, with reduced immunoreactivity of CNP in anterior frontal cortex of schizophrenics (Flynn et al. 2003).

The precise biological function of CNP is unclear. The two forms of CNP mRNA produce membrane-bound enzymes found abundantly in the cytoplasmic compartments of central nervous system (CNS) myelin (Esposito et al. 2008; O'Neill and Braun 2000). Developmentally, CNP is one of the earliest myelination-specific polypeptides synthesized by oligodendrocytes and its synthesis persists into adulthood, suggesting a role in the synthesis and maintenance of the myelin sheath (O'Neill and Braun 2000). In CNP deficient mice features identical to the pathology of schizophrenia are observed (Lappe-Siefke et al. 2003), and these mice have disrupted axon–glia interactions in the CNS, which may be involved in the aetiology of schizophrenia (Rasband et al. 2005). This schizophrenia pathology is followed by delayed onset neurodegenerative disease indicating the importance of CNP to the maintenance of myelin and axonal integrity (Rasband et al. 2005). Over-expression of human CNP in transgenic mice produces abnormalities of oligodendrocytes and their myelin sheaths (Gravel et al. 1996; Yin et al. 1997). Similar structural myelin abnormalities are observed in aged monkeys (Hinman et al. 2006), where CNP accumulates and CNP proteolysis is significantly increased (Hinman et al. 2007). This CNP accumulation is hypothesised to be a result of impairment of the ubiquitin proteasomal system (Hinman et al. 2007; Sandell and Peters 2003; Sloane et al. 2000). Changes in brain white matter are prominent features of the aging brain and include glial cell activation, disruption of myelin membranes and loss of myelinated fibers associated with inflammation and oxidative stress (Hinman et al. 2007). Abnormal changes of white matter are common in patients with AD (Brun and Englund 1986; Englund and Brun 1990; Fazekas et al. 1996; Scheltens et al. 1992; Scheltens et al. 1995; Steingart et al. 1987), although the mechanisms for the development of these changes are not fully understood (Lee et al. 2006). Specific white matter changes have shown association with symptoms of psychosis in AD, particularly delusional misidentification (Binetti et al. 1995; Lee et al. 2006). CNP bioactivity is similar to that of other proteins implicated in neurodegenerative diseases, in particular a malfunction of CNP as a membrane-interacting peptide could reduce membrane plasticity (Esposito et al. 2008). Numerous groups have explored expression and activity of CNP in AD brains, with decreased levels found in frontal cortex of AD

brains (Vlkolinsky et al. 2001). CNP activity, amount of myelin proteins and lipids have also been shown to be decreased in AD brains (Reinikainen et al. 1989; Svennerholm and Gottfries 1994; Wallin et al. 1989), indicating loss of myelin and thus myelinated axons in the affected brain regions (Reinikainen et al. 1989).

3.10.1. Literature Summary

Following evidence of suggestive linkage to chromosomal region 17p11.2-q25.1 with schizophrenia, association with the *CNP* locus was identified (Peirce et al. 2006). The associated marker is correlated with decreased *CNP* expression in peripheral blood (Peirce et al. 2006), and DLPFC grey matter (Mitkus et al. 2008). The genetic association with rs2070106 did not replicate in successive studies (Tang et al. 2007; Usui et al. 2006) probably due to ethnic differences across studies. CNP is a membrane-bound enzyme found predominantly in central nervous system myelin, where it is suggested to function in the onset and maintenance of the myelin sheath. Accumulation of CNP is noticeable in the aging brain. In animal models deficiency of CNP leads to a schizophrenia like pathology and neurodegeneration (Rasband et al. 2005). Over-expression of CNP produces altered cell morphology and oligodendrocyte and myelin abnormality (Gravel et al. 1996; Yin et al. 1997). CNP functions in white matter maintenance and shows similarity to neurodegenerative proteins making CNP a putative functional candidate for AD. No genetic investigations into the role(s) of CNP in the aetiology of AD have been pursued, and the decrease in CNP levels and activity in AD brains are probably secondary to neuronal loss. However, CNP remains an interesting putative functional candidate gene for AD due to the similarity of its bioactivity to that of other proteins implicated in neurodegenerative diseases and its function in white matter maintenance, a process thought to be dysfunctional in the AD brain (Lee et al. 2006).

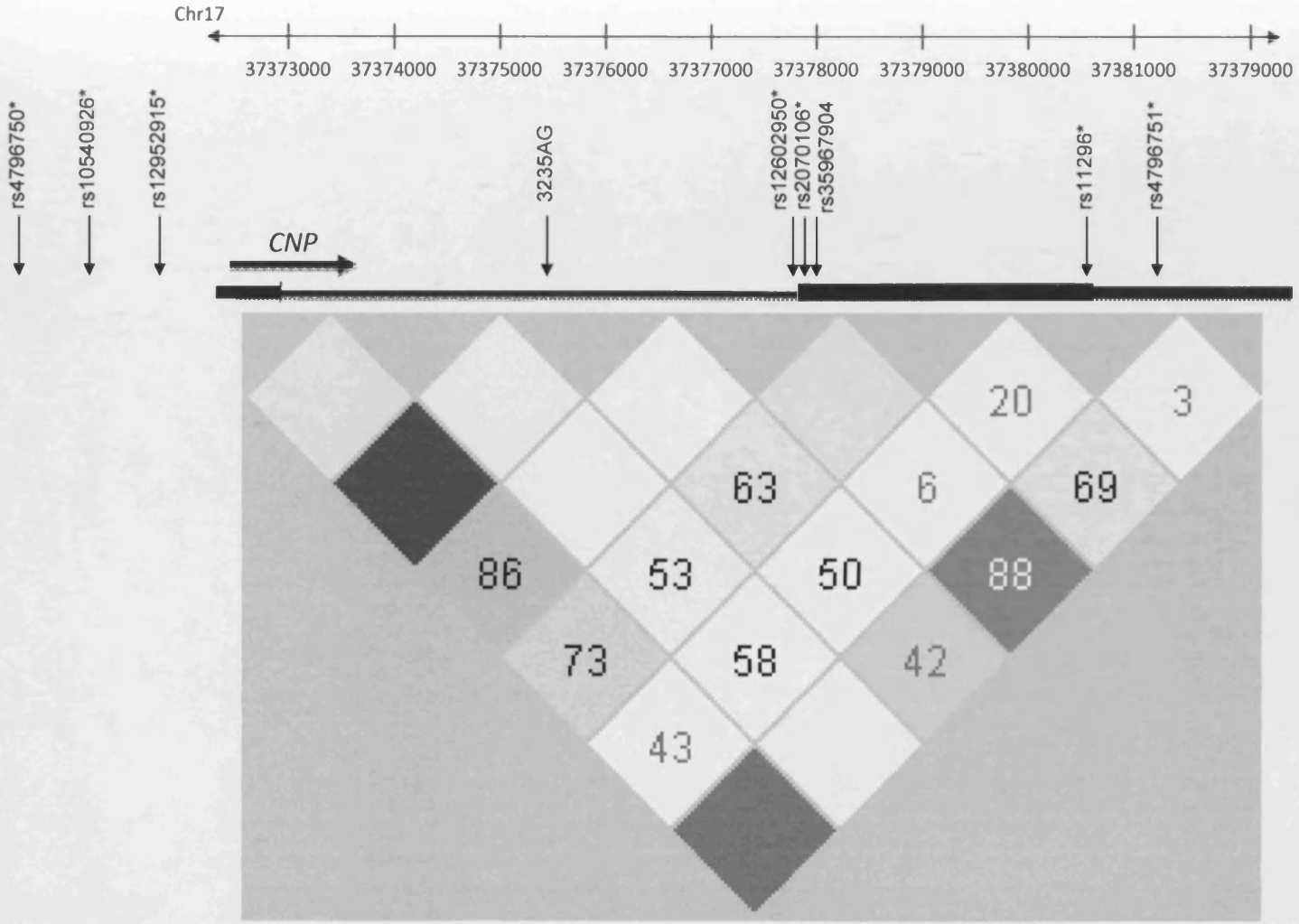


Figure 3.10. The *CNP* locus. Schematic shows chromosomal position (bp), the SNPs genotyped in this study (* indicates database SNP), with their position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *CNP* gene with exons/UTR indicated by black bars, (long/short bars respectively) and finally the LD plot of the region (r^2).

3.10.2. Study Design

Ten SNPs at the *CNP* locus were examined for association with LOAD+P and LOAD, including marker rs2070106 which has previously shown association with schizophrenia (Peirce et al. 2006). Markers were identified from a previous study of *CNP* in Caucasian schizophrenics (Peirce et al. 2006). Variants included in the previous study were examined for LD across *CNP* (11 kb) in CEPH individuals using the HAPLOVIEW software (Barrett et al. 2005). Based on $r^2 > 0.8$ and minor allele frequency (MAF) > 0.05, non-redundant markers were chosen for genotyping. Nine of the SNPs are available from public databases, the remaining SNP was identified by DNA screening of 14 schizophrenia patients in the previous study. All markers were genotyped using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, California, USA) as described in chapter 2.7.3. Relative PCR and extension sequences are provided in Appendix Table 3.19.

3.10.3. Results

One *CNP* marker, rs4796750 showed deviation from HWE ($P = 0.02$) in the LOAD with psychosis dataset. Neither this variant nor any other variant tested showed a significant allelic or genotypic association with LOAD+P under either disease model (see Tables 3.10a and 3.10b). Haplotypic analysis of the *CNP* locus provided no further evidence for association with LOAD+P (global $P = 0.6$ and 0.55 for LOAD+P vs. LOAD-P and LOAD+P vs. control analysis, respectively), see Appendix Table 3.20. None of the SNPs showed an allelic, genotypic or haplotypic (global $P = 0.67$) association with LOAD.

Table 3.10a. MAF and genotype counts of tested *CNP* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	**MA**	**LOAD+P**	**LOAD-P**	**LOAD**	**Control**	**LOAD+P**	**LOAD-P**	**LOAD**	**Control**
rs4796750	A	0.27	0.25	0.27	0.26	32/105/177	12/79/117	75/329/482	70/384/568
rs10540926	-	0.2	0.18	0.19	0.18	15/99/201	8/59/142	31/252/580	29/261/649
rs12952915	A	0.19	0.15	0.18	0.17	14/92/208	5/54/149	35/245/606	38/278/706
3235AG	G	0.27	0.26	0.26	0.26	27/125/183	12/93/124	65/395/539	72/445/613
rs12602950	G	0.27	0.25	0.27	0.26	31/108/175	12/80/116	72/334/480	71/288/563
rs2070106	A	0.35	0.32	0.33	0.33	42/136/136	24/87/97	103/386/397	102/464/456
rs11079028	A	0.19	0.19	0.2	0.22	12/97/205	9/60/139	38/272/576	57/327/638
rs35967904	A	0.14	0.13	0.14	0.14	10/66/238	2/51/155	23/198/665	17/248/757
rs11296	C	0.05	0.06	0.05	0.06	0/33/281	1/23/184	3/91/792	4/112/906
rs4796751	T	0.14	0.13	0.15	0.14	8/70/236	2/50/156	16/225/645	17/258/747

Table 3.10b. Individual genotyping of *CNP* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*P*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs4796750	0.6	0.44	1.12 (0.84-1.49)	3.64	0.16	0.41	0.52	1.07 (0.87-1.31)	4.67	0.1	0.95	0.33	1.07 (0.93-1.24)	1.77	0.41
rs10540926	0.6	0.75	1.08 (0.7-1.42)	0.7	0.64	1.98	0.15	1.14 (0.76-1.56)	1.7	0.39	0.19	0.84	1.02 (0.87-1.19)	1.86	0.4
rs12952915	2.39	0.12	1.3 (0.93-1.81)	2.48	0.29	1.05	0.3	1.13 (0.9-1.42)	1	0.61	0.14	0.71	1.03 (0.87-1.22)	0.13	0.94
3235AG	0.19	0.66	1.06 (0.81-1.39)	1.95	0.38	0.06	0.8	1.03 (0.84-1.25)	1.12	0.57	0.03	0.87	1.01 (0.88-1.16)	0.03	0.99
rs12602950	0.55	0.46	1.11 (0.84-1.48)	3.13	0.21	0.32	0.57	1.06 (0.87-1.3)	3.54	0.17	0.53	0.47	1.06 (0.91-1.22)	0.96	0.62
rs2070106	0.74	0.39	1.12 (0.86-1.46)	0.71	0.7	1.2	0.27	1.11 (0.92-1.34)	2.9	0.24	0.23	0.63	1.03 (0.9-1.18)	1.56	0.46
rs11079028	0.04	0.83	1.03 (0.75-1.42)	0.3	0.86	1.54	0.21	1.15 (0.92-1.44)	1.84	0.4	2.17	0.14	1.13 (0.96-1.32)	2.33	0.31
rs35967904	0.05	0.83	1.04 (0.72-1.5)	3.4	0.18	0	0.95	1.01 (0.78-1.31)	3.92	0.14	0.0006	0.98	1 (0.83-1.21)	2.78	0.25
rs11296	0.27	0.6	1.15 (0.68-1.97)	1.56	0.46	0.34	0.56	1.12 (0.76-1.67)	1.29	0.52	0.28	0.6	1.08 (0.82-1.42)	0.28	0.87
rs4796751	0.11	0.74	1.06 (0.74-1.53)	1.81	0.4	0.14	0.71	1.05 (0.81-1.36)	2	0.37	0.04	0.85	1.02 (0.85-1.22)	0.07	0.97

3.10.4. Discussion

CNP has previously show significant association with schizophrenia in a Caucasian case-control sample (Peirce et al. 2006), which correlates with decreased gene expression (Mitkus et al. 2008; Peirce et al. 2006). The ten markers genotyped in the Peirce and colleagues study, which tag the *CNP* locus, were investigated for association with LOAD+P. No significant association was found. It is unlikely that common genetic variation at the CNP locus contributes to the aetiology of psychosis in LOAD. However, rare variation may contribute to the aetiology of LOAD+P and the CNP locus should not be excluded from this type of future analysis.

3.11. Oligodendrocyte Lineage Transcription Factor 2

The oligodendrocyte lineage transcription factor 2 (*OLIG2*) was originally identified in rodents. The gene is located on the long arm of chromosome 21 at q22.11, a band commonly called the Down syndrome region, as translocation of chromosome 21 at q22.11 is sufficient to produce the Down syndrome phenotype. Most linkage studies do not support the involvement of the region containing the *OLIG2* gene (21q22) in schizophrenia; but structural chromosomal aberrations in this region have been detected in schizophrenics (Demirhan and Tastemir 2003). Analysis of *OLIG2* in a UK case-control sample (n = 1,400) revealed several SNPs to be associated with schizophrenia (minimum $P = 0.0001$, gene-wide $P = 0.0009$) (Georgieva et al. 2006). Genetic interaction between *OLIG2* and two genes of relevance to oligodendrocyte function has also been identified (Georgieva et al. 2006). Evidence for association of these genes with schizophrenia has previously been reported (Norton et al. 2006; Peirce et al. 2006). Thus, providing strong evidence that variation in *OLIG2* confers susceptibility to schizophrenia alone and as part of a network of genes implicated in oligodendrocyte function. Two schizophrenia associated SNPs rs1059004 and rs9653711 predict low mRNA expression in DLPFC white matter. Post-hoc comparisons showed that these SNPs are in perfect linkage disequilibrium ($r^2 = 1$), and that individuals carrying the minor A allele at SNP rs1059004 and the C allele at SNP rs9653711 (enriched in cases)

had lower OLIG2 mRNA levels than subjects homozygous for the major allele (Mitkus et al. 2008). The data remained significant after correcting for multiple comparisons. Exonic variant rs762178 replicated its significant genetic association to schizophrenia in a small sample set (n = 617; $P = 0.005$, Bonferroni corrected $P = 0.015$) (Huang et al. 2008). However, the associated allele was not consistent with that reported by Georgieva and colleagues (Georgieva et al. 2006); this may be attributed to genetic heterogeneity since the studies were carried out in distinct ethnic populations. A significantly associated haplotype was identified in the Huang and colleagues study (Bonferroni corrected $P = 0.009$), which may influence *OLIG2* expression through regulation of mRNA activity (Kuersten and Goodwin 2003). A negative finding of *OLIG2* has been reported in a Japanese schizophrenia sample (Usui et al. 2006). Down-regulation of oligodendrocyte and myelination genes has been shown by 1·65-fold ($P = 0·0027$) in schizophrenia and 2·46-fold ($P = 0·0003$) in bipolar disorder brains compared to controls (Tkachev et al. 2003).

OLIG2 (Figure 3.11a) encodes a 32 kDa basic helix-loop-helix (bHLH) transcription factor. Members of the bHLH transcription factor super-family are indicated to be important regulatory components in transcriptional networks, controlling a diversity of processes from cell proliferation to cell lineage establishment. *OLIG2* is expressed exclusively in both the developing and mature vertebrate CNS (Ligon et al. 2006a), specifically in cells of the oligodendrocyte lineage (Tabu et al. 2006). It plays a key role in the cell fate specification of oligodendrocytes, the myelinating cell of the central nervous system (Ligon et al. 2006a; Ligon et al. 2006b; Miller 2002; Pfeiffer et al. 1993). Overexpression of OLIG2 has been found in a wide spectrum of glial cell originating malignant cell lines, including leukaemia, non-small cell lung carcinoma, melanoma, and breast cancer cell lines (Lin et al. 2005b). The protein has also been implicated in the repair process after a traumatic injury (Magnus et al. 2007).

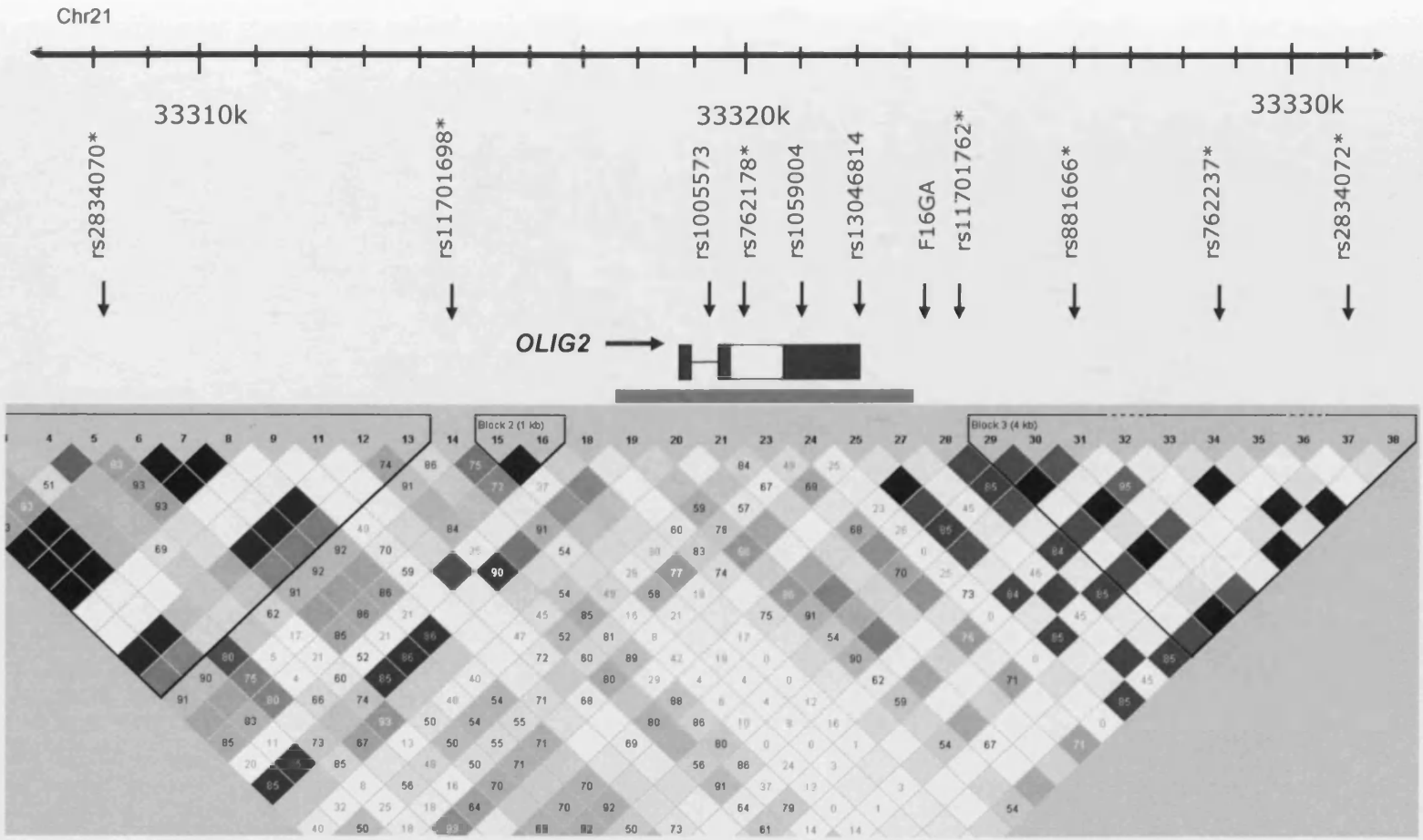


Figure 3.11a. Schematic of *OLIG2* and marker position relative to position (in bases) given in HapMap. * indicates db SNPs. Black indicates untranslated region. White indicates translated region. The line under *OLIG2* depicts region screened for polymorphisms in Georgieva et al. (2006) study. LD plot of the region (r^2) provided at bottom.

Decreased expression of myelin and oligodendrocyte-related genes has been demonstrated in post-mortem prefrontal cortex samples of schizophrenic individuals (Hakak et al. 2001; Hof et al. 2003; Tkachev et al. 2003). Thus, suggesting that there is a disruption of oligodendrocyte function and/or a loss of oligodendrocytes in schizophrenia (Hof et al. 2003). Neuronal cell death following down-regulation of *OLIG2* has been reported in AD models (Uchida et al. 2007; Waldau and Shetty 2008) and reduced expression of other myelination genes has been reported in AD (Katsel et al. 2005). In an AD mouse model, a decrease in OLIG2 cells can be observed in the cerebral cortex (Uchida et al. 2007), hypothesised to result from Aβ42 cells down-regulating OLIG2, and switching the cell fate to death (Uchida et al 2007). Abnormal changes in white matter are common in patients with AD (Scheltens et al. 1992; Scheltens et al. 1995; Fazekas et al. 1996) and specific white matter changes have been shown to significantly correspond with psychosis score in AD (Lee et al. 2006), with a previous computerised tomography (CT) study suggesting a relationship between delusions and lacunar infarcts of white matter and AD (Binetti et al. 1995).

3.11.1. Literature Summary

OLIG2 plays a key role in the cell fate specification of oligodendrocytes, (Miller et al. 2002; Pfeiffer et al. 1993), acting in the development and maintenance of the myelin sheath (Ligon et al. 2006a; Ligon et al. 2006b). Several SNPs within *OLIG2* have been found to be associated with schizophrenia (Georgieva et al. 2006), as have variants within *OLIG2* interacting genes (Georgieva et al. 2006; Huang et al. 2008; Norton et al. 2006; Peirce et al. 2006). The polymorphic variants identified have been shown to significantly affect gene expression (Mitkus et al. 2008). Neuronal cell death following down-regulation of *OLIG2* has been reported in AD models (Uchida et al. 2007; Waldau and Shetty 2008) and reduced expression of other myelination genes has been reported in AD (Katsel et al. 2005). White matter changes in the AD brain have been shown to significantly correlate with degree of psychosis (Binetti et al. 1995; Lee et al. 2006)

3.11.2. Study Design

Eleven SNPs were examined in *OLIG2*, some of which have previously shown association to schizophrenia (Georgieva et al. 2006), were tested for association with LOAD+P and LOAD. Markers were identified from the previous study of *OLIG2* in Caucasian schizophrenics (Georgieva et al. 2006). Variants included in the previous study were examined for LD across *OLIG2* (3.2 kb) plus ± 20 kb in CEPH individuals using the HAPLOVIEW software (Barrett et al. 2005). Based on $r^2 > 0.8$ and minor allele frequency (MAF) > 0.05, non-redundant markers were chosen for genotyping in this study. Seven of the SNPs are available from public databases. The remaining 4 SNPs were identified by DNA screening of 14 schizophrenia patients in the previous study (Georgieva et al. 2006). All markers were genotyped using the Sequenom™ MassARRAY™ system (Sequenom, San Diego, California, USA) as described in chapter 2.7.3. Relative PCR and extension sequences are provided in Appendix Table 3.21.

3.11.3. Results

Four markers show significant evidence for association with LOAD+P under the disease modifier model (Tables 3.11a and 3.11b). Marker rs762237 showed the most significant evidence for association ($P = 0.002$, OR = 1.42) which remained significant after correction for multiple testing ($P = 0.04$).This variant is located 6.7 kb from the 3' end of *OLIG2*. It has a minor T allele frequency of 36% (according to HapMap CEPH data), with the C allele associated with LOAD+P status. Polymorphism rs2834072 ($P = 0.004$, OR = 1.41) also shows significant evidence for association with LOAD+P which withstands correction for multiple testing ($P = 0.05$). It has a minor G allele frequency of 48% (according to HapMap CEPH data), with the A allele being associated with LOAD+P status. Markers rs13046814 ($P = 0.03$, OR = 1.30) and rs62216115 ($P = 0.04$, OR = 1.41) show association, but these do not withstand correction for multiple testing ($P = 0.3$ and $P = 0.39$ respectively). Variant rs13046814 is located within the 3' UTR of *OLIG2* and polymorphism rs62216115 is located 1 kb 3' of the gene. Disease subtype analysis yielded no significant association with any of the tested SNPs and none of the SNPs showed an allelic or genotypic association with LOAD. Haplotype

analysis of each dataset revealed no greater evidence for association at the *OLIG2* locus than that seen with marker rs762237 in the disease modifier analysis (disease modifier global $P = 0.004$; disease subtype global $P = 0.17$; LOAD global $P = 0.11$) (Appendix Table 3.22).

The LD structure across the *OLIG2* locus was investigated using the HAPLOVIEW software (Barrett et al. 2005) and data from the HapMap project (The International HapMap Consortium 2005). All markers showing $r^2 > 0.3$ with an associated variant were investigated (Table 3.11c). The Evolutionary Conservation of Genomes (ECR) browser (Figure 3.11b), and the UCSC most conserved track (Figure 3.11c), were used to highlight regions of high conservation, in conjunction with Cluster Buster (http://zlab.bu.edu/cluster-buster/cbust.html) to identify transcription factor binding sites (Figure 3.11d). No associated variant, nor a LD proxy, reside in a promoter region, a region of high conservation (>80%) or a predicted transcription factor binding site.

Table 3.11a. MAF and genotype counts of tested *OLIG2* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		Alleles				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs2834070	T	0.34	0.31	0.32	0.33	151/165/38	115/117/20	473/459/98	522/530/123
rs11701698	G	0.23	0.23	0.22	0.23	209/140/13	149/96/10	631/369/49	693/430/61
rs1005573	C	0.3	0.29	0.3	0.3	176/145/36	131/99/23	502/430/98	564/502/102
rs762178	A	0.42	0.41	0.41	0.41	116/172/62	84/121/40	345/478/174	400/539/202
rs1059004	C	0.43	0.44	0.44	0.43	108/183/62	76/123/48	320/501/191	390/564/221
rs13046814	G	0.38	0.32	0.35	0.36	130/179/47	112/111/24	426/475/122	472/556/146
F16GA	A	0.17	0.22	0.2	0.19	248/107/10	153/93/9	662/340/42	778/366/46
rs11701762	A	0.11	0.1	0.11	0.12	283/71/2	205/41/5	819/202/13	915/244/24
rs881666	C	0.45	0.4	0.42	0.42	113/174/76	92/121/41	352/494/194	416/565/218
rs762237	T	0.34	0.42	0.38	0.36	160/161/41	80/139/38	407/492/146	491/565/150
rs2834072	G	0.55	0.47	0.52	0.54	110/179/71	50/136/64	276/498/239	334/589/243

Table 3.11b. Individual genotyping of *OLIG2* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs2834070	1.11	0.29	1.13 (0.89-1.46)	1.5	0.47	0.11	0.74	1.03 (0.86-1.24)	0.2	0.9	0.75	0.39	1.06 (0.93-1.2)	0.81	0.67
rs11701698	0.01	0.92	1.01 (0.77-1.32)	0.1	0.95	0.23	0.63	1.05 (0.86-1.29)	1.38	0.5	1.15	0.28	1.09 (0.96-1.25)	1.31	0.52
rs1005573	0.66	0.42	1.1 (0.85-1.4)	0.41	0.81	0.38	0.54	1.06 (0.88-1.28)	1.6	0.45	0.01	0.91	1.01 (0.89-1.15)	0.59	0.75
rs762178	0.15	0.7	1.05 (0.83-1.33)	0.22	0.9	0.09	0.76	1.03 (0.86-1.23)	0.44	0.8	0.004	0.95	1.01 (0.89-1.13)	0.11	0.95
rs1059004	0.13	0.72	1.04 (0.82-1.3)	0.4	0.82	0.37	0.54	1.06 (0.89-1.26)	1.2	0.55	0.3	0.59	1.05 (0.93-1.18)	0.67	0.72
rs13046814	4.81	0.03	1.3 (1.03-1.67)	5.2	0.07	0.62	0.43	1.07 (0.9-1.28)	0.81	0.67	0.45	0.5	1.07 (0.95-1.21)	0.49	0.78
F16GA	4.26	0.04	1.36 (1.03-1.82)	5.2	0.07	1.63	0.2	1.16 (0.92-1.45)	1.64	0.44	0.79	0.37	1.07 (0.92-1.24)	0.95	0.62
rs11701762	0.03	0.87	1.04 (0.72-1.52)	3.74	0.15	1.7	0.19	1.2 (0.91-1.58)	3.74	0.15	1.85	0.17	1.08 (0.9-1.29)	2.54	0.28
rs881666	2.93	0.09	1.23 (0.97-1.54)	2.98	0.23	2.3	0.13	1.14 (0.96-1.36)	2.31	0.32	0.2	0.66	1.03 (0.91-1.16)	0.2	0.9
rs762237	**10.1**	**0.002**	**1.42 (1.13-1.8)**	**10.9**	**0.004**	1.83	0.18	1.14 (0.94-1.37)	1.98	0.37	1.31	0.25	1.07 (0.95-1.21)	1.45	0.49
rs2834072	**8.61**	**0.004**	**1.41 (1.12-1.79)**	**9.55**	**0.008**	0.65	0.42	1.07 (0.9-1.28)	0.78	0.68	1.88	0.17	1.1 (0.98-1.23)	2.44	0.3

Table 3.11c. Marker and chromosomal position of all markers in $r^2 > 0.3$ with either rs2834072 or rs762237, ranged by r2, according to the HapMap.

Marker 1	Position 1	Marker 2	Position 2	D'	r2
rs2834072	33332334	rs9984627	33393473	0.748	0.3
rs762237	33330047	rs2834076	33338971	1	0.3
rs2834072	33332334	rs2834088	33388132	0.741	0.302
rs2834065	33301570	rs762237	33330047	0.801	0.306
rs881666	33327136	rs2834072	33332334	0.714	0.307
rs2834072	33332334	rs928736	33361759	0.711	0.31
rs4817516	33307299	rs2834072	33332334	0.859	0.311
rs762237	33330047	rs2834081	33354662	0.674	0.336
rs762237	33330047	rs7276171	33385508	0.609	0.345
rs762237	33330047	rs2834088	33388132	0.633	0.359
rs881666	33327136	rs762237	33330047	1	0.373
rs2834072	33332334	rs2834076	33338971	1	0.486
rs762237	33330047	rs12233311	33331127	0.952	0.57
rs762237	33330047	rs6517138	33334005	1	0.614
rs2834072	33332334	rs2834077	33344608	1	0.619
rs762237	33330047	rs11088236	33338057	1	0.619
rs762237	33330047	rs12053728	33338217	1	0.619
rs762237	33330047	rs12482007	33335893	1	0.619
rs762237	33330047	rs2834072	33332334	1	0.619
rs13051692	33327883	rs762237	33330047	1	0.639
rs7275842	33327713	rs762237	33330047	1	0.641
rs743309	33329131	rs2834072	33332334	1	0.661
rs762237	33330047	rs12481989	33335770	1	0.673
rs13051692	33327883	rs2834072	33332334	0.851	0.698
rs7275842	33327713	rs2834072	33332334	0.851	0.699
rs762237	33330047	rs1892658	33336472	1	0.755
rs762237	33330047	rs2834074	33337172	1	0.757
rs2834072	33332334	rs915534	33347504	1	0.76
rs762237	33330047	rs12053659	33339412	1	0.776
rs2834072	33332334	rs12053659	33339412	1	0.782
rs762237	33330047	rs915534	33347504	1	0.807
rs2834072	33332334	rs1892658	33336472	1	0.815
rs2834072	33332334	rs2834074	33337172	1	0.817
rs743309	33329131	rs762237	33330047	1	0.961
rs2834072	33332334	rs12481989	33335770	1	0.965
rs12233311	33331127	rs2834072	33332334	1	0.966
rs2834072	33332334	rs11088236	33338057	1	1
rs2834072	33332334	rs12053728	33338217	1	1
rs2834072	33332334	rs12482007	33335893	1	1
rs2834072	33332334	rs6517138	33334005	1	1
rs743331	33329836	rs2834072	33332334	1	1
rs762237	33330047	rs2834077	33344608	1	1

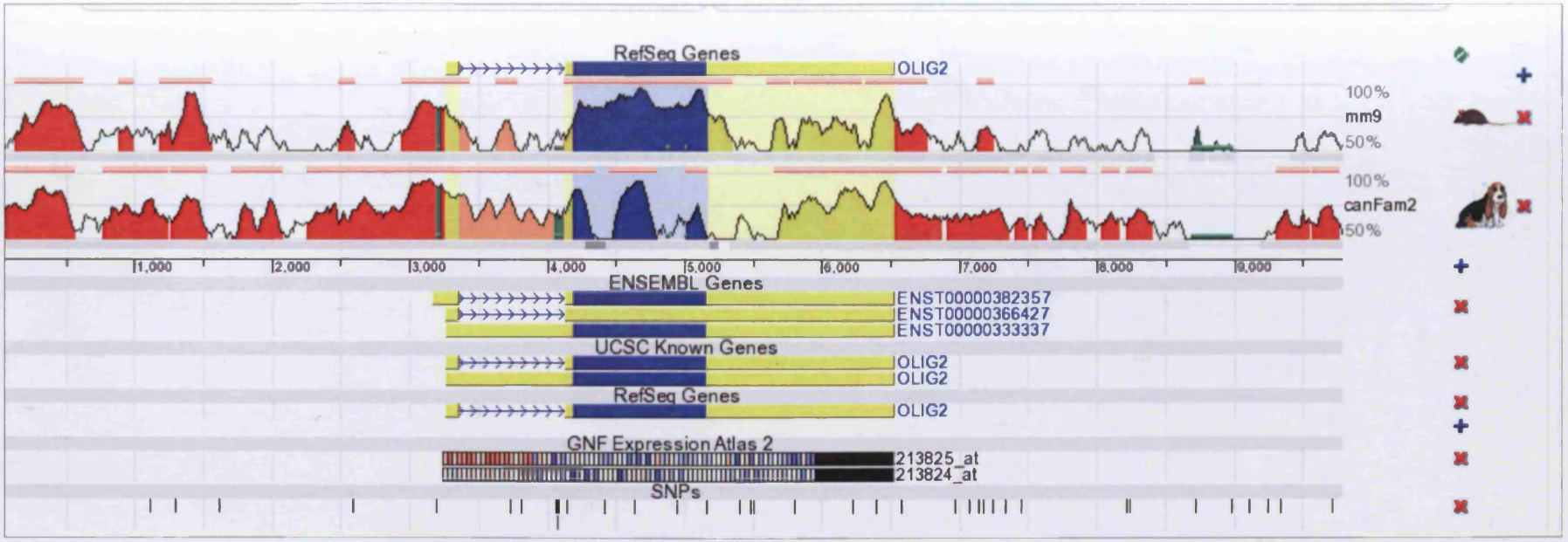


Figure 3.11b. Representation of ECR browser output for the *OLIG2* region, comparing human, canine and murine conservation. Height of peaks indicates degree of conservation, colour of peaks indicates function of region, e.g. Blue indicates exons, Pink indicates ECR, Green indicates transposons and simple repeats, Salmon indicates intronic regions, Red indicates intergenic regions and Yellow indicates UTR.

Figure 3.11c. Output from UCSC most conserved track for the *OLIG2* gene and surrounding sequence. Peak height on conservation track indicates the degree of conservation overlap across all species listed on left of figure.

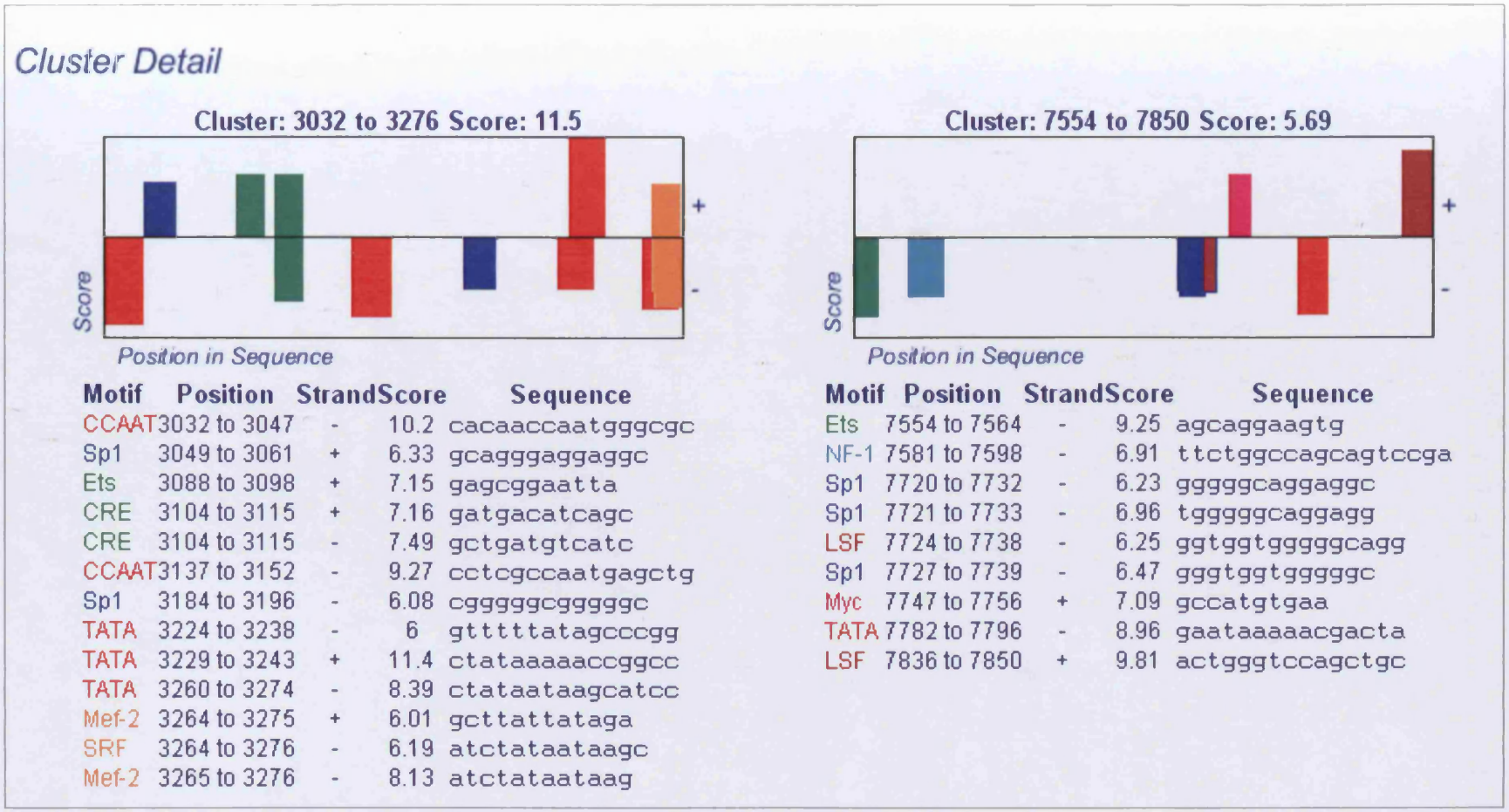


Figure 3.11d. Output from Cluster Buster transcription factor motif identifying software for the *OLIG2* gene and surrounding region. Coloured blocks relate to the specific binding motifs listed below. Position relates to *OLIG2* sequence, starting at -20kb.

3.11.4. Further Work

To decipher the functional relevance of the genetic association to marker rs762237 the study was extended to genotype tag markers for the oligodendrocyte lineage transcription factor 1 (*OLIG1*) gene (Figure 3.11e). This gene resides 34.4 kb distal to the associated *OLIG2* variant and 41.1 kb from *OLIG2*. OLIG1 is a bHLH transcription factor which has a similar role to OLIG2 (Lu et al. 2000; Zhou et al. 2000). The two genes are differentially expressed during development and are thought to act in unison to specify motoneuron and oligodendrocyte fate (Zhou and Anderson 2002), with OLIG1 having specific action within the brain (Lu et al. 2002). Fifteen SNPs were genotyped to tag the *OLIG1* gene + 20 kb 3 prime and 35 kb 5 prime of the gene with parameters set at $r^2 \geq 0.8$ and MAF ≥ 0.05. This methodology tags the *OLIG1* locus plus the region immediately neighbouring the *OLIG2* association signal. All markers except rs11088236 were genotyped using the Sequenom™ MassARRAY™ genotyping system (chapter 2.7.3). Variant rs11088236 was genotyped using Amplifluor™ chemistry on a 60°C program with 22 cycles (chapter 2.7.2), the relative PCR and extension primers are provided in Appendix Table 3.23. All markers passed the stringent quality control measures (see chapter 2.7.4) (Table 3.11d). Four significant allelic associations with markers rs11088236 ($P = 0.007$; OR = 0.72), rs2834076 ($P = 0.04$; OR = 1.29), rs2834083 ($P = 0.02$; OR = 0.75) and rs8131457 ($P = 0.03$; OR = 0.77) were seen in the disease modifier analysis (Tables 3.11e and 3.11f). Only the association with variant rs11088236 survives correction for multiple testing (permuted $P = 0.02$) (rs2834076 permuted $P = 0.4$; rs2834083 permuted $P = 0.15$; rs8131457 permuted $P = 0.39$). Upon further investigation marker rs11088236 was found to be a proxy SNP for rs762237 (D' = 1 and $r^2 = 1$ in the CEPH). Therefore rs762237 remains the most significantly associated marker. In the disease subtype analysis rs7276171 showed a significant association (allelic $P = 0.006$; OR = 0.77, genotypic P = 0.005), with the minor G allele and GG genotype under-represented in LOAD+P cases. However, this association did not survive permutation correction for multiple testing (allelic $P = 0.07$; genotypic $P = 0.06$). No variant showed significant association to LOAD and no haplotypic analysis produced a significant association (disease modifier global $P = 0.57$; disease subtype global $P = 0.68$; LOAD global $P = 0.86$) (Appendix Table 3.24).

Table 3.11d. *OLIG1* variants genotyped in this study. Table provides SNP ID, base position, nucleotide change, % genotyped, and HWE p-values for each tested dataset.

				HWE			
SNP	BP		%Geno	LOAD+P	LOAD-P	LOAD	Control
rs17632819	33336721	G/A	99.5	0.80	0.27	0.82	1.00
rs11088236	33338057	C/T	99.3	0.50	0.24	0.35	0.45
rs2834076	33338971	G/A	97.6	0.67	1.00	0.69	0.57
rs10483016	33339071	T/C	97.4	0.67	0.17	0.47	0.73
rs2834079	33352040	A/G	96.9	0.74	0.48	0.10	0.77
rs2834083	33358843	A/G	96.4	0.32	1.00	0.80	0.10
rs928736	33361759	T/C	95.1	0.56	0.49	0.88	0.50
rs7278735	33366952	C/T	98.2	1.00	0.71	1.00	0.62
rs11088237	33372448	T/C	99.5	0.90	0.47	0.63	0.40
rs8131457	33375767	T/C	98	0.73	0.68	0.38	0.16
rs7276171	33385508	A/G	99.8	1.00	1.00	0.43	0.08
rs4817527	33385955	G/A	97.6	0.75	0.16	0.85	0.07
rs2834086	33387137	C/T	96.9	0.20	0.89	0.69	1.00
rs2834087	33387301	G/A	98.5	0.92	0.60	0.25	0.92
rs12481815	33387990	T/C	99.7	0.91	0.89	0.62	0.63

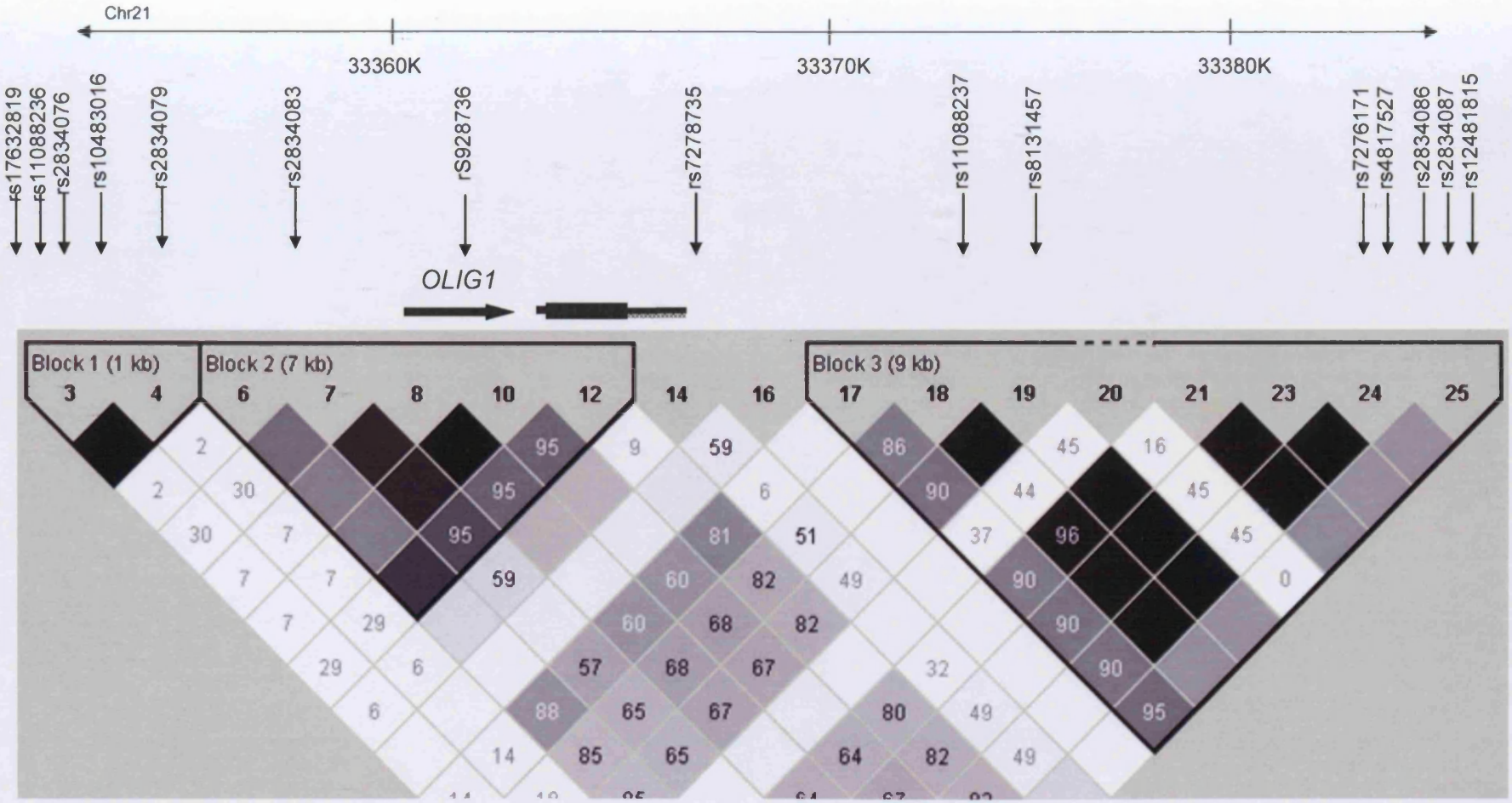


Figure 3.11e. The *OLIG1* locus. Schematic shows chromosomal position (bp), the SNPs genotyped in this study, with their position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *OLIG1* gene with exons/UTR indicated by black bars (tall/short bars respectively), and finally the LD plot of the region (r^2).

Table 3.11e. MAF and genotype counts of tested *OLIG1* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs17632819	A	0.07	0.07	0.07	0.07	2/41/293	1/30/202	6/143/888	5/145/949
rs11088236	T	0.43	0.52	0.47	0.46	62/167/106	58/124/51	223/532/280	228/548/324
rs2834076	A	0.4	0.34	0.37	0.36	54/156/124	24/107/100	142/466/405	146/491/448
rs10483016	C	0.06	0.04	0.05	0.05	1/35/300	0/20/213	3/89/938	1/96/999
rs2834079	G	0.1	0.1	0.11	0.11	2/64/269	0/48/185	6/205/815	13/217/863
rs2834083	G	0.42	0.49	0.45	0.44	58/167/111	55/118/58	209/505/316	206/562/327
rs928736	C	0.29	0.34	0.31	0.31	26/140/166	24/109/98	98/440/480	113/461/522
rs7278735	T	0.19	0.19	0.18	0.18	11/105/219	9/69/153	34/304/683	37/316/744
rs11088237	C	0.04	0.04	0.03	0.03	0/26/310	0/17/216	0/71/964	0/75/1025
rs8131457	C	0.43	0.5	0.46	0.46	58/173/105	62/107/64	220/495/311	236/525/336
rs7276171	G	0.29	0.34	0.32	0.35	22/150/164	27/103/103	102/466/471	142/476/482
rs4817527	A	0.46	0.43	0.44	0.43	70/172/94	42/118/73	197/497/323	218/519/363
rs2834086	T	0.37	0.36	0.36	0.35	47/155/133	32/103/97	140/470/418	134/497/459
rs2834087	A	0.21	0.16	0.18	0.18	14/110/210	6/64/162	40/297/688	39/335/720
rs12481815	C	0.04	0.03	0.03	0.03	0/25/311	0/15/217	0/66/972	0/78/1112

Table 3.11f. Individual genotyping of *OLIG2* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP	χ^2	P	OR	χ^2	P	χ^2	P	OR	χ^2	P	χ^2	P	OR	χ^2	P
rs17632819	0.01	0.91	0.97 (0.61-1.56)	0.13	0.94	0.1	0.75	0.95 (0.67-1.33)	0.32	0.85	0.67	0.41	1.1 (0.88-1.38)	0.73	0.69
rs11088236	**7.19**	**0.007**	**0.72 (0.57-0.92)**	**7.69**	**0.02**	1.01	0.32	0.91 (0.77-1.09)	1.04	0.6	0.6	0.44	1.05 (0.93-1.18)	0.65	0.72
rs2834076	**4.17**	**0.04**	**1.29 (1.01-1.66)**	4.62	0.1	2.59	0.11	1.16 (0.97-1.38)	2.55	0.28	0.3	0.58	1.04 (0.91-1.17)	0.31	0.86
rs10483016	0.85	0.36	1.3 (0.74-2.27)	1.24	0.54	1.23	0.27	1.25 (0.84-1.84)	1.66	0.44	0.02	0.88	1.02 (0.77-1.36)	1.14	0.57
rs2834079	0.01	0.93	0.98 (0.67-1.45)	1.56	0.46	0.49	0.48	0.9 (0.68-1.2)	0.99	0.61	0.29	0.59	0.95 (0.78-1.15)	1.75	0.42
rs2834083	**5.79**	**0.02**	**0.75 (0.59-0.95)**	5.88	0.05	1.17	0.28	0.91 (0.76-1.08)	1.31	0.52	0.05	0.83	1.01 (0.9-1.14)	1.97	0.37
rs928736	3.27	0.07	0.79 (0.61-1.02)	3.45	0.18	1.41	0.24	0.89 (0.74-1.08)	1.91	0.39	0.03	0.87	0.99 (0.87-1.12)	0.39	0.82
rs7278735	0.003	0.96	1.01 (0.74-1.37)	0.26	0.88	0.48	0.49	1.08 (0.87-1.35)	0.8	0.67	0.13	0.72	1.03 (0.88-1.2)	0.25	0.88
rs11088237	0.04	0.85	1.06 (0.57-1.98)	0.04	0.84	0.32	0.57	1.14 (0.72-1.8)	0.33	0.56	0.03	0.86	0.97 (0.7-1.34)	0.001	0.97
rs8131457	**4.78**	**0.03**	**0.77 (0.61-0.97)**	**7.23**	**0.03**	1.23	0.27	0.91 (0.76-1.08)	2.99	0.22	0.02	0.9	0.99 (0.88-1.12)	0.12	0.94
rs7276171	3	0.08	0.8 (0.62-1.03)	4.69	0.1	**7.48**	**0.006**	**0.77 (0.64-0.93)**	**10.66**	**0.005**	2.51	0.11	0.9 (0.8-1.02)	5.7	0.06
rs4817527	1.06	0.3	1.13 (0.89-1.44)	1.09	0.58	1.9	0.17	1.13 (0.95-1.34)	3.03	0.22	0.12	0.72	1.02 (0.91-1.15)	1.2	0.55
rs2834086	0.16	0.69	1.05 (0.82-1.35)	0.26	0.88	0.96	0.33	1.09 (0.91-1.31)	0.99	0.61	1.34	0.25	1.08 (0.95-1.22)	1.4	0.5
rs2834087	3.27	0.07	1.33 (0.98-1.81)	3.28	0.19	1.05	0.31	1.12 (0.9-1.39)	1.05	0.59	0.001	0.97	1 (0.86-1.17)	0.56	0.76
rs12481815	0.19	0.66	1.16 (0.6-2.22)	0.2	0.66	0.39	0.53	1.16 (0.73-1.84)	0.4	0.53	0.03	0.85	0.97 (0.69-1.35)	0.04	0.85

3.11.5. Discussion

Four polymorphisms have been identified at the *OLIG2* locus which associate with psychosis in LOAD. Interestingly the most significantly associated LOAD+P markers rs762237 ($P = 0.002$) and rs2834072 ($P = 0.004$) do not show an association with schizophrenia ($P = 0.35$ and $P = 0.78$ respectively), and the associated schizophrenia polymorphisms, rs1059004 ($P = 0.0001$) and rs762178 ($P = 0.0005$), do not show an association with LOAD+P ($P = 0.72$ and $P = 0.7$ respectively) (Georgieva et al. 2006). This study has power of 0.71 to detect the previously observed associations (OR = 1.33, MAF = 0.47) Polymorphism rs13046814 shows association in the same direction to schizophrenia ($P = 0.002$) and LOAD+P ($P = 0.03$). While variant rs62246115 shows no association with schizophrenia ($P = 0.75$) and a nominal association with LOAD+P ($P = 0.04$). Independent loci within the same gene have previously been shown to increase risk for, or cause distinct diseases. For example, multiple differing mutations within the gene (*GJB3*) encoding connexin 31 (Cx31) have been shown to increase risk for skin disease (Di et al. 2002) including erythrokeratodermia variabilis (Richard et al. 1998), and hearing impairment (Rabionet et al. 2002). While, mutations in the gene encoding tripartite motif-containing 32 (*TRIM32*) independently cause Bardet–Biedl syndrome (BBS) (Chiang et al. 2006) and Limb-girdle muscular dystrophy type 2H (LGMD2H) (Frosk et al. 2005). While noting that replication in independent samples is essential, the data provide preliminary evidence that genetic variation in *OLIG2* modifies psychosis in LOAD, albeit by a varying mechanism to that seen in schizophrenia, and suggests a possible role for white matter abnormalities in the aetiology of a psychosis subtype of LOAD, but not LOAD *per se*. The significant association seen with a variant in *OLIG1* does not exceed that seen at the *OLIG2* locus and owing to LD across the region indirectly supports the association with rs762237 in this dataset. On a cautionary note, *OLIG2* is located 6.8 Mb telomeric of the amyloid precursor protein (*APP*) gene. *APP* is well established as a disease risk gene for early onset Alzheimer's disease (EOAD). The genetic associations of *APP* and *OLIG2* are unlikely to be related as their chromosomal locations lie far outside the range of LD, however, the possibility that these genes interact cannot be excluded. No functional variant could be identified at the *OLIG2* locus that explains the association findings; therefore

further exploration of the *OLIG2* region is warranted. Future work at this locus will include analysis of *OLIG2* in the LOAD+P GWAS described in chapter 5. Following replication of the genetic association of *OLIG2* with LOAD+P, future work could entail exploration of the locus to identify a coding variant or an effect on gene expression. The examination of other OMR genes with respect to LOAD+P could prove fruitful in further dissecting the aetiology of this disease.

3.12. Catechol-*O*-methyl Transferase

Catechol-*O*-methyl transferase (COMT) is an enzyme involved in the breakdown of the catecholamine neurotransmitters (dopamine, epinephrine and norepinephrine). These neurotransmitters are paramount to the signaling pathways thought to be perturbed in numerous psychotic disorders. The *COMT* gene (Figure 3.12) is located at chromosome 22q11.21-11.23 a region renowned for a common microdeletion (Singh et al. 2002) which has shown association to psychosis. Several early schizophrenia and bipolar disorder analyses found positive linkage to 22q11–q12 (Gill et al. 1995; Lachman et al. 1997; Lasseter et al. 1995; Hovatta et al. 1998), Suggestive evidence for linkage to chromosome 22q11 with psychosis was identified by Hamshere and colleagues (Hamshere et al. 2005b) in UK patients with schizoaffective disorder (LOD = 1.96). No linkage was observed in this region in the group's original schizophrenia or bipolar disorder scans, suggesting the existence of loci that influence susceptibility to a broad functional psychosis, rather than distinct phenotypes. However, linkage of the region to schizophrenia and bipolar disorder is supported by multiple meta-analyses (Badner and Gershon 2002; Holmans et al. 2009a; Lewis et al. 2003). Most association studies have focused upon the valine to methionine change at codon 158 of the brain-predominant membrane-bound form of *COMT* and codon 108 of the soluble form. The valine allele confers higher activity and thermal stability (Chen et al. 2004a) and has been fairly consistently associated with reduced performance in tests of frontal lobe function (Egan et al. 2001; Malhotra et al. 2002). The findings in schizophrenia are mixed, the vast majority of case–control studies have failed to find evidence for association (Williams et al. 2005a), and meta-analyses report no overall evidence for association (Fan et al. 2005; Glatt et al. 2003; Munafo et al.

2005). Some studies find stronger evidence for association between haplotypes at *COMT* than for the val/met polymorphism alone (Chen et al. 2004b; Handoko et al. 2005; Sanders et al. 2005; Shifman et al. 2002), with the most studied being the 'Shifman haplotype'. This haplotype shows association to schizophrenia in at least two studies (Chen et al. 2004b; Handoko et al. 2005). The finding that this haplotype shows greater association to disease than the functional polymorphism is difficult to explain, given that activity at the *COMT* locus is largely dictated by this marker. The val/met variant may confer a small effect on susceptibility or have a role in phenotype modification. It also remains possible that variation elsewhere in *COMT* or in a neighbouring gene confers psychosis susceptibility (Craddock et al. 2006). The *COMT* gene has not been studied extensively in bipolar disorder. Borderline evidence for association with the met allele was found in one meta-analysis (OR = 1.18) (Craddock et al. 2001) which did not replicate in a later meta-analysis (Craddock et al. 2006). The *COMT* locus has been studied in a AD case-control sample of 373 individuals. The val/met variant showed modest association to AD+P in females and a 4 marker haplotype showed significant evidence for association irrespective of sex (Sweet et al. 2005). It is likely that genetic variation in this region influences susceptibility across the psychosis spectrum, although whether *COMT* is the only (or the major) susceptibility gene at this locus is unclear. If it is, the mechanism is complex and the phenotype not yet adequately defined (Craddock et al. 2006).

The catechol-O-methyl transferase (COMT) enzyme degrades catecholamines including dopamine. Two main COMT protein isoforms are known, in most tissues the soluble cytoplasmic form predominates, whereas in brain the longer membrane bound form is the major species (Tenhunen et al. 1994). Although expressed widely, COMT is a minor player in dopamine clearance compared with neuronal synaptic uptake by the dopamine transporter (Huotari et al. 2002), except in the prefrontal cortex where expression of the dopamine transporter is low (Sesack et al. 1998), subsequently increasing the importance of COMT (Gogos et al. 1998; Tunbridge et al. 2004). Differential expression of the COMT transcripts in different tissues maybe explained by the identification of a number of putative regulatory elements within and neighbouring the gene (Tenhunen et al. 1994), which include numerous oestrogen response elements (Xie et al. 1999).

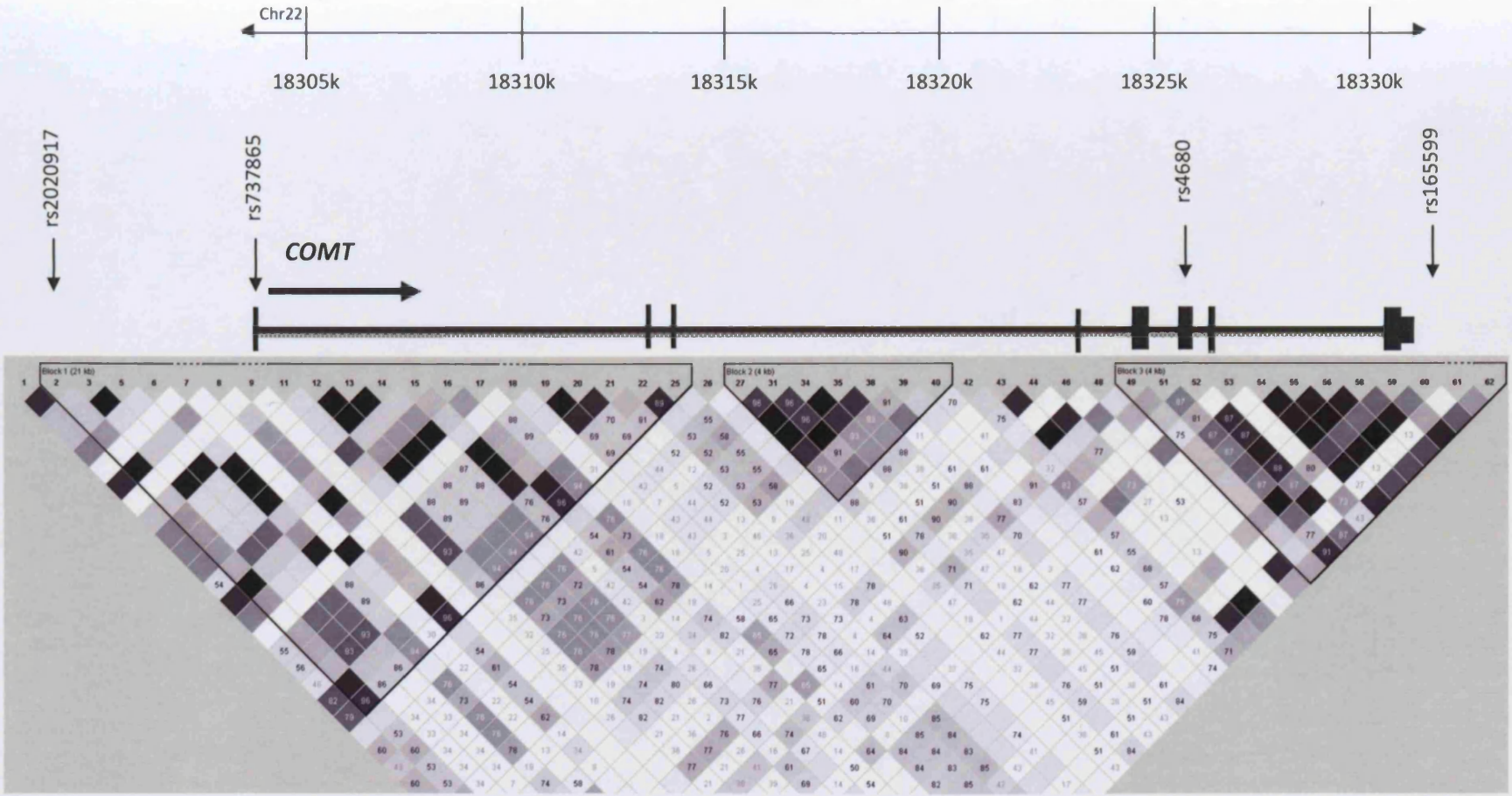


Figure 3.12. The *COMT* locus. Schematic shows chromosomal position (bp), the SNPs and genotyped in this study, with their position within the gene indicated by an arrow, the direction of transcription indicated by horizontal arrow, the *COMT* gene with exons/UTR indicated by black bars, and finally the LD plot of the region (r2).

3.12.1. Literature Summary

Catechol-*O*-methyl transferase (COMT) is an enzyme involved in the breakdown of the catecholamine neurotransmitters (dopamine, epinephrine and norepinephrine). These neurotransmitters are paramount to the signaling pathways thought to be perturbed in numerous psychotic disorders. Multiple schizophrenia, schizoaffective disorder and bipolar disorder analyses have found positive linkage to the 22q11–q12 region (Gill et al. 1996; Lachman et al. 1997; Lasseter et al. 1995; Hamshere et al. 2005b; Hovatta et al. 1998), but the results of association analyses have been mixed. Most studies have focused upon the val/met polymorphism, the valine allele of which confers higher activity and thermal stability (Chen et al. 2004a) Some studies find stronger evidence for association between haplotypes at *COMT* than for the val/met polymorphism alone (Chen et al. 2004b; Handoko et al. 2005; Sanders et al. 2005; Shifman et al. 2002), with the most studied being the 'Shifman haplotype' which shows association to schizophrenia in at least 2 studies (Chen et al. 2004b; Handoko et al. 2005). The *COMT* locus has been studied in AD+P with the val/met variant showing modest association to AD+P in females and a 4 marker haplotype showing significant evidence for association irrespective of sex (Sweet et al. 2005).

3.12.2. Study Design

In an attempt to replicate the previous association of *COMT* with psychosis in AD (Sweet et al. 2005), 4 SNPs were genotyped and analysed. Three of these SNPs represent the 'Shifman' haplotype (rs4680, rs165599 and rs73765), and include the val/met SNP (rs4680). The additional variant, rs2020917 is a promoter variant that is adjacent to an oestrogen response element (ERE6) which is hypothesised to be involved in regulation of gene expression (Tenhunen et al. 1994). All SNPs were genotyped using the Sequenom™ MassArray™ platform (chapter 2.7.3), the relevant PCR and extension primer sequences are provided in Appendix Table 3.25.

3.12.3. Results

One variant (rs165599) showed nominal deviation from HWE in the LOAD+P dataset (P = 0.01). This variant is essential to complete the investigated haplotype therefore it was not excluded from further analysis. Neither of the LOAD+P association analyses undertaken yielded a significant association with any of the tested SNPs (Tables 3.12a and 3.12b), with the val/met variant (rs4680) having a p-value of 0.65 (OR= 1.06) in the disease modifier analysis. No variant showed a significant association with LOAD alone. Haplotype analyses of the 4 variants in all datasets also failed to generate a significant association (global P = 0.77 in the disease modifier analysis, global P = 0.96 in the disease subtype analysis and global P = 0.71 in the LOAD analysis (Appendix Table 3.30). Due to the sex differences previously observed at the COMT locus in LOAD+P, the datasets were analysed by sex. No significant association was seen in allelic, genotypic or haplotypic analyses (see Appendix Tables 3.26 - 3.29 and 3.31 - 3.32).

3.12.4. Discussion

The val/met marker and a 4 marker haplotype have previously shown association with LOAD+P (Sweet et al. 2005). These markers were genotyped in this study. This study does not replicate the findings of Sweet and colleagues (Sweet et al. 2005), despite using a more powerful dataset. However, this dataset is still underpowered to detect the associations. The findings of Sweet and colleagues may prove to be false positive. However, one of the genotyped markers shows deviation from HWE in this study. Also, confounding factors such as population differences between the studies have not been accounted for. The 3 marker "Shifman haplotype" was also analysed in this study with no significant findings.

Table 3.12a. MAF and genotype counts of tested *COMT* SNPs. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs2020917	T	0.28	0.29	0.29	0.28	28/125/166	26/81/120	85/366/481	90/410/570
rs737865	C	0.29	0.3	0.29	0.28	30/123/166	27/81/119	88/365/479	91/413/566
rs4680	G	0.48	0.46	0.48	0.48	78/148/93	51/108/68	227/434/271	249/526/295
rs165599	G	0.3	0.29	0.3	0.29	27/136/156	13/107/107	82/402/448	102/425/543

Table 3.12b. Individual genotyping of *OLIG2* SNPs through LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*
rs2020917	0.11	0.74	1.05 (0.8-1.36)	1.41	0.49	0.16	0.69	1.04 (0.85-1.27)	0.16	0.92	0.69	0.41	1.06 (0.92-1.22)	0.67	0.72
rs737865	0.14	0.71	1.05 (0.81-1.37)	1.09	0.58	0.19	0.66	1.04 (0.86-1.27)	0.26	0.88	0.73	0.39	1.06 (0.93-1.22)	0.75	0.69
rs4680	0.21	0.65	1.06 (0.83-1.35)	0.29	0.87	0.01	0.93	1.01 (0.84-1.2)	0.75	0.69	0.02	0.89	1.01 (0.89-1.14)	1.35	0.51
rs165599	0.03	0.86	1.02 (0.79-1.33)	2.05	0.36	0.04	0.85	1.02 (0.84-1.24)	0.98	0.61	0.45	0.5	1.05 (0.91-1.2)	2.42	0.3

3.13. Discussion

In this chapter specific markers that have been directly or indirectly associated with psychosis were investigated for association with LOAD+P. These variants were tested under the hypothesis that psychosis susceptibly genes may act across diseases, modifying psychotic symptoms in multiple distinct disorders. The markers were also investigated for association with LOAD+P as a sub-form of LOAD. Markers at 11 loci, *DISC1*, *DTNBP1*, *GRIK2*, *GRM3*, *NRG1*, *BDNF*, *DAOA*, *CNP*, *OLIG2*, *OLIG1* and *COMT*, were investigated for association with LOAD+P and LOAD. Two previously published associations of *COMT* and *NRG1* with AD+P failed to replicate in this study. Variant rs12525702 at the *DTNBP1* locus shows nominal evidence for a role in the aetiology of LOAD+P, with a 4% difference in allele frequency and a trend towards association in the disease modifier analysis. One synonymous *GRM3* variant (rs2228595) shows evidence for association to LOAD+P under both disease models, but also shows deviation from HWE. Four *GRM3* variants showed significant association to LOAD, two of which survived correction for multiple testing. A haplotype of these markers shows the strongest association, however, variant rs6465084, which is included in the multi-marker haplotype, shows deviation from HWE. The *BDNF* val66met polymorphism does not show an association to LOAD+P, but does show significant evidence for association with LOAD alone ($P = 0.01$, OR = 1.2). This variant may reduce protein function of the gene, whose activity fits well with the oxidative stress hypothesis of LOAD aetiology. Four polymorphisms in the *OLIG2* gene showed significant association with psychosis in LOAD, two of which survive correction for multiple testing. These markers differ to those associated in schizophrenia (Georgieva et al. 2006), but provide preliminary evidence that genetic variation in *OLIG2* modifies psychosis in LOAD, albeit by a varying mechanism to that seen in schizophrenia, and suggests a possible role for white matter abnormalities in the aetiology of a psychosis subtype of LOAD.

This study used a sample set of 1,205 LOAD patients, 379 of which have experienced psychosis and 269 who have never had a psychotic episode, plus 1,305 matched controls. The disease modifier analysis had a limited power of 34% to detect an association with an effect size of 1.3 with a risk variant frequency of

30%, while the disease subtype analysis had an increased power of 57%. The power of the sample set is a limitation of this study, which means that a true association may be missed. A larger sample-set would greatly increase the study power. However, this would require recruitment of around 5000 LOAD patients with data on occurrence of psychotic symptoms to produce a more suitably powered dataset. This limitation is partially assessed in chapter 5, where genotype data from 543 LOAD+P cases, 417 LOAD-P cases and 4,706 control individuals is investigated. This dataset provides an increase in power of 13% and 21% for the disease modifier and disease subtype analyses respectively. At the time of study commencement the sample utilised in this chapter was the largest LOAD+P sample of any published study, and it remains substantial in comparison to other LOAD+P datasets (Wilkosz et al. 2007). The methodology of marker selection in this study was not ideal as for numerous loci the coverage of the locus was poor meaning that risk factors for disease susceptibility may be missed. However, this study took a novel approach to investigate multiple putative psychosis susceptibility genes. Common functional polymorphisms and polymorphisms previously associated with psychosis were prioritised for investigation. It is also worth remembering that rare variation at the tested loci may have an effect on psychosis susceptibility but that this study is not designed or powered to detect such an association.

Future work will include the analysis of the investigated loci, specifically *OLIG2*, in the LOAD+P GWAS described in chapter 5. Other oligodendrocyte and myelination related genes will also be prioritised for investigation in this dataset which could prove fruitful in further dissecting the aetiology of this disease. Further exploration of the *OLIG2* region is also warranted as the associated variants have no apparent functional role. This work may include studies to identify novel variants at the locus through screening and sequencing, and investigation of *cis*-acting variants through allelic expression assays.

While noting that replication in independent samples is essential, the identification of a novel association of *OLIG2* to LOAD+P is extremely interesting. This data suggests a possible role for white matter abnormalities in the aetiology of a psychosis subtype of LOAD, support for which is provided by functional studies

which show that white matter changes show significant correspondence with psychosis score in LOAD (Lee et al. 2006). This finding also supports the methodology of investigating schizophrenia and bipolar disorder susceptibility genes across disorders in which psychosis is a symptom.

4. Replication of Schizophrenia GWAS

4.1 Introduction

In the previous chapter the hypothesis that specific susceptibility variants for neurodevelopmental psychosis may be associated with risk of developing psychosis in LOAD was tested. The investigation of these putative psychosis candidate polymorphisms led to the successful identification of *OLIG2* as a psychosis modifier gene in LOAD (see chapter 3.11). Following this finding it seemed logical to extend this approach and investigate polymorphisms recently found to be associated with schizophrenia in a large individual case-control genome-wide association study (GWAS) of the disease (O'Donovan et al. 2008) (referred to hereafter as the O'Donovan GWAS). This genome-wide association study of schizophrenia is discussed along with other published schizophrenia GWAS.

Numerous GWA studies have utilised a DNA pooling methodology (Baum et al. 2008; Butcher and Plomin 2008; Hui et al. 2008; Kirov et al. 2009; Shifman et al. 2008). Pooled DNA genotyping offers the possibility of applying the same technologies to large samples at a fraction of the cost. Evidence shows that the pooling methodology is accurate (Norton et al. 2004), and that the accuracy of estimating differences in allele frequency between pools using ultra-high throughput platforms is comparable with lower throughput platforms (Kirov et al. 2006). Two groups have applied the DNA pooling methodology to GWAS of schizophrenia. Kirov and colleagues (Kirov et al. 2009) used a parent–offspring trio design with pools constructed from 605 unaffected controls, 574 schizophrenia patients and a third pool from the parents of the patients. Sixty-three SNPs were selected for individual genotyping in 574 trios and the results analysed with the transmission disequilibrium test (Kirov et al. 2009). No result reached genome-wide significance, although forty were significant at $P < 0.05$. The best result was with rs11064768 ($P = 1.2 \times 10^{-6}$), a SNP within the gene coiled-coil domain containing 60 gene (*CCDC60*) on chromosome 12. The third best SNP ($P = 0.00016$) was rs893703. This SNP lies within AT rich interactive domain 4A (*ARID4A*) an inhibitor

of the PI3K/Akt signalling pathway, members of which have been implicated in schizophrenia (Kalkman 2006).

Shifman and colleagues (Shifman et al. 2008) performed a genome-wide association (GWA) scan for schizophrenia using the Affymetrix 5.0 SNP array in an Ashkenazi Jewish population of 419 male case, 241 female case, 1,807 male control and 964 female control pools. One hundred and ninety four SNPs were selected for individual genotyping, 167 SNPs passed quality control measures, 52 of these showed p-values below 0.05 in at least one of the tests (male, female and combined), and 9 SNPs had p-values below 0.005 (Shifman et al. 2008). A female-specific association was found with rs7341475, ($P = 2.9 \times 10^{-5}$) which replicated ($P = 2.1 \times 10^{-3}$) in 4 additional populations, totalling 2,274 cases and 4,401 controls. Meta-analysis drives the association close to genome-wide statistical significance with an estimated relative risk of 1.58 ($P = 8.8 \times 10^{-7}$) for women carrying the common genotype (Shifman et al. 2008). Polymorphism rs7341475 is located in the fourth intron of the reelin (*RELN*) gene on chromosome 7. RELN is a serine protease known to be part of the apolipoprotein E (APOE) biochemical pathway that is involved in the pathogenesis of Alzheimer's disease (Seripa et al. 2008). The gene has previously shown association to AD specifically in females (Seripa et al. 2008). Although, rs7341475 was not an associated variant and is not in LD with any of the identified AD associated variants.

At the time of study commencement 4 individually genotyped GWAS of schizophrenia had been published including the O'Donovan GWAS. The study by Mah and colleagues (Mah et al. 2006) used a modest schizophrenia sample of European descent (320 cases and 325 matched controls) to genotype over 25,000 SNPs located within approximately 14,000 genes. Despite the small number of variants tested, 62 markers were found to be nominally associated with disease status. The most consistent finding was for a candidate locus (*PLXNA2*) on chromosome 1q32. The marker rs752016 showed suggestive association ($P = 0.006$; OR = 1.49) (Mah et al. 2006), and a trend towards association in an independent case-control sample of European Americans ($P = 0.07$; OR = 1.38) (combined $P = 0.035$; OR = 1.38). Similar genetic effects were observed in smaller subsets of Latin Americans (OR = 1.26) and Asian Americans (OR = 1.37) but these did not reach statistical significance ($P = 0.35$ and $P = 0.22$ respectively), and there was no supporting evidence from two family-based collections ($P = 0.34$,

OR = 1.31 and $P = 0.52$, OR = 0.87) (Mah et al. 2006). The plexin A2 (*PLXNA2)* gene is a member of the transmembrane semaphorin receptor family that is involved in axonal guidance during development and may modulate neuronal plasticity and regeneration. Interestingly, a *PLXNA2* ligand, semaphorin 3A, has been shown to be upregulated in the cerebellum of individuals with schizophrenia (Eastwood et al. 2003). However, the p-values identified by Mah and colleagues (Mah et al. 2006) are small, and are unlikely to survive correction for multiple testing.

Lencz and colleagues (Lencz et al. 2007) conducted a GWAS, examining approximately 500,000 markers in a small Caucasian sample of 178 cases and 144 controls. A strong effect ($P = 3.7 \times 10^{-7}$) of a novel locus (rs4129148) was revealed near the colony stimulating factor, receptor 2 alpha gene (*CSF2RA*) in the pseudo-autosomal region PAR1 of the X and Y chromosomes. However, this association did not replicate in an additional 102 subjects, and no neighbouring SNPs on the genotyping platform supported the association (Lencz et al. 2007). Sequencing of *CSF2RA* and its neighbour, interleukin 3 receptor alpha (*IL3RA*), in an independent case-control cohort revealed common intronic haplotypes and novel rare missense variants which associated with schizophrenia (Lencz et al. 2007). However, the haplotypic global p-values are not striking (best $P = 0.016$) and no correction for multiple comparisons was made.

Sullivan and colleagues (Sullivan et al. 2008) genotyped 738 cases with DSM-IV schizophrenia and 733 group-matched controls for 492,900 SNPs using an Affymetrix two-chip platform plus a custom fill-in chip. No SNP or multimarker combination of SNPs achieved genome-wide statistical significance (Sullivan et al. 2008). The lack of genome-wide significant association is not unexpected due to the moderate sample size employed by this study.

O'Donovan and colleagues (O'Donovan et al. 2008) used a multistage association study founded upon a GWAS (O'Donovan et al. 2008). The initial sample consisted of 642 UK cases, 479 of which were genotyped with the Affymetrix GeneChip® 500K Mapping Array allowing comparison of allele frequencies with 2937 controls from the Wellcome Trust Case Control Consortium study (WTCCC 2007). Three-hundred and sixty-two thousand, five hundred and thirty-two SNPs passed the stringent quality control measures. In concordance with the WTCCC, loci reaching thresholds of $P < 1 \times 10^{-5}$ and $P < 5 \times 10^{-7}$ were considered

to show suggestive and moderately strong evidence for association respectively. Twelve loci surpassed the threshold for suggestive association ($P < 1x10^{-5}$) and were selected for follow up genotyping using the Sequenom™ MassARRAY™ system. One SNP per loci was tested in replication sample 1 which comprised 1664 cases and 3541 controls. For 6 of the 12 SNPs the association was in the same direction as that seen in the GWAS (O'Donovan et al. 2008). These 6 SNPs were tested in an additional 4,143 cases and 6,515 controls, named replication sample 2, giving a total replication data set of 6,666 cases and 9,897 controls. Replication sample 2 included out-bred European populations, individuals from China, Japan, and Ashkenazi Jews. The full replication dataset provided strong independent support for susceptibility variants for schizophrenia at three loci; zinc finger protein 804A (*ZNF804A*) on 2q32.1 and intergenic regions on 11p14.1 at 29.1 Mb and 16p13.12 at ~13 Mb. Two additional loci remained nominally significant; 16q12.2 in retinitis pigmentosa GTPase regulator interacting protein 1 – like (*RPGRIP1L*) and 11q25 in opioid binding protein/cell adhesion (*OPCML*).

4.2. Study Design

This study is based on the hypothesis that psychosis susceptibly genes may act across disease divides, thus modifying psychotic symptoms in multiple distinct disorders. This design of this study is to investigate polymorphisms found to be associated with schizophrenia in the large individual case-control O'Donovan GWAS of the disease (O'Donovan et al. 2008). The primary hypothesis is that these polymorphisms may contribute to the aetiology of psychosis in LOAD under a 'disease modifier' model, i.e. that these variants modify the psychosis phenotype in LOAD independent of the susceptibility variants for neurodegenerative disease. An alternate hypothesis is that these putative schizophrenia susceptibility variants may be involved in the aetiology of LOAD+P under a 'disease subtype' model i.e. that these markers increase susceptibility of developing a form of LOAD with psychotic symptoms. The replication samples used in the O'Donovan GWAS utilised individuals from numerous populations, including Chinese, Japanese, out bred Europeans and Ashkenazi Jews. Differences in allele frequencies across

populations is a well documented phenomena that can create false positive or false negative associations (discussed in chapter 1.4.6), Therefore, in this study the SNPs identified in the initial UK stage of the O'Donovan GWAS were genotyped. This strategy aimed to avoid missing a true association due to differences in allele frequencies across populations. Twelve loci were identified as showing suggestive and moderately strong evidence ($P < 0.01$) for association to schizophrenia in the UK sample of the O'Donovan GWAS. A total of 21 polymorphisms across these 12 loci were genotyped. These variants are listed in Table 4.2.

4.3 Genotyping Strategy

All SNPs were genotyped using the Sequenom™ iPlexGOLD™ MassARRAY™ system as previously described (see chapter 2.7.3). Details of the marker flanking sequences can be found in Appendix Table 4.1. The PCR and extension primers designed for each variant can be found in Appendix Table 4.2. Quality control measures were as stated in chapter 2.7.4. SNPs were tested for deviation from HWE and statistically significant association using the methods described in chapter 2.7.4 and 2.9 respectively. Conserved regions and binding motifs were determined as described in chapter 2.11. Linkage disequilibrium between markers was determined using the HapMap LD Data download facility (www.hapmap.org). Markers were considered to be in "high LD" if they met threshold criteria of D' = 1 and $r^2 \geq 0.7$. Power was calculated using the PS program described in chapter 2.9.6.

Table 4.2. The 21 SNPs genotyped in this study from the 12 chromosomal loci, identified in the UK stage of the O'Donovan GWAS. Table includes the relative chromosome, gene, base position (BP), and p-value (*P*) and odds ratio (OR) from the O'Donovan GWAS.

Chr	SNP ID	Gene	BP	UK P	UK OR
1	rs7546928	AGBL4	73023563	1.29E-04	0.76
1	rs11162231	ST6GALNAC5	77040318	3.22E-04	0.71
2	rs16984718	intergenic	18430882	5.94E-04	2.1
2	rs2890738	intergenic	144390753	1.73E-08	1.44
2	rs12613195	ZNF804a	185314727	2.05E-05	0.72
2	rs1344706	ZNF804a	185603934	1.47E-06	0.7
3	rs12629685	TRIM71	32855082	5.39E-05	1.33
3	rs9870579	CLSTN2	141162314	4.72E-05	0.56
8	rs10103330	CSMD1	4167498	4.03E-06	1.47
9	rs2210539	c9orf39	17158867	1.38E-03	0.73
9	rs10869675	PCSK5	75819589	1.29E-05	1.38
10	rs17101921	FGFR2	123143285	6.00E-03	1.73
11	rs10835482	intergenic	29079278	7.83E-05	1.45
11	rs1602565	intergenic	29118712	9.04E-06	1.55
11	rs3016384	OPCML	132078600	7.83E-06	0.73
12	rs6490121	NOS1	116192578	4.72E-07	1.43
13	rs4238270	IRS2	109268029	2.87E-05	0.72
15	rs3784397	PLCB2	38384136	2.05E-05	1.35
16	rs7192086	intergenic	12969112	1.15E-06	1.45
16	rs9922369	RPGRIP1L	52205983	2.59E-07	2.26
18	rs1893146	intergenic	8977427	6.37E-07	1.55

4.4. Results

Twenty-one putative schizophrenia susceptibility variants were tested for association with LOAD+P through genotyping of 379 LOAD+P cases, 269 LOAD-P cases, and 1,361 controls. For a common genetic variant (MAF 30%) and a fairly large effect size of 1.3 the disease modifier analysis has power of 34% and the disease subtype analysis 57% at a significance level of $P \leq 0.05$. The LOAD (n=1,205) versus control analysis has a power of 88% to detect the same association. All SNPs had a genotyping success rate of > 95% in the full sample (Table 4.4a). Two SNPs were found to deviate from HWE in one dataset (Table 4.4a). SNP rs1344706 showed deviation from HWE in the control sample (P = 0.004; χ^2 = 8.18). The raw genotypes for this marker may not be trust worthy and the association analysis results for rs1344706 in the LOAD+P vs. control and the LOAD vs. control analysis were therefore taken with caution. Variant rs1893146 shows deviations from HWE in the LOAD+P subgroup (P = 0.02; χ^2 = 5.64). Therefore the association findings for rs1893146 in the LOAD+P vs. LOAD-P and the LOAD+P vs. control analyses were taken with caution. HWE states that both allele and genotypes frequencies in a population remain constant or are in equilibrium from generation to generation. Possible explanations for the deviation from HWE at these SNPs were explored. No genotyping error was found, genotypes of HapMap samples included on the genotyping plates were checked and confirmed against HapMap data. Neither of the SNPs were found to have a non-specific assay (where a primer shows high homology to multiple genomic regions) or a primer covering a known variant. Therefore, no reasons for the observed deviations from HWE could be identified. The MAF and genotype counts for each of the tested variants are provided in Table 4.4b.

Table 4.4a. Quality control Measures for all tested variants. Table includes chromosome, SNP name, gene name, base position, nucleotide change, percentage genotyped in full sample, and HWE p-values for each data subset.

						HWE			
Chr	**SNP ID**	**Gene**	**BP**		**% Gen**	**LOAD+P**	**LOAD-P**	**LOAD**	**Control**
1	rs11162231	ST6GALNAC5	77040318	T/G	98.3	0.20	0.85	0.57	0.60
1	rs7546928	AGBL4	73023563	G/A	97.2	0.74	0.61	0.90	0.31
2	rs12613195	ZNF804A	185197466	C/G	98.4	0.08	0.36	0.06	0.42
2	rs1344706	ZNF804A	185603934	A/C	96.6	0.17	0.79	1.00	**0.004**
2	rs16984718	Intergenic	18430882	G/A	98.8	1.00	1.00	1.00	1.00
2	rs2890738	Intergenic	144390753	C/T	97.8	0.56	0.88	0.94	0.59
3	rs12629685	TRIM71	32855082	G/A	96.9	0.48	0.18	0.09	0.17
3	rs9870579	CLSTN2	141162306	T/C	98.2	0.80	0.38	0.36	0.20
8	rs10103330	CSMD1	4167498	T/A	97.7	0.85	0.83	0.74	0.75
9	rs2210539	c9orf39	17158867	G/A	98.0	0.06	1.00	0.84	0.85
9	rs10869675	PCSK5	77779855	A/G	96.5	0.49	0.74	0.48	0.23
10	rs17101921	FGFR2	123143285	G/A	98.5	0.23	1.00	0.36	0.56
11	rs10835482	Intergenic	29079278	C/T	97.0	1.00	0.27	0.50	0.89
11	rs1602565	Intergenic	29118712	T/C	98.5	1.00	0.33	1.00	1.00
11	rs3016384	OPCML	132078600	T/C	97.6	0.67	0.30	0.65	0.81
12	rs6490121	NOS1	116192578	A/G	96.6	1.00	0.45	0.94	0.29
13	rs4238270	IRS2	109268029	G/T	97.4	1.00	0.47	0.62	0.58
15	rs3784397	PLCB2	38384136	T/C	98.3	0.82	0.34	0.84	0.32
16	rs7192086	Intergenic	12969112	A/T	95.4	0.67	0.12	0.15	0.46
16	rs9922369	RPGRIP1L	52205983	G/A	98.8	1.00	1.00	0.53	1.00
18	rs1893146	Intergenic	8977427	G/A	98.7	**0.02**	0.78	0.05	0.48

Table 4.4b. Chromosome number, SNP name, minor allele, minor allele frequency and genotype counts for LOAD+P, LOAD-P, LOAD and Control datasets.

			MAF				Genotype Counts			
Chr	SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
1	rs11162231	G	0.22	0.22	0.21	0.22	20/108/216	11/84/144	48/325/615	51/391/677
1	rs7546928	A	0.45	0.48	0.47	0.47	72/166/104	53/124/62	221/488/274	237/572/304
2	rs12613195	G	0.32	0.3	0.33	0.35	26/162/153	19/105/104	90/452/422	125/509/464
2	rs1344706	C	0.39	0.41	0.4	0.42	58/150/132	38/118/82	159/471/351	219/492/395
2	rs16984718	A	0.02	0.02	0.02	0.02	0/13/331	0/8/231	0/34/954	0/44/1075
2	rs2890738	T	0.36	0.32	0.36	0.33	41/164/139	24/107/108	125/450/410	127/488/504
3	rs12629685	A	0.36	0.4	0.37	0.37	40/164/138	32/123/80	123/484/374	158/492/457
3	rs9870579	C	0.13	0.08	0.1	0.09	6/74/263	0/39/200	6/179/802	12/173/932
8	rs10103330	A	0.17	0.18	0.18	0.17	10/95/237	7/73/157	32/281/668	31/322/763
9	rs2210539	A	0.17	0.21	0.19	0.2	5/109/227	10/77/141	34/303/627	45/361/692
9	rs10869675	G	0.27	0.27	0.28	0.31	27/127/184	15/95/125	82/384/503	96/490/523
10	rs17101921	A	0.03	0.03	0.02	0.03	1/17/326	0/13/225	1/40/945	1/58/1060
11	rs10835482	T	0.11	0.13	0.14	0.13	4/70/267	2/60/166	15/234/715	19/246/833
11	rs1602565	C	0.11	0.11	0.12	0.11	4/65/275	1/51/187	14/214/760	14/223/882
11	rs3016384	C	0.48	0.46	0.48	0.49	78/176/89	54/110/74	228/483/272	270/562/282
12	rs6490121	G	0.33	0.3	0.33	0.34	37/152/154	19/106/113	105/431/447	139/484/487
13	rs4238270	T	0.32	0.34	0.33	0.32	35/150/158	30/102/107	105/445/434	115/475/528
15	rs3784397	C	0.4	0.38	0.39	0.41	55/162/127	38/105/96	153/467/367	180/558/381
16	rs7192086	T	0.26	0.29	0.28	0.24	25/128/186	25/87/123	84/369/513	68/393/640
16	rs9922369	A	0.03	0.03	0.03	0.03	0/18/326	0/16/223	1/52/935	0/63/1058
18	rs1893146	A	0.15	0.13	0.13	0.12	2/98/244	3/56/180	10/240/738	19/233/868

Polymorphism rs9870579 showed an allelic (P = 0.02; OR = 1.61) and genotypic (P = 0.009) association to LOAD+P under the disease modifier model. The minor C allele showed a 5% over representation to LOAD cases with psychosis when compared to LOAD cases without psychosis (Table 4.4c). This variant also showed an allelic (P = 0.004; OR = 1.48) and genotypic (P = 0.02) association to LOAD+P under the disease subtype model. The minor C allele had an increased frequency of 4% in LOAD cases with psychosis compared to controls. The most significant allelic association was with the disease subtype model. However, marker rs9870579 appears to have the largest effect on LOAD+P under the disease modifier model, with a greater over representation of the minor C allele. None of the observed associations survive correction for multiple testing. Polymorphism rs9870579 is an intronic marker in the calsyntenin 2 *CLSTN2* gene. *CLSTN2* is a large gene of 632 kb situated on chromosome 3q23. There is one known gene transcript which is expressed exclusively in brain. The calsyntenin family of proteins are predominantly postsynaptic membrane proteins of excitatory CNS synapses with putative calcium binding capacity (Hintsch et al. 2002). However, the highest levels of CLSTN2 protein are found in GABAergic neurons (Hintsch et al. 2002), i.e. neurons that produce GABA as their output. GABA (Gamma-aminobutyric acid) is the chief inhibitory neurotransmitter in the mammalian CNS. Disrupted GABAergic signalling has been implicated in numerous and varied neurological and psychiatric pathologies including schizophrenia. The 3q23 region has previously shown linkage to LOAD and the *CLSTN2* gene has shown significant association to cognitive function (Liu et al. 2007). Thus, *CLSTN2* can be considered a putative functional and positional candidate gene for LOAD and psychosis. The functional relevance of the observed association was investigated. The marker is not within a conserved region or any known binding motif according to the UCSC most conserved track, ECR browser (http://ecrbrowser.dcode.org/) or cluster buster databases (http://zlab.bu.edu/cluster-buster/index.html) (Figure 4.4a). Variants in high LD with rs9870579, according to the HapMap CEU data (Table 4.4d), were investigated for functional relevance, location in conserved regions or known binding motifs. One SNP rs11927384, in D' = 1.0 and r^2 = 0.83 with variant rs9870579, appears to lie in a conserved region according to the UCSC most conserved track and ECR browser (Figure 4.4a). This SNP also lies within intron 1 of the *CLSTN2* gene.

Conservation suggests that this variant or the region in which it lies may have a functional role. For example, the conserved region may act as a binding site for transcription factors involved in regulation of gene expression. However, there is no known function of this specific region of conservation.

Variant rs1893146 showed significant genotypic association with LOAD+P in the disease subtype analysis ($P = 0.005$), and a trend towards allelic association ($P = 0.06$; OR = 1.26) with a 3% increase in the minor A allele in the LOAD+P subgroup compared to controls. This association does not survive correction for multiple comparisons and variant rs1893146 shows deviation from HWE in the LOAD+P subgroup ($P = 0.02$; $\chi^2 = 5.64$), meaning that this finding may not be trustworthy.

Polymorphism rs7192086 showed an allelic ($P = 0.01$; OR = 1.22) and genotypic ($P = 0.02$) association to LOAD, with the minor T being over represented in cases with an allele frequency difference of 4%. However, the association did not survive permutation correction for multiple testing (allelic $P = 0.3$; genotypic $P = 0.21$). SNP rs7192086 is an intergenic SNP on chromosome 16. According to the UCSC conserved region track the SNP lies in a region of low conservation (see Figure 4.4b). No other tested polymorphism was shown to associate with LOAD.

Table 4.4c. Chromosome number, SNP name, allelic and genotypic statistics for LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control analyses.

		LOAD+P vs. LOAD-P					LOAD+P vs. control					LOAD vs. control				
		Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
Chr	SNP ID	χ^2	*P*	OR	χ^2	*P*	χ^2	*P*	OR	χ^2	*P*	χ^2	*P*	OR	χ^2	*P*
1	rs11162231	0.07	0.79	0.96 (0.73-1.28)	1.14	0.57	0.09	0.77	0.97 (0.79-1.19)	2.07	0.36	0.34	0.56	0.96 (0.83-1.11)	1.04	0.6
1	rs7546928	0.88	0.35	0.89 (0.71-1.13)	1.38	0.5	0.55	0.46	0.94 (0.79-1.11)	1.32	0.52	0.05	0.81	1.02 (0.9-1.15)	0.77	0.68
2	rs12613195	0.2	0.65	1.06 (0.82-1.37)	0.25	0.88	1.88	0.17	0.88 (0.73-1.06)	2.21	0.33	1.13	0.29	0.93 (0.82-1.06)	1.44	0.49
2	rs1344706	0.31	0.58	0.93 (0.74-1.19)	1.72	0.42	1.77	0.18	0.89 (0.74-1.06)	1.66	0.44	1.36	0.24	0.93 (0.82-1.05)	4.93	0.08
2	rs16984718	0.07	0.79	1.13 (0.47-2.75)	0.08	0.78	0.02	0.9	0.96 (0.51-1.79)	0.02	0.9	0.35	0.55	0.87 (0.56-1.37)	0.36	0.55
2	rs2890738	1.39	0.24	1.16 (0.91-1.49)	1.46	0.48	1.61	0.2	1.12 (0.94-1.34)	2.38	0.3	2.66	0.1	1.11 (0.98-1.26)	2.74	0.25
3	rs12629685	2.02	0.16	0.84 (0.66-1.07)	2.42	0.3	0.17	0.68	0.96 (0.81-1.15)	2.02	0.36	0.21	0.65	1.03 (0.91-1.17)	5.06	0.08
3	rs9870579	**5.63**	**0.02**	**1.61 (1.08-2.4)**	**9.52**	**0.009**	**8.25**	**0.004**	**1.48 (1.13-1.94)**	**8.14**	**0.02**	0.9	0.34	1.11 (0.9-1.36)	3.81	0.15
8	rs10103330	0.46	0.5	0.9 (0.66-1.22)	0.63	0.73	0.05	0.83	0.97 (0.78-1.23)	0.14	0.93	0.12	0.73	1.03 (0.88-1.21)	0.42	0.81
9	rs2210539	3.15	0.08	0.77 (0.57-1.03)	5.96	0.05	3.49	0.06	0.81 (0.65-1.01)	5.83	0.05	0.6	0.44	0.94 (0.81-1.1)	0.62	0.73
9	rs10869675	0.005	0.95	1.01 (0.77-1.32)	0.82	0.66	3.87	0.05	0.82 (0.68-1)	5.53	0.06	2.99	0.08	0.89 (0.78-1.02)	4.84	0.09
10	rs17101921	0.001	0.98	1.01 (0.49-2.07)	1.18	0.56	0.01	0.91	1.03 (0.61-1.74)	0.81	0.67	1.36	0.24	0.79 (0.53-1.18)	1.52	0.47
11	rs10835482	0.61	0.43	0.87 (0.6-1.24)	0.78	0.68	0.98	0.32	0.87 (0.67-1.14)	1.36	0.51	0.68	0.41	1.08 (0.9-1.29)	1.06	0.59
11	rs1602565	0.07	0.8	0.95 (0.65-1.38)	1.39	0.5	0.2	0.65	0.94 (0.71-1.24)	0.21	0.9	1.06	0.3	1.1 (0.91-1.33)	1.09	0.58
11	rs3016384	0.76	0.38	1.11 (0.88-1.4)	2.07	0.36	0.24	0.63	0.96 (0.81-1.14)	0.33	0.85	1.21	0.27	0.93 (0.83-1.06)	1.49	0.47
12	rs6490121	0.94	0.33	1.13 (0.88-1.46)	1.35	0.51	0.46	0.5	0.94 (0.78-1.13)	0.76	0.69	1.43	0.23	0.92 (0.81-1.05)	1.86	0.4
13	rs4238270	0.42	0.52	0.92 (0.72-1.18)	0.78	0.68	0.08	0.78	1.03 (0.85-1.23)	0.19	0.91	1.5	0.22	1.08 (0.95-1.23)	2.14	0.34
15	rs3784397	0.33	0.57	1.07 (0.84-1.36)	0.7	0.71	0.45	0.5	0.94 (0.79-1.12)	1.04	0.6	1.43	0.23	0.93 (0.82-1.05)	2.23	0.33
16	rs7192086	1.17	0.28	0.87 (0.67-1.13)	1.88	0.39	1.36	0.24	1.13 (0.92-1.37)	1.34	0.51	**7.56**	**0.01**	**1.22 (1.06-1.4)**	**7.56**	**0.02**
16	rs9922369	0.53	0.47	0.78 (0.39-1.54)	0.55	0.46	0.08	0.78	0.93 (0.55-1.58)	0.08	0.78	0.02	0.88	0.97 (0.67-1.4)	1.8	0.41
18	rs1893146	0.8	0.37	1.17 (0.83-1.64)	2.49	0.29	3.49	0.06	1.26 (0.99-1.62)	**10.61**	**0.005**	1.05	0.31	1.1 (0.92-1.32)	5.14	0.08

Table 4.4d. Markers in high LD with variant rs9870579. The relevant gene, function, nucleotide change and base position are shown. D' and r^2 values are for the HapMap CEU population. SNP rs11927384 is highlighted in bold font as it has been found to lie in a region of high conservation.

SNP	Gene	Function		BP	D'	r^2
rs7355825		Intergenic	A/G	141118491	1	0.69
rs980337		Intergenic	A/G	141126259	1	0.69
rs9821898	CLSTN2	Intronic	A/G	141146198	1	0.69
rs9824931	CLSTN2	Intronic	C/T	141142693	1	0.69
rs9835329	CLSTN2	Intronic	C/T	141144636	1	0.69
rs17397077	CLSTN2	Intronic	A/C	141177598	1	0.69
rs9824300	CLSTN2	Intronic	A/T	141148245	1	0.71
rs9821561	CLSTN2	Intronic	A/C	141169098	1	0.82
rs10935363	CLSTN2	Intronic	A/T	141178433	1	0.83
rs11927306	CLSTN2	Intronic	C/G	141166429	1	0.83
rs11927384	**CLSTN2**	**Intronic**	**A/G**	**141166481**	**1**	**0.83**
rs6439886	CLSTN2	Intronic	A/G	141165193	1	0.83
rs7637853	CLSTN2	Intronic	C/G	141167105	1	0.83
rs9859388	CLSTN2	Intronic	A/G	141168906	1	0.83
rs7617850	CLSTN2	Intronic	A/G	141167334	1	0.87
rs7356017	CLSTN2	Intronic	A/G	141161986	1	1
rs9852679	CLSTN2	Intronic	C/G	141157433	1	1
rs9860322	CLSTN2	Intronic	G/T	141160536	1	1
rs9864420	CLSTN2	Intronic	C/T	141160744	1	1
rs9870376	CLSTN2	Intronic	C/T	141162167	1	1
rs9870392	CLSTN2	Intronic	C/T	141162204	1	1
rs9813092	CLSTN2	Intronic	A/G	141162442	1	1

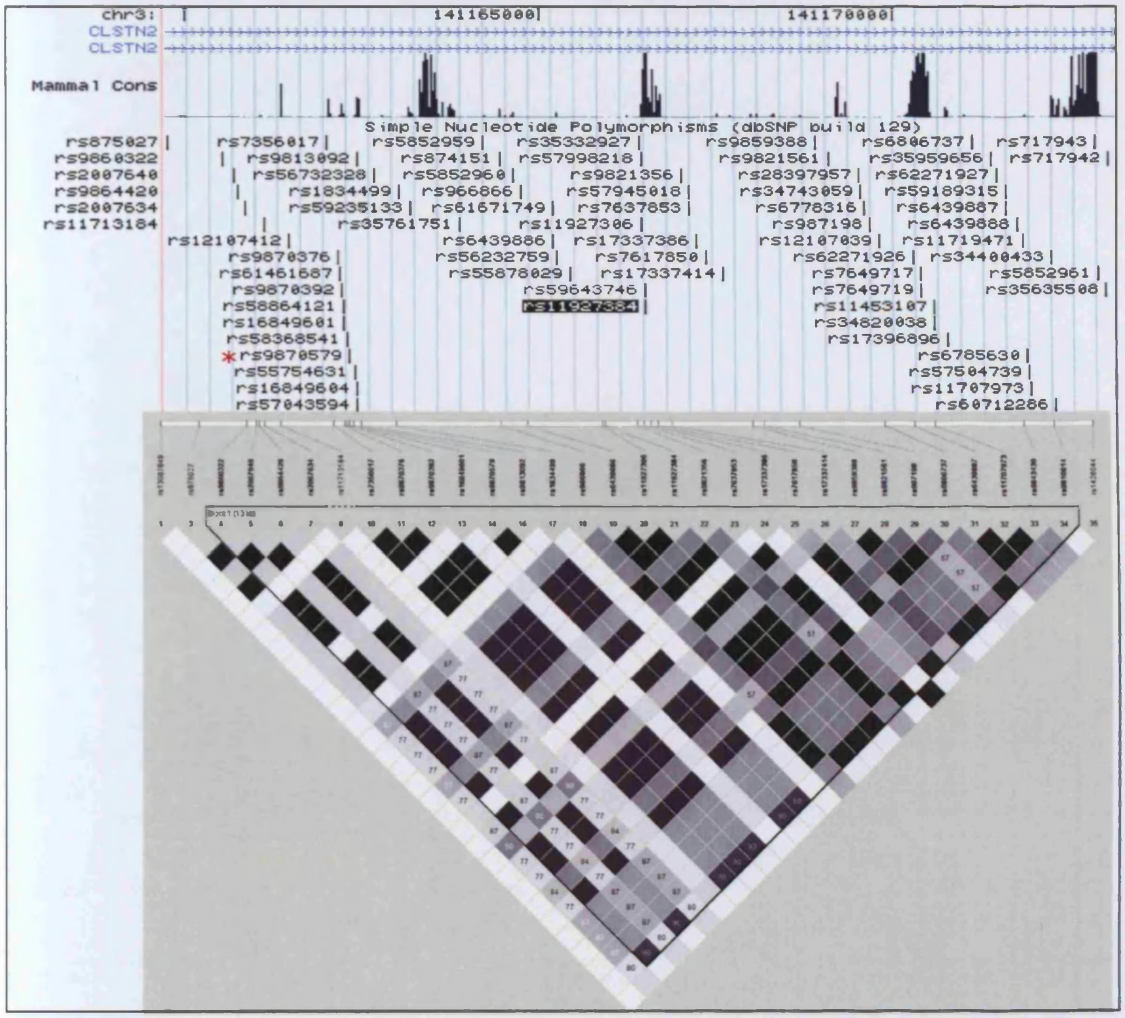


Figure 4.4a. Schematic of variant rs11927384 (highlighted in black with white font) which is in high LD with rs9870579 (indicated by *) and resides in a region of mammalian conservation. Diagram shows the chromosomal position of both variants, their locations in intron 1 of *CLSTN2*, the mammalian conservation in the region and the neighbouring SNPs. Lower part of diagram shows LD across the region, the blacker the square the higher the r^2 between SNPs [Figure produced using the UCSC genome browser, HapMap data and HAPLOVIEW HapMap format software].

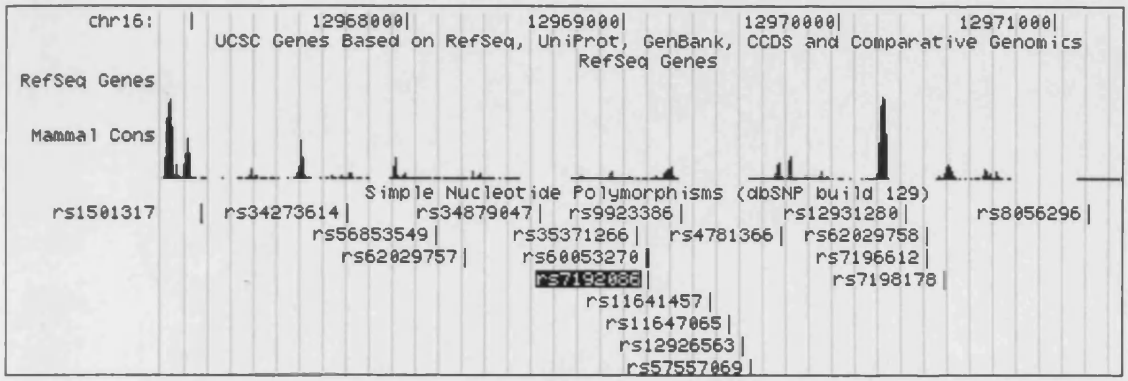


Figure 4.4b. Schematic of variant rs7192086; highlighted in black with white font. Diagram shows the chromosomal position of the variant, its location in an intergenic region, the mammalian conservation in the region and the neighbouring SNPs. [Figure produced using the UCSC genome browser].

4.4.1. Comparison with O'Donovan GWAS

The O'Donovan GWAS revealed one loci *ZNF804A* (rs1344706; $P = 1.61 \times 10^{-7}$) that surpassed the $P < 5 \times 10^{-7}$ benchmark corresponding to strong evidence for association in genome-wide studies (O'Donovan et al. 2008). A secondary analysis, added additional data from 1865 bipolar disorder patients from the WTCCC study (WTCCC 2007). There was no evidence for shared risk for most of the loci, but for the *ZNF804A* locus, the evidence for association substantially strengthened (rs1344706; $P = 9.96 \times 10^{-9}$), suggesting alleles in the vicinity of *ZNF804A* influence risk to a broad psychosis phenotype. *ZNF804A* maps to chromosome 2 at 185.1-185.5 Mb. The encoded protein is uncharacterized, has no known function, but contains predicted zinc ion and DNA binding domains, suggesting a possible role as a regulator of gene expression. Neither of the *ZNF804A* variants tested in this study, rs1344706 and rs12613195, showed any evidence for association to LOAD+P. In fact, marker rs1344706 showed deviation from HWE in the control sample of this study ($P = 0.004$).

The only marker to show association to LOAD with psychosis is rs9870579. In the O'Donovan GWAS discovery sample this marker had a significant p-value of 4.72×10^{-5}. However, the association did not survive meta-analysis of the discovery sample plus the independent replication samples (O'Donovan et al. 2008). In the O'Donovan GWAS the major T allele was over-represented in the case sample. The allele associated to LOAD+P is the minor C allele. Therefore, the association seen in LOAD+P is in the opposite direction to that seen in schizophrenia. A possible explanation for this finding could be that rs9870579 influences susceptibility to psychosis in neurodevelopmental and neurodegenerative disease by varying mechanisms. Alternatively, at least one of the observed rs9870579 associations could be a false positive.

Marker rs7192086 shows nominal association to LOAD. The associated allele is the minor T allele. This variant was the 5th most significant marker in the O'Donovan GWAS discovery sample, the 3rd most significant finding in the meta-analysis of the discovery and replication samples and the 5th most significant finding in the combined analysis with data from the WTCCC study of bipolar disorder (WTCCC 2007). The associated allele in all analyses was the minor T allele. Therefore, the T allele is associated with psychosis in a schizophrenia and

bipolar disorder dataset, but with LOAD and not psychosis in LOAD in this sample. It is noteworthy that the association seen in this LOAD dataset is nominal and does not survive correction for multiple tests. Therefore, it is possible that this association is a type 1 error and that the T allele is likely to be associated with a broad phenotype of psychosis as the data from the O'Donovan GWAS suggests.

4.5. Discussion

This study tested 21 markers for association with LOAD+P. These variants were identified from the O'Donovan schizophrenia GWAS. No marker showed a significant association, in any analysis that survived correction for multiple testing. The lack of significant evidence for association is not unexpected due to the limited power of this study. The most significant association was seen with variant rs9870579 in the LOAD+P vs. control analysis (allelic $P = 0.004$; OR = 1.48). However, rs9870579 appears to have a greater effect on LOAD+P under a disease modifier model (OR = 1.61 versus 1.48 for the disease subtype analysis), with an increase in the over representation of the minor C allele in LOAD+P (5% compared to 4% in the disease subtype analysis). Marker rs9870579 is located in intron 1 of the calsyntenin 2 gene. *CLSTN2* is a large gene situated on chromosome 3q23. There is one known gene transcript which is expressed exclusively in brain. The calsyntenin family of proteins are predominantly postsynaptic membrane proteins of excitatory CNS synapses with putative calcium binding capacity (Hintsch et al. 2002). However, the highest levels of CLSTN2 protein are found in GABAergic neurons (Hintsch et al. 2002), i.e. neurons that produce GABA as their output. GABA (Gamma-aminobutyric acid) is the chief inhibitory neurotransmitter in the mammalian CNS. Disrupted GABAergic signalling has been implicated in numerous and varied neurological and psychiatric pathologies including schizophrenia. The 3q23 region has previously shown linkage to LOAD and the *CLSTN2* gene has shown significant association to cognitive function (Liu et al. 2007). Thus, *CLSTN2* can be considered a putative functional and positional candidate gene for LOAD and psychosis. Marker rs9870579 appears to have no functional relevance and does not lie in a region of conservation or in a binding

motif. However the variant is in high LD (D' = 1.0 and r^2 = 0.83) with polymorphism rs11927384 which resides within a conserved region of intron 1 of the *CLSTN2* gene. Conservation suggests that this variant or the region in which it lies may have a functional role. For example, the conserved region may act as a binding site for transcription factors involved in regulation of gene expression. In the O'Donovan discovery sample this marker had a significant p-value of 4.72×10^{-5}. However, this association did not survive a meta-analysis of the discovery sample plus independent replication samples (O'Donovan et al. 2008). The association of rs9870579 with LOAD+P is in the opposite direction to that seen in schizophrenia. This suggests that rs9870579 influences susceptibility to psychosis in neurodevelopmental and neurodegenerative disease by varying mechanisms, or that at least one of the observed rs9870579 associations is a false positive finding.

Variants at the *ZNF804A* gene were identified as the 'top hits' in the O'Donovan GWAS. Neither of the *ZNF804A* variants tested in this study, rs1344706 and rs12613195, showed any evidence for association with LOAD+P. However, rs1344706 has not been adequately investigated as a susceptibility variant for LOAD with psychosis as a sub-form of LOAD as the marker deviates from HWE in the control sample.

Marker rs7192086 showed association with LOAD (allelic P = 0.01; OR = 1.22 and genotypic P = 0.02). However, the association does not survive correction for multiple testing. The marker is located on 16p13.12 in an intergenic region at least 56 kb from the nearest known gene *AK000877*. There is low conservation across the region and no recognised binding motifs.

It is likely that there are true schizophrenia susceptibility loci yet to be identified that may associate with psychosis in LOAD. None of the published schizophrenia GWAS identify a schizophrenia susceptibility locus which replicates in independent studies. It is probable that the moderately sized samples used across the studies, and therefore the resultant power of each study, is inadequate to identify a novel susceptibility gene for schizophrenia with definitive confidence. Power calculations show that to achieve a significant association with an effect size of 1.2 in a complex genetic disorder a minimum sample size of 1300 cases and 1300 controls is required (Faul et al. 2007). Power is further reduced in the pooled DNA studies by the additional inaccuracy, albeit small, of the pooled DNA methodology. Significant association to disease, identified by GWAS, is often the

result of very small differences in allele frequencies. Therefore, despite the mean error rate of the pooled DNA methodology being extremely small (Hoogendoorn et al. 2000; Le Hellard et al. 2002; Meaburn et al. 2005; Norton et al. 2002; Sham et al. 2002), in GWAS this can prove to be a highly substantial inaccuracy and lead to the production of both false positive and false negative associations.

Additional genotyping of the *CLSTN2* gene should be undertaken. The aim of this would be to identify a functional variant associated with disease. Depending on the results of this genotyping, additional work maybe to screen and sequence regions of the gene to identify novel variants which would then be tested for association with disease. Investigation of gene expression differences which associate with disease could be undertaken by cis-acting allelic expression assays (Bray et al. 2003b). The *CLSTN2* gene plus other putative psychosis susceptibility genes, including those identified by the O'Donovan GWAS, will be explored further in the first GWAS of LOAD+P described in chapter 5. The marker rs9870579 is not included on the Illumina HD Human610 beadchip, however, a suitable proxy can be identified.

This is the first study to utilise the novel approach of using the results of a schizophrenia GWAS to interrogate psychosis susceptibility variants for association with psychosis in LOAD. This is a conservative hypothesis being tested. However, it seems a sensible approach to undertake in one of the early genetic association studies of psychosis in AD, drawing vital information from the established field of schizophrenia genetics to inform the choice of genetic variants to test. Due to the lack of consistency across schizophrenia GWAS, and the lack of identification of any loci which show replicated genome-wide significant association to schizophrenia, this study utilises the results of the O'Donovan GWAS, whose sample set most closes matches the LOAD+P sample used in this study. This approach means that true positive associations with schizophrenia, identified by other schizophrenia GWAS, were excluded from this analysis. Also, only specific gene variants have been tested, assuming that either these polymorphisms or markers in LD with them are associated with disease. It is feasible that a common gene(s) is involved in the aetiology of schizophrenia and LOAD+P, but that alternate polymorphisms at the loci increase the risk for alternate phenotypes. Using this strategy this theory cannot be tested and these genes cannot be excluded as susceptibility loci for LOAD+P. A preferable approach would be to

genotype 'tag' SNPs across the gene(s) of interest with the aim of identifying either direct or indirect association. However, this methodology is time consuming and not financially viable. Also, this sample size is small and therefore insufficiently powered to detect susceptibility variants of moderate effect size hypothesised to act in complex diseases. Future meta-analyses of multiple LOAD+P studies investigating overlapping genetic markers may prove more fruitful in identifying susceptibility factors for this form of LOAD.

5. Genome-Wide Association Study of LOAD with Psychosis

5.1. Introduction

Since 2005, genome-wide association studies (GWAS) have produced strongly significant evidence that common DNA sequence variation influences genetic susceptibility to more than 40 different common phenotypes (Manolio et al. 2008). Many of these findings implicate previously unsuspected candidate genes and new pathophysiological hypotheses (Cichon et al. 2009). Findings for type 2 diabetes (T2D) particularly highlight the success of GWAS and illustrate the importance of large sample size in identifying common genetic variants with small to moderate effect size. For example, rs7903146 at the *TCF7L2* gene locus was found to be associated with T2D. This SNP has an overall odds ratio of 1.37 (Frayling 2007), and was detected by most but not all previous T2D association studies. The other 10 loci identified (with odds ratios from 1.05 to 1.25) that contribute to the aetiology of T2D were missed by most single studies due to a lack of sample power. Allelic odds ratios between 1.1 and 1.2 require from 10,000 to well over 20,000 total subjects for 80% power (Cichon et al. 2009). In the Wellcome Trust Case Control Consortium study, the T2D susceptibility loci were ranked from 2 to 26,017 in their strength of association (Frayling 2007). A meta-analysis combining over 60,000 subjects implicated eight genes achieving p-values < $5x10^{-8}$ (with odds ratios from 1.09 to 1.15), which had not previously reached genome-wide significance in individual studies (Zeggini et al. 2008).

There have been 9 published GWAS of LOAD that analysed individual genotypes (Lambert et al. 2009; Beecham et al. 2009; Bertram et al. 2008; Carrasquillo et al. 2009; Coon et al. 2007; Feulner et al. 2009; Harold et al. 2009; Li et al. 2008; Reiman et al. 2007), a report of a study that genotyped pooled samples (Abraham et al. 2008) and a study which used a pooling strategy to investigate potentially functional SNPs across the genome (Grupe et al. 2007). All studies identified the *APOE* association, proving the power of the approach under the 'common disease - common variant' hypothesis (Avramopoulos 2009), at least

for relatively large effect sizes. Excluding Lambert et al. (2009) and Harold et al. (2009), none of the studies exceeded a sample size of 2,500 (cases and controls) making them of modest size for the purpose of a GWAS. None of these studies found a genome-wide significant association (except with *APOE*) in their primary analysis. The study by Carrasquillo and colleagues did identify genome-wide significance with rs5984894 at the *PCDH11X* locus when stage 1 and stage 2 datasets were combined and adjusted for sex ($P = 3.9 \times 10^{-12}$) (Carrasquillo et al. 2009). One of the two larger studies is a two-stage LOAD GWAS (Harold et al. 2009). This drew upon on a combined sample of 19,000 subjects, and produced evidence for two novel susceptibility loci: rs11136000 in the *CLU* gene ($P = 1.4 \times 10^{-9}$) and rs3851179, a SNP 5' to the *PICALM* gene ($P = 1.9 \times 10^{-8}$) (Harold et al. 2009). Both novel associations were supported in an independent replication sample producing compelling evidence for association with LOAD (Harold et al. 2009). The study of Lambert and colleagues (Lambert et al. 2009) supports the identification of *CLU* as a susceptibility gene for AD. With genome-wide significant evidence for association of rs11136000 ($P = 7.5 \times 10^{-9}$; OR = 0.86) in the same direction. The study of Lambert and colleagues also identifies the *CR1* gene (rs6656401; $P = 3.7 \times 10^{-9}$; OR = 1.21) as a novel susceptibility gene for AD (Lambert et al. 2009). This finding is supported by the study of Harold and colleagues (rs1408077; $P = 8.3 \times 10^{-6}$) (Harold et al. 2009). In addition to SNPs meeting stringent criteria for genome wide significance a significant excess of loci showing 'sub-threshold' association ($p < 1 \times 10^{-5}$) with AD were also observed, providing strong evidence that there are several genes associated with LOAD that remain to be identified (Harold et al. 2009; Lambert et al. 2009).

GWAS in numerous disorders have shown that traditional psychiatric diagnostic phenotypes might not provide the most powerful means of mapping disease loci (Sabb et al. 2009). For example, it is becoming increasingly apparent that within diagnostic categories, such as bipolar disorder and schizophrenia, extensive aetiological and genetic heterogeneity operates (O'Donovan et al. 2009). Secondary analysis of a major depressive disorder GWAS with a narrower, more homogeneous phenotype produced genome-wide significant association with a variant located in the piccolo (*PCLO*) gene which replicated in additional samples (Sullivan et al. 2009). As a result, there is increasing emphasis on the use of endophenotypes and sub phenotypes to elucidate genotype-phenotype

relationships. As psychotic symptoms in LOAD have been proposed as a marker for a purer form of the disease suitable for gene mapping efforts (Sweet et al. 2003), the sub-phenotype of LOAD with psychosis was used here to search for susceptibility genes. There are at least two models which could explain how genetic variation relates to psychosis in LOAD (Sweet et al. 2003). First, disease modifier genes could influence susceptibility to psychotic symptoms in the presence of neurodegeneration caused by LOAD resulting from other environmental or genetic factors ('disease modifier' model). Given such a scenario, one would expect to observe differences between LOAD cases with and without psychotic symptoms, but not necessarily between LOAD cases with psychosis and healthy controls. Second, LOAD characterized by the presence of psychotic symptoms maybe a biologically distinct phenotype, for which one or more susceptibility genes may exist (LOAD+P 'disease subtype' model). The comparison of LOAD+P cases with healthy controls will allow for the detection of such associations. Using the data from the Harold and colleagues (Harold et al. 2009) GWAS of LOAD (referred to hereafter as the main LOAD GWAS), variation that may influence susceptibility to psychotic symptoms in the presence of AD was investigated. Given the two possible aetiological models of LOAD+P, two separate analyses were undertaken. First the disease modifier hypothesis was explored by testing for genetic association with LOAD+P in a within LOAD case analysis, thus comparing LOAD cases with psychosis against LOAD cases without psychosis (LOAD+P vs. LOAD-P). Secondly the disease subtype model was tested by analysing LOAD cases with psychosis against both elderly screened and population controls (LOAD+P vs. control). The aim of these analyses was to identify LOAD+P modifier and/or susceptibility genes, and attempt to elicit the biological functions which contribute to the aetiology of LOAD+P, a severe form of LOAD. These primary analyses will be presented in Section 1 of this chapter.

The hypothesis-free nature of GWAS analysis must be considered one of its advantages; nevertheless, incorporating prior biological knowledge can prove fruitful (Holmans et al. 2009b). Therefore, the results of the LOAD+P analyses were examined to determine if significant associations existed at biologically plausible candidates. For example, the 'top results' from the schizophrenia forum 'szgene' database (Allen et al. 2008). These secondary analyses will be presented in Section 2 of this chapter.

Section 1: LOAD+P GWAS

5.2. Materials and Methods

5.2.1. Sample Ascertainment and Diagnostic Criteria

Due to the unavailability of complete phenotypic data, only a subset of the sample used in the main LOAD GWAS that had complete NPI data for delusions and hallucinations was used in these analyses. This study comprised a total of 1,671 LOAD cases and 5,167 controls. All individuals were drawn from the United Kingdom, were of Caucasian ancestry and included 1,208 cases and 1,044 screened controls from the MRC genetic resource for LOAD (Cardiff University; Institute of Psychiatry, London; Cambridge University; Trinity College, Dublin) (Morgan et al. 2007) previously used in this thesis. Additional samples were recruited by Queen's University Belfast (Carson et al. 2008), University of Southampton (McCulley et al. 2004) and the London and the South East Region LOAD project (LASER-AD) (Livingston et al. 2007). LOAD cases met criteria for either probable (NINCDS-ADRDA (McKhann et al. 1984}, DSM-IV) or definite (CERAD) (Mirra et al. 1991) AD. A total of 1,135 elderly screened controls were ascertained from the MRC Genetic resource for LOAD and by Queen's University, Belfast. All elderly screened controls were 60 years or above and were screened for cognitive decline using the Mini-Mental State Examination (Folstein et al. 1975). In addition, 4,032 population controls were drawn from the 1958 British Birth Cohort (1958BBC) (http://www.b58cgene.sgul.ac.uk). Psychotic symptoms were assessed using the Neuropsychiatric Inventory (NPI) (Cummings 1994) whose reliability and validity are well established (Cummings et al. 1997), and is comprehensively described in chapter 2.2. The threshold domain score for psychosis in LOAD implemented in the LOAD+P GWAS is slightly different from that used in the rest of this thesis. LOAD+P was defined as either the presence of delusions and hallucinations, or where only one symptom was present a delusion domain score greater than or equal to 4 or a hallucination domain score greater than or equal to 2. A more stringent cut off for delusions was adopted because

delusional behaviour among LOAD sufferers is often a form of confabulation secondary to amnesia and is therefore likely to have a different aetiology to other psychotic features (Hollingworth et al. 2006). LOAD cases who had not displayed any hallucinations or delusions were coded as LOAD with no psychosis (LOAD-P). As psychotic symptoms typically emerge in the moderate stages of LOAD (Hollingworth et al. 2006; Paulsen et al. 2000b) those LOAD-P cases in the mild stages of disease were considered to be at substantial risk of going on to develop delusional or hallucinatory behaviour and were therefore excluded from the analysis. Subjects with a known previous history of mood disorders, bipolar disease, unipolar disease, or an anxiety disorder were also excluded from all analyses. A total of 645 cases met criteria for LOAD+P, 505 individuals were characterised as LOAD-P.

5.2.2. Sample Sets

One thousand six hundred and seventy one LOAD cases and 1,135 elderly screened controls included in this study were genotyped on the Illumina 610-quad chip. The remaining 4,032 population controls from the 1958BBC were previously genotyped using the Illumina HumanHap550. The following sample preparation and laboratory quality control measures refer to the samples genotyped on the Illumina 610-quad chip only.

5.2.3. DNA Extraction, Sample Preparation and Laboratory Quality Control

DNA was obtained from blood samples from each participant and extraction was completed by phenol/chloroform methodology as previously described (chapter 2.4). DNA quantification and concentration dilution was also as described (chapter 2.4), with a final sample dilution of 50 ng/µl. DNA quality was assessed by 2% agarose gel electrophoresis under standard conditions (described in chapter 2.6). Samples showing no evidence of degradation were then genotyped in a panel of 30 SNPs using the Sequenom™ MassARRAY™ and iPlex™ systems (Sequenom™, San Diego, CA) (chapter 2.7.3). This allowed sex to be checked and permitted sample identity checks after re-aliquoting for GWAS genotyping.

5.2.4. Genotyping

Genotyping on the Illumina 610-quad Beadchips was performed at the Sanger Institute, UK. All samples were re-aliquoted for GWAS genotyping using a Biomek® FX Laboratory Automation Workstation (Beckman Coulter®, Inc., Fullerton, CA) into 96 well plate formats. 200 ng of input DNA per sample was used and prepared for genotyping using the Illumina Infinium™ system (Illumina® Inc., San Diego, CA, USA). Manufacturer's protocols were followed throughout. Briefly, DNA was isothermally amplified overnight then enzymatically fragmented, alcohol precipitated and resuspended. The Illumina Human 610-Quad BeadChips (Illumina® Inc., San Diego, CA, USA) were prepared for hybridisation in a capillary flow-through chamber. The amplified and fragmented DNA samples were hybridised to the bead chips using a Tecan robot and an enzymatic base extension used to confer allele specificity. The chips were subsequently stained and scanned using an iSCAN reader (Illumina® Inc., San Diego, CA, USA) to detect fluorescence at each bead. Data were loaded into Beadstudio and final call reports containing X, Y, X-Raw and Y-Raw outputted. The Illuminus algorithm for cluster analysis was used for genotype calling (Teo et al. 2007). In addition, 4,032 population controls from the 1958 birth cohort (1958BBC) previously genotyped using the Illumina HumanHap550 were included in this study. The 1958BBC were genotyped by the Wellcome Trust Case Control Consortium (WTCCC) (WTCCC 2007) or the Type 1 Diabetes Genetics Consortium (T1DGC), who used version 1 and version 3 of the Illumina HumanHap550 array, respectively. There was no sample overlap across the WTCCC and T1DGC studies meaning that the 1958BBC genotypes from the WTCCC and T1DGC were treated as independent samples.

5.2.5. Individual Quality Control

The main LOAD GWAS had already undergone stringent quality control (QC) (Harold et al. 2009), meaning that individual QC was already complete for the sub-sample employed in this study. This individual QC for the main LOAD GWAS is described as follows. Genotypes were derived from 8 separate datasets/studies, making the application of stringent QC filters important, as differential genotyping error rates between studies could result in spurious associations when the data are

combined (Clayton et al. 2005; Moskvina et al. 2006). These filters were applied separately to each of these 8 datasets to remove poorly performing samples using tools implemented in PLINK (v1.05) (http://pngu.mgh.harvard.edu/~purcell/plink) (Purcell et al. 2007). Briefly, a missing genotyping rate of > 0.01 was applied excluding 1,469 individuals; a filter based on mean autosomal heterozygosity was applied excluding 578 individuals with values above or below empirically determined thresholds. The specific QC thresholds applied and the breakdown of samples excluded in this study are given in Tables 5.2a and 5.2b, respectively. Seventy-one individuals with inconsistencies between reported sex and genotype-determined sex and 22 individuals with ambiguous genotype-determined sex were removed from the main LOAD GWAS. Potential genetic relatedness was examined by calculating identity by descent (IBD) estimates for all possible pairs of individuals in PLINK, and removing one of each pair with an IBD estimate ≥ 0.125 (the level expected for first cousins). IBD estimates were calculated in the main LOAD GWAS using SNPs that were common to all Illumina chips used in the study, with a genotype missing data rate ≤ 0.01, Hardy-Weinberg Equilibrium $P \geq 1x10^{-5}$ and a minor allele frequency ≥ 0.01. As a result, 506 individuals were excluded from these analyses. Non-European ancestry was detected by merging genotype data from SNPs typed in all cohorts with genotypes at the same SNPs from 210 unrelated European (CEU), Asian and Yoruban samples from the HapMap project. Subsequent to removing SNPs in extensive regions of linkage disequilibrium (chr5: 44-51.5 Mb; chr6: 25-33.5 Mb; chr8: 8-12 Mb; chr11: 45-57 Mb) (Price et al. 2008), SNPs were pruned if any pair within a 50-SNP window had $r^2 > 0.2$. Genome-wide average identity by state (IBS) distance was calculated in PLINK between each pair of individuals in the resulting dataset, based on 57,966 SNPs (all with a genotype missing data rate ≤ 0.01, Hardy-Weinberg Equilibrium $P \geq 1x10^{-5}$ and a minor allele frequency ≥ 0.01). The resulting matrix of IBS distances was used as input for classical multi-dimensional scaling (MDS) in R (v2.7.1) (http://www.r-project.org). When the first two dimensions were extracted and plotted against each other, three clusters were observed corresponding to the European, Asian and Yoruban samples. Sixteen samples which appeared to be ethnic outliers from the European cluster were excluded. Population structure was assessed within the data using principal components analysis (PCA) as implemented in EIGENSTRAT (Price et al. 2006) to infer continuous axes of

genetic variation. Eigenvectors were calculated based on the previously described LD-pruned subset of 57,966 SNPs common to all arrays. The EIGENSTRAT program also identifies genetic outliers, which are defined as individuals whose ancestry is at least 6 standard deviations from the mean on one of the top ten axes of variation, these outliers were identified and excluded. Following individual QC 543 LOAD+P cases, 417 LOAD-P cases and 4,706 controls were included in the analysis.

5.2.6. SNP Quality Control

Due to unresolved genotype-calling issues with a proportion of SNPs on the sex chromosomes, only autosomal SNPs were included in this analysis. Individuals included in this study were genotyped on either the Illumina 610-quad array specifically for the main LOAD GWAS, or were previously genotyped on the Illumina HumanHap550, and the genotypes made available to us. SNPs in the 1958BBC datasets were filtered prior to inclusion in this study. This filtering included expulsion of SNPs not common to both versions of the Illumina HumanHap550 chip (v1 and v3), or not also present on the Illumina 610-quad chip. The 610-quad data and the 1958BBC data were merged, and were subsequently QC-filtered prior to the association analyses presented here. While SNP QC had been performed as part of the main LOAD GWAS, additional QC was employed here to remove poorly performing SNPs specific to the LOAD+P, LOAD-P and control datasets. Harold and colleagues (Harold et al. 2009) assessed the effects of different missing data rate and Hardy-Weinberg filters, and the filters chosen were also applied to the sub-sample used here. For each of the SNP categories, markers were excluded if they had a minor allele frequency (MAF) < 0.01 or a Hardy-Weinberg Equilibrium $P \leq 1x10^{-5}$, in either cases or controls. SNPs with a MAF ≥ 0.05 were excluded if they had a genotype missing rate of > 0.03 in either cases or controls; for SNPs with a MAF between 0.01 and 0.05, a more stringent genotype missing rate threshold of 0.01 was employed. As a result of this basic SNP QC 43,542 SNPs were excluded. SNPs included in this study fell into 2 categories: 1) 456,449 SNPs common to both the Illumina HumanHap550 and Illumina 610-quad arrays, 2) 72,756 SNPs with genotypes only from the Illumina

610-quad array. As a result of the additional SNP QC, an extra 2,651 SNPs were excluded. Ten principal components (PCs) were extracted using EIGENSTRAT, on the main LOAD GWAS dataset (Harold et al. 2009). To determine if these PCs could assuage any population structure within the sample, logistic regression tests of association with LOAD+P were performed, sequentially including between 0 and 10 of the top PCs as covariates. The impact of including the PCs was evaluated by calculating the genomic control inflation factor, λ (Devlin and Roeder 1999). Including the first 1 PC as a covariate had the maximum impact on λ in both the within case and case-control analysis (see Tables 5.6c and 5.6d, respectively). To minimise inter-chip and inter-cohort differences that could result in an inflation of type I error rate, minor allele frequencies had previously been compared between controls in the different groups using logistic regression analysis (Harold et al. 2009). For each of the SNP categories, a quantile-quantile (QQ) plot was produced for each cohort control comparison, and the significance threshold employed to exclude SNPs was based on where the observed χ^2 statistics departed from the null expectation (see Appendix Table 5.1 and Appendix Figure 5.1). A total of 9,828 SNPs were excluded as a result of these comparisons. Thus, the final 'LOAD+P versus LOAD-P' and 'LOAD+P versus control' GWAS included 526,554 SNPs.

5.2.7. Study Power

Study power was calculated using the PS power and sample size program (v3.0) (Dupont and Plummer 1998), freely available at http://biostat.mc.vanderbilt.edu/PowerSampleSize.

Table 5.2a. Table shows quality control filters applied to individuals in each cohort. N = number of individuals; Chr = chromosome.

Cohort	N	Chip	Missing Genotype	Autosomal Heterozygosity	X-Chr Heterozygosity: Males	X-Chr Heterozygosity: Females
610	2806	610	≤0.01	0.325-0.337	<0.02	0.25-0.4
1958BBC (T1DGC)	2596	550	≤0.01	0.33-0.34	<0.007	0.294-0.37
1958BBC (Sanger)	1436	550	≤0.01	0.329-0.34	<0.011	0.295-0.38

Table 5.2b. Table shows samples excluded from each cohort following application of the quality control measures listed in Table 5.2a. N = number of individuals. Auto Het = mean autosomal heterozygosity. X-Chr Het = mean X chromosome heterozygosity. *Population controls were not excluded if there was a discrepancy between reported sex and genotype-determined sex. Outliers identified by EIGENSTRAT.

Group	N before QC	Missing Genotype	Auto Het	X-Chr Het	Sex	Relatedness	Non-European	Genetic Outlier	N after QC
610	2304	248	39	2	46	47	0	7	1915
1958BBC (T1DGC)	2596	57	2	2	1*	4	0	12	2519
1958BBC (Sanger)	1436	87	108	4	0	1	0	4	1232

Table 5.2c. LOAD+P vs. LOAD-P. Table shows the effect of varying the number of principal component (PCs), extracted from EIGENSTRAT, on the genomic control inflation factor, λ. These values are based on analysis of SNPs common to the Illumina 610-quad and HumanHap550 chips.

Principal Components Included	λ
0	1.004
1	1.002
1-2	1.003
1-3	1.004
1-4	1.005
1-5	1.007
1-6	1.006
1-7	1.007
1-8	1.009
1-9	1.010
1-10	1.011

Table 5.2d. LOAD+P vs. control. Table shows the effect of varying the number of principal component (PCs), extracted from EIGENSTRAT, on the genomic control inflation factor, λ. These values are based on analysis of SNPs common to the Illumina 610-quad and HumanHap550 chips.

Principal Components Included	λ
0	1.021
1	1.006
1-2	1.007
1-3	1.007
1-4	0.994
1-5	0.993
1-6	0.994
1-7	0.993
1-8	0.993
1-9	0.992
1-10	0.992

5.2.8. Association Analyses.

SNPs were tested for association with LOAD+P using logistic regression, assuming an additive model. Following analysis, 140 cluster plots were visually inspected for SNPs with a p-value ≤ $1x10^{-5}$ (7 SNPs were represented in both analyses). One SNP showed poorly formed clusters and was excluded. A conservative genome-wide significance threshold of 0.05/526554 = 9.5×10^{-8} was employed. To detect statistical significance at this threshold this study has power of 0.001 and 0.007 to detect an association with MAF of 0.3 and effect size of 1.3 in the disease modifier and disease subtype analysis, respectively. To detect a significant association of the same effect size at $P \leq 0.05$ this study has 47% and 78% power for the disease modifier and disease subtype analysis, respectively. The study design is illustrated in Figure 5.2a.

A set based analysis was undertaken to determine if the top hits were significant at the gene-wide level. The WGAViewer program (Ge et al. 2008) was used to identify SNPs within the locus of interest (the gene + 40 kb of flanking sequence), genotyped in this study. For each of the two LOAD+P GWAS datasets, a set-based logistic regression analysis was run using PLINK (v1.05) (Purcell et al. 2007), including the 1 PC included in the primary association analysis. Parameters were set at maximum set = 1, and number of permutations = 1,000,000. Gene coverage was determined by download of phase 1 and 2 CEPH genotype data, for the gene locus of interest, from the HapMap database (The International HapMap Consortium 2007), this was loaded into the HAPLOVIEW (v4.0) program (Barrett et al. 2005), with the list of the SNPs genotyped at the locus force included into the tagger tab of the software at MAF ≥ 0.05 and $r^2 \geq 0.8$.

Overlap analysis was undertaken to determine if any identified SNP showed evidence for association in a previous schizophrenia (O'Donovan et al. 2008) and bipolar (WTCCC 2007) dataset. The top 100 hits from both 'disease modifier' and 'disease subtype' GWAS were taken and where possible an Affymetrix proxy for each SNP was determined using the SNP Annotation and Proxy Search (SNAP) (v2.1) from the Broad Institute (http://www.broadinstitute.org/mpg/snap/) (Johnson et al. 2008). These SNP IDs were used to mine the schizophrenia and bipolar disorder GWAS datasets. It was then determined if any investigated SNP showed evidence for association with schizophrenia or bipolar disorder at $P \leq 0.1$.

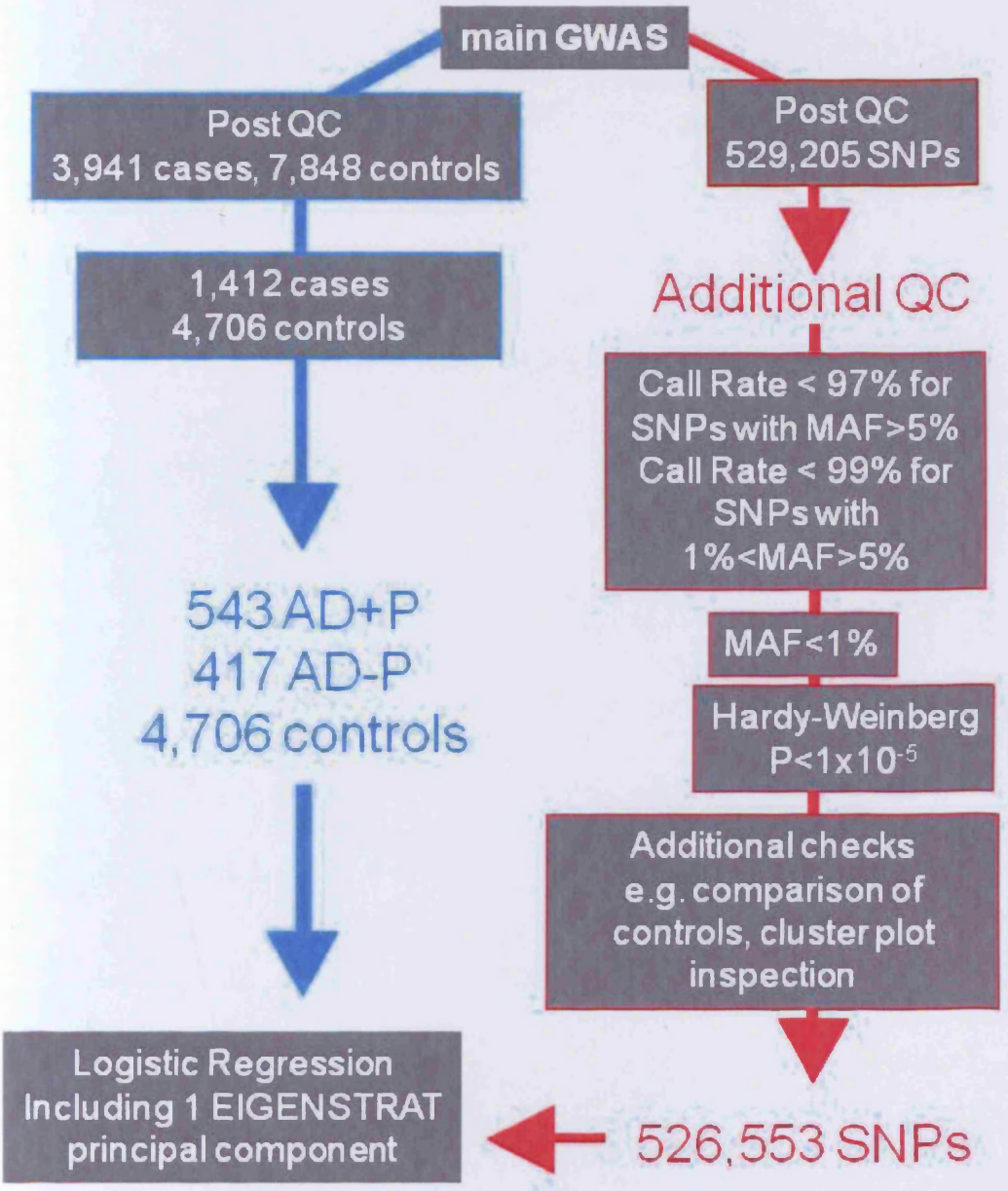


Figure 5.2a Schematic of study design. Diagram shows number of samples (with complete NPI data) and SNPs taken from main GWAS and the additional SNP QC employed resulting in the loss of 2,651 SNPs.

5.3. Results

Following individual QC 543 LOAD+P cases, 417 LOAD-P cases and 4,706 controls were included in further analysis. Clinical characteristics of the sample can be found in Table 5.3a, and a full breakdown of psychosis classification in LOAD cases by study can be seen in Appendix Table 5.2. A total of 526,554 SNPs were analysed. The quantile-quantile (QQ) plots for the 'LOAD+P versus LOAD-P' and 'LOAD+P versus controls' analyses can been seen in Figures 5.3a and 5.3b respectively. The genomic control inflation factors (λ) (Devlin and Roeder 1999) were 1.002 and 1.006 respectively, suggesting little evidence for residual stratification. Tables 5.3b and 5.3c list the top 20 hits in the LOAD+P vs. LOAD-P and LOAD+P vs. controls analysis, respectively. Figures 5.3c and 5.3d show the Manhattan plots for the LOAD+P vs. LOAD-P and LOAD+P vs. control analyses, respectively. Results for all SNPs with p-values ≤ $1x10^{-4}$ can be found for LOAD+P versus LOAD-P and LOAD+P versus control analyses in Appendix Tables 5.3 and 5.4, respectively. A breakdown of minor allele frequencies in all datasets is shown for SNPs with a p-value ≤ $1x10^{-4}$ in Appendix Table 5.5. Note that there was no significant difference in allele frequencies between elderly, screened controls and population controls for any SNP with a p-value ≤ $1x10^{-4}$, and the QQ plots of the different control sets (screened vs. WTCCC population controls, screened vs. T1DGC population controls, WTCCC population controls vs. T1DGC population controls) shows no deviation of the observed chi-square from that expected, see Appendix Figure 5.2.

Table 5.3a. Basic clinical characteristics and description of sample.

	LOAD Cases*	LOAD+P	LOAD-P	Screened Controls	Population Controls
n (n before QC)	1412 (1671)	543 (645)	417 (505)	937 (1135)	3751 (4032)
Sex, % Female	71.1	73.1	68.3	61.9	50.8
Mean age at assessment (years)	80.87	80.85	80.69	75.92	44.50
Mean age at onset (years)	75.85	75.54	76.13	-	-
Mean MMSE	11.33	9.93	12.25	29.19	-

* Total number of LOAD cases with available Neuropsychiatric Inventory and genotype data.

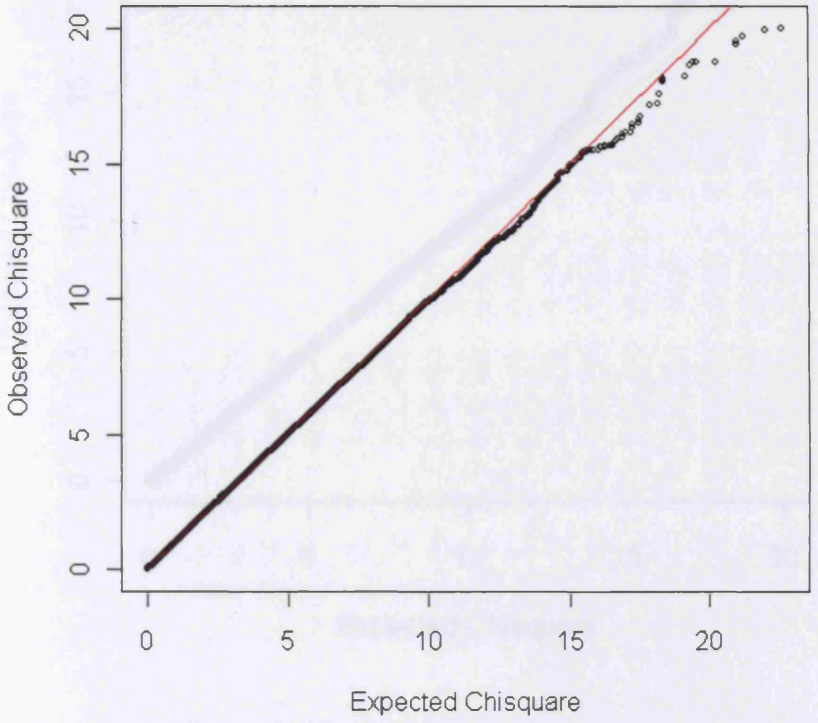
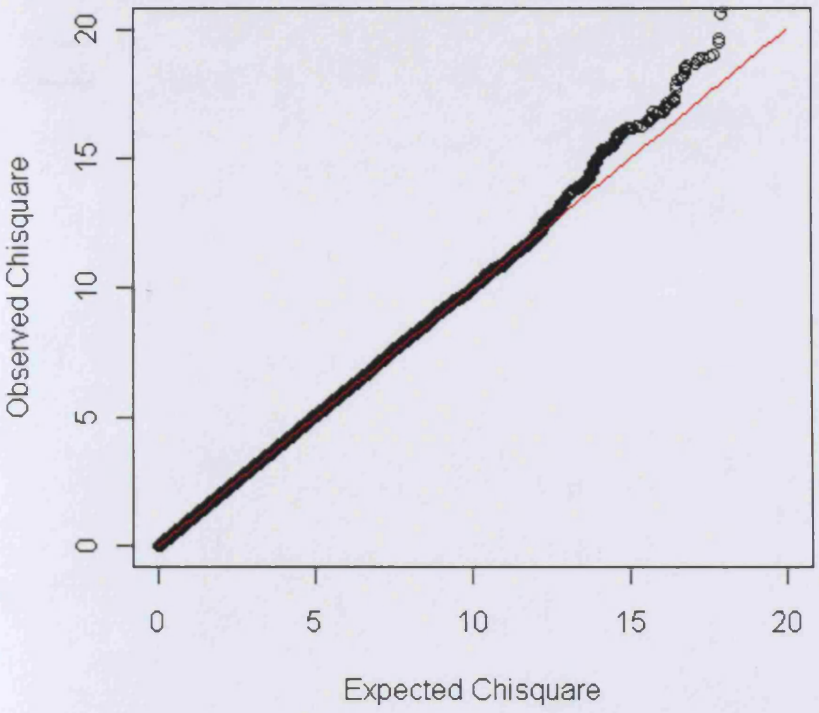


Figure 5.3a. LOAD+P vs. LOAD-P. Quantile-quantile (QQ) plot of 526,554 observed genome wide association χ^2 test statistics (y-axis) against those expected under the null expectation (x-axis). The line of equality is colored red. (λ = 1.002).

a)



b)

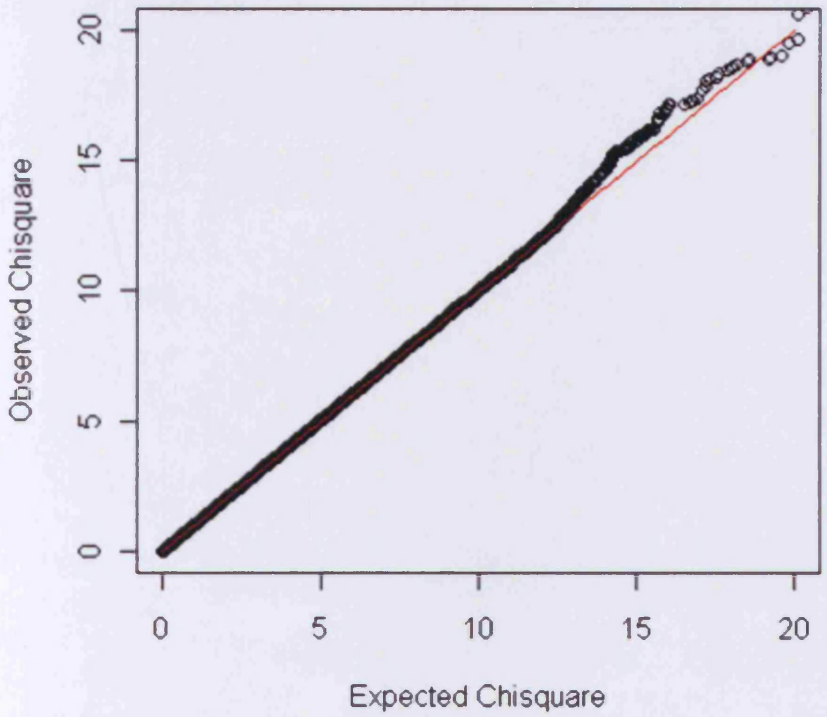


Figure 5.3b. LOAD+P vs. control. a) Quantile-quantile (QQ) plot of 526,554 observed genome wide association χ^2 test statistics (y-axis) against those expected under the null expectation (x-axis). The line of equality is colored red. ($\lambda = 1.006$). b) QQ plot after removal of 168 SNPs at the *APOE* locus. 526,386 SNPs remain ($\lambda = 1.006$).

Table 5.3b. Top 20 hits in the LOAD+P vs. LOAD-P analysis. Table shows SNP rank, chromosome, SNP ID, base position, gene name, SNP type, associated allele, p-value and OR in LOAD+P vs. LOAD-P and LOAD+P vs. control analysis.

Rank	Chr	SNP	BP	Gene	Type	Allele	*P*	OR	LOAD+Pvs. Control P	LOAD+Pvs. Control OR
1	8	rs2034140	6224172	MCPH1	INTERGENIC	G	7.62E-06	0.33	1.93E-04	0.46
2	6	rs11756091	39390784	KCNK16	NON-SYNONYMOUS	T	8.06E-06	1.53	2.76E-03	1.21
3	8	rs10956535	131623943	ASAP1	INTERGENIC	C	8.79E-06	1.54	5.71E-05	1.30
4	4	rs753129	56363188	AC110611.3-1	DOWNSTREAM	G	9.98E-06	1.62	2.04E-05	1.36
5	9	rs960644	105060805	RP11-341A22.1	INTERGENIC	G	1.04E-05	0.55	5.67E-04	0.70
6	12	rs2682778	117544535	SUDS3	INTERGENIC	T	1.45E-05	0.60	3.65E-02	0.84
7	10	rs1444400	52904161	PRKG1	INTRONIC	G	1.48E-05	1.58	7.23E-03	1.25
8	10	rs1444401	52904934	PRKG1	INTRONIC	A	1.50E-05	1.58	4.51E-03	1.21
9	10	rs6480276	52907458	PRKG1	INTRONIC	G	1.59E-05	1.57	6.09E-03	1.20
10	2	rs11680774	158954231	CCDC148	INTRONIC	C	2.00E-05	0.63	1.74E-02	0.83
11	2	rs2883854	158947298	CCDC148	INTRONIC	C	2.07E-05	0.64	1.29E-02	0.83
12	6	rs3734618	39392162	KCNK16	SYNONYMOUS	C	2.09E-05	1.50	3.36E-03	1.21
13	1	rs12045777	29301591	EPB41	INTRONIC	G	2.18E-05	1.52	1.12E-01	0.90
14	12	rs1520780	117524108	SUDS3	INTERGENIC	A	2.75E-05	0.62	4.57E-02	0.85
15	4	rs2695234	24413336	SOD3	DOWNSTREAM	T	3.39E-05	0.47	2.03E-03	0.65
16	4	rs12648438	188865648	AC115540.3	INTERGENIC	A	3.40E-05	1.66	2.57E-02	1.19
17	6	rs13201744	6071844	F13A1	INTERGENIC	T	4.31E-05	0.55	2.18E-02	0.77
18	4	rs2100889	88917369	IBSP	INTERGENIC	A	4.51E-05	0.66	4.68E-02	0.87
19	2	rs3771599	159105674	PK4P;PKP4	INTRONIC	G	4.88E-05	0.67	3.12E-01	0.92
20	2	rs11674284	159081135	PK4P;PKP4	INTRONIC	C	4.90E-05	0.65	1.04E-01	0.89

Table 5.3c. Top 20 hits in the LOAD+P vs. control analysis. Table shows SNP rank, chromosome, SNP ID, base position, gene name, SNP type, associated allele, p-value and OR in LOAD+P vs. control, LOAD+P vs. LOAD-P and LOAD analysis.

Rank	Chr	SNP	BP	Gene	Type	Allele	OR	*P*	LOAD GWAS *P*	LOAD GWAS OR	LOAD+Pvs. LOAD-P *P*	LOAD+Pvs. LOAD-P OR
1	19	rs2075650	50087459	TOMM40	INTRONIC	C	2.47	3.60E-34	1.8E-157	2.53	7E-01	1.04
2	19	rs157580	50087106	TOMM40	INTRONIC	G	0.62	1.96E-11	9.62E-54	0.63	3.6E-01	0.91
3	19	rs6859	50073874	PVRL2	3PRIME_UTR	T	1.54	3.54E-11	6.95E-41	1.46	8.3E-0.1	0.98
4	19	rs439401	50106291	APOE;PKP2	UPSTREAM	T	0.68	5.94E-08	2.65E-23	0.72	4.6E-01	1.16
5	19	rs8106922	50093506	TOMM40	INTRONIC	G	0.69	6.05E-08	5.37E-39	0.68	8.5E-01	0.98
6	15	rs8038077	58791939	RORA	INTRONIC	G	1.78	1.56E-07	2.1E-02	1.13	4.5E-03	1.63
7	19	rs405509	50100676	APOE;PKP2	UPSTREAM	A	1.37	1.20E-06	4.88E-37	0.7	5.6E-02	1.21
8	7	rs868055	129222427	MIRN183	INTERGENIC	T	1.62	1.80E-06	4.11E-04	1.21	2E-03	1.65
9	12	rs1906950	25408885	AC092451.12-2	INTERGENIC	A	1.35	4.88E-06	9.07E-04	1.1	9E-03	1.29
10	8	rs4527852	125542605	TRMT12	INTERGENIC	T	1.61	5.56E-06	1.85E-01	1.07	1E-02	1.5
11	2	rs1430170	133749869	AC010974.1	INTRONIC	G	0.71	9.29E-06	1.09E-02	0.92	3E-03	0.73
12	8	rs10094093	4493322	AC019176.4-2	INTERGENIC	T	0.68	9.93E-06	6.03E-02	0.94	2E-02	0.76
13	3	rs9289666	144727672	SLC9A9	INTRONIC	A	1.49	1.31E-05	2.48E-02	1.11	1.7E-03	1.57
14	19	rs10402948	490266	CDC34	INTRONIC	T	1.63	1.35E-05	1.49E-01	1.09	3.8E-02	1.42
15	2	rs4671328	58788786	IK	INTERGENIC	A	0.75	1.39E-05	4.18E-03	0.92	7E-02	0.84
16	7	rs4726443	140618341	AC005692.1	INTERGENIC	T	2.51	1.39E-05	1.68E-02	1.46	5E-02	1.6
17	8	rs16899656	125602072	TATDN1	INTRONIC	G	1.58	1.42E-05	6.99E-02	1.11	3E-02	1.42
18	16	rs11648322	10082553	GRIN2A	INTRONIC	T	1.33	1.56E-05	2.51E-02	1.07	1E-01	1.17
19	10	rs6482252	23397218	AL139815.12-3	INTERGENIC	G	1.33	1.58E-05	3.29E-03	1.09	6E-03	0.77
20	10	rs7902903	23398083	AL139815.12-3	INTERGENIC	C	1.33	1.69E-05	8.54E-03	1.08	3E-03	0.76

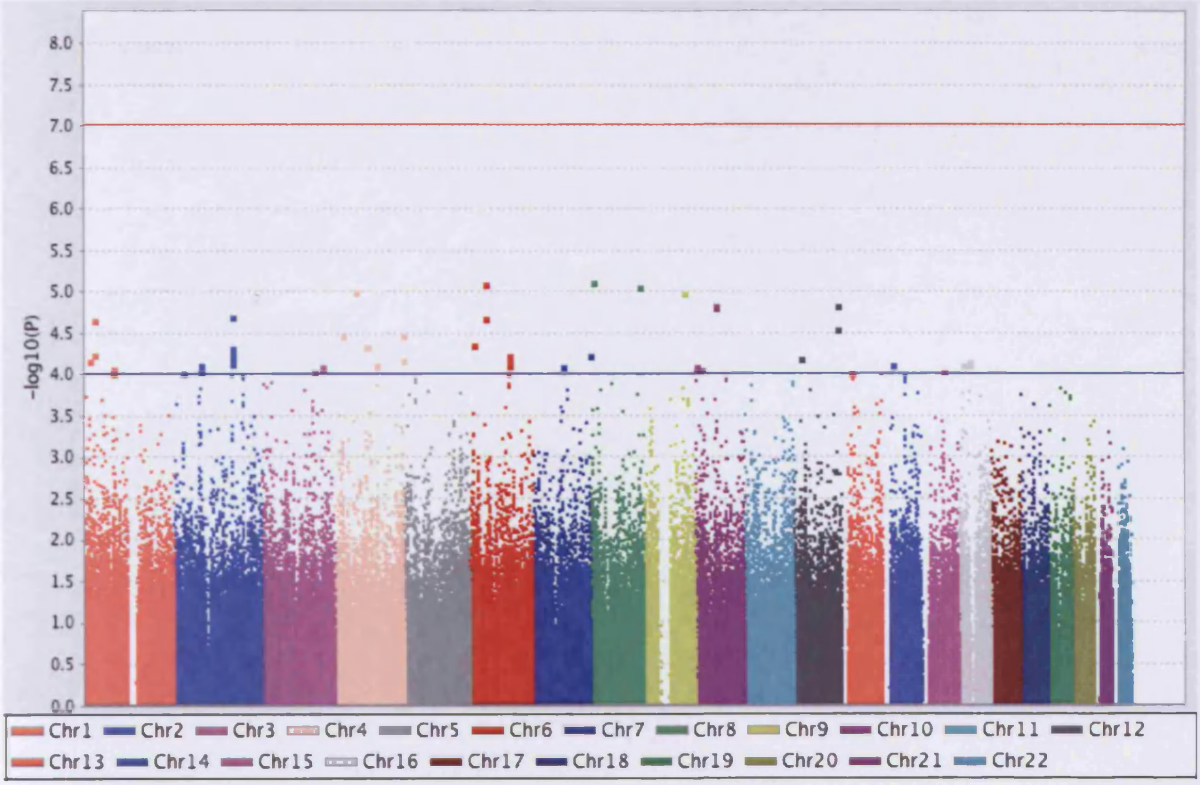


Figure 5.3c. Manhattan plot of chromosomal position (x-axis) against –log 10 GWAS p-value (y-axis) for LOAD+P vs. LOAD-P analysis. The threshold for genome-wide significance ($P \leq 9.5\text{x}10^{-8}$) is indicated by the red horizontal line. 55 SNPs with $P \leq 1\text{x}10^{-4}$ lie above the blue horizontal line and are listed in Appendix Table 5.3. Plot produced using HAPLOVIEW (v4.0) (http://www.broad.mit.edu/mpg/haploview/).

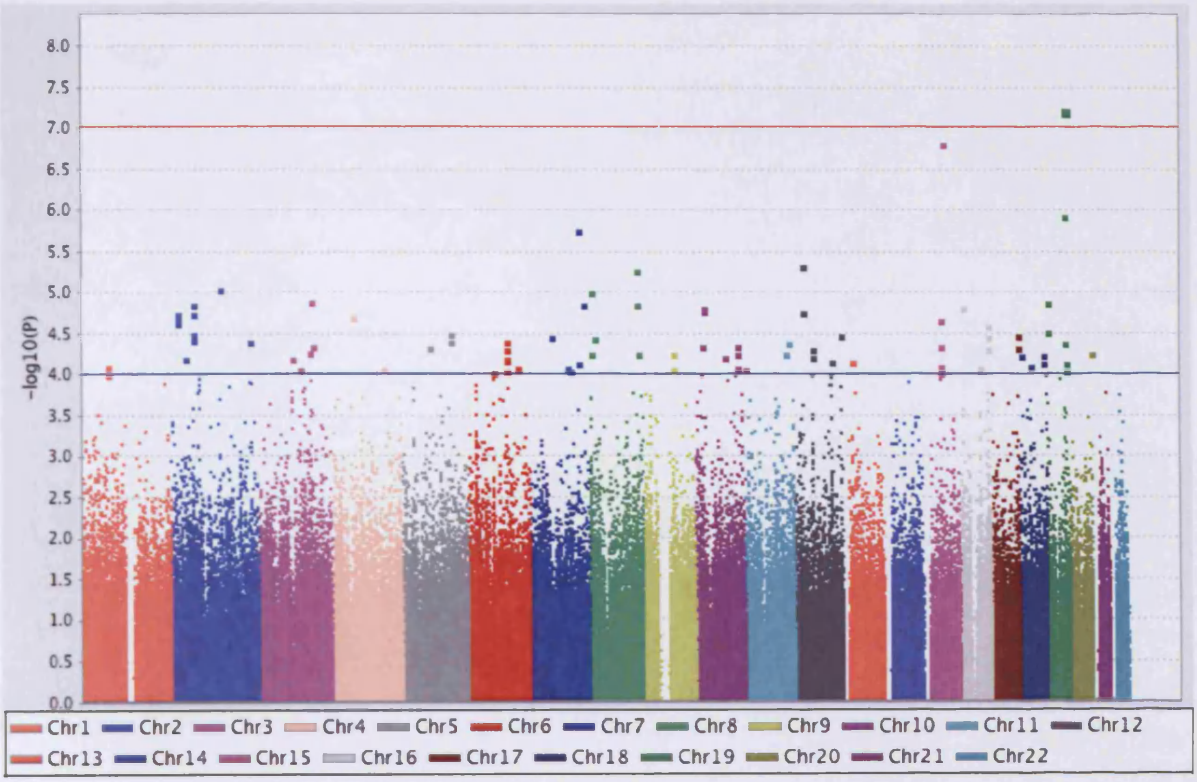


Figure 5.3d. Manhattan plot of chromosomal position (x-axis) against –log 10 GWAS p-value (y-axis) for LOAD+P vs. control. The y-axis scale has been limited to 8 ($P = 1.0 \times 10^{-8}$) although highly significant association was observed with SNPs in the vicinity of the *APOE* locus. The threshold for genome-wide significance ($P \leq 9.5 \times 10^{-8}$) is indicated by the red horizontal line. Ninety-one SNPs with $P \leq 1 \times 10^{-4}$ lie above the blue horizontal line and are listed in Appendix Table 5.4. Plot produced using HAPLOVIEW (v4.0) (http://www.broad.mit.edu/mpg/haploview/).

Genome wide significance (GWS) was observed with five SNPs when comparing LOAD+P cases with controls (p-values ranging from $6.05 \times 10^{-8} - 3.59 \times 10^{-34}$). These markers, and another showing a p-value close to the GWS threshold, were located in close proximity to the *APOE* locus. All of these markers showed similar patterns of effect and more compelling evidence of association in the main LOAD GWAS (p-values ranging from $2.65 \times 10^{-23} - 1.75 \times 10^{-157}$) (Harold et al. 2009). Furthermore none of these SNPs showed evidence for association with LOAD+P when compared to LOAD-P ($P > 0.163$), indicating that the *APOE* locus is associated with LOAD, unconditional of psychosis status. Excluding the *APOE* region, the most significant SNP (rs8038077) had a p-value of 1.56×10^{-7} (OR = 1.78) and was associated with LOAD+P when compared to controls. This marker rs8038077 is within an intron of the RAR-related orphan receptor A (*RORA*) gene on chromosome 15q. The same SNP showed some evidence for association in analysis of 'LOAD+P versus LOAD-P' ($P = 0.005$; OR= 1.63). Polymorphism rs17237486, located 200 kb distal to rs8038077 (LD; D' = 0.08, $r^2 = 0$) within the *RORA* gene, also showed association at $P = 9.03 \times 10^{-5}$ (OR= 1.63) in the LOAD+P vs. LOAD-P analysis. Other SNPs of interest highlighted by the LOAD+P vs. control analysis include rs1430170 ($P = 9.29 \times 10^{-6}$; OR = 0.71), an intronic SNP within the Nck associated protein 5 (*NAP5*) gene on chromosome 2 and polymorphism rs9289666 ($P = 9.29 \times 10^{-6}$; OR = 1.49) which resides within an intron of the solute carrier family 9 (*SLC9A9*) gene. The most significant SNP in LOAD+P versus LOAD-P analysis was rs2034140 located 27 kb 5 prime of the microcephalin (*MCPH1*) gene, $P = 7.02 \times 10^{-6}$; OR = 0.33. This SNP also showed evidence of association with LOAD+P when compared to controls, $P = 1.93 \times 10^{-4}$; OR = 0.45. A potential functional risk variant is marker rs11756091 ($P = 8.06 \times 10^{-6}$; OR = 1.53), a non-synonymous SNP within the potassium channel, subfamily k, member 16 (*KCNK16*) gene. The rs11756091 variant converts amino acid 301 from a proline to a histidine which may have an effect on structure and charge of the protein. This SNP is also in high linkage disequilibrium (LD) (D' = 1, $r^2 = 0.967$) with a synonymous variant rs3734618 predicted to be an exon splicing enhancer (Conde et al. 2006). The third most significant result is with marker rs10956535 ($P = 8.79 \times 10^{-6}$; OR = 1.54), 140 kb 5 prime of the development and differentiation enhancing factor (*ASAP1*). Despite none of these associations reaching genome-

wide significance, *KCNK16*, *MCPH1* and *RORA* are all significantly associated with LOAD+P at the gene-wide level (Table 5.3d).

Of the top 100 hits from this study, two SNPs showed overlap between LOAD+P, schizophrenia (O'Donovan et al. 2008) and bipolar disorder (WTCCC 2007) (Table 5.3e). Polymorphism rs17443484 (proxy rs17502679; D' = 1, r^2 = 1) is an intergenic marker on chromosome 3 which shows association at $P = 8.26 \times 10^{-5}$ (OR = 0.6) in the within case analysis. In the bipolar disorder and schizophrenia datasets the Affymetrix proxy for this SNP showed association, in the same direction, at $P = 2 \times 10^{-2}$ (OR = 1.16) and $P = 4 \times 10^{-3}$ (OR = 1.33), respectively. Variant rs11122300 (proxy rs11122275; D' = 1, r^2 =0.817) is an intronic SNP in the polypeptide N-acetylgalactosaminyltransferase 2 (*GALNT2*) gene on chromosome 1. This SNP shows association to LOAD+P as a subtype of disease $P = 1.3 \times 10^{-4}$ (OR = 1.34). The affymetrix proxy for this SNP showed association, in the same direction, at $P = 2.2 \times 10^{-3}$ (OR = 0.84) and $P = 9 \times 10^{-2}$ (OR = 0.85), for bipolar disorder and schizophrenia respectively.

In the main LOAD GWAS, SNPs at two novel loci showed genome-wide significant association to LOAD; rs11136000 in the *CLU* gene ($P = 1.4 \times 10^{-9}$) and rs3851179, a SNP 5' to the *PICALM* gene ($P = 1.9 \times 10^{-8}$) (Harold et al. 2009). In the LOAD+P vs. control subset of that data, representing 44.5% of the original sample, a similar pattern of effect ($P = 0.016$, OR = 0.85; $P = 0.005$, OR = 0.83, for rs11136000 and rs3851179, respectively) was noted. When comparing LOAD+P and LOAD-P cases neither rs1113600 nor rs3851179 showed evidence for association, $P = 0.74$ and $P = 0.94$, respectively.

Table 5.3d. Gene-wide analysis of the 6 genes showing most evidence for association to LOAD+P in either LOAD+P vs. LOAD-P or LOAD+P vs. LOAD-P analysis. Table shows gene name, gene coverage, number of SNPs genotyped, number of significant SNPs, number of independent SNPs, empirical p-value, and associated SNP for each analysis.

			LOAD+P vs. LOAD-P				LOAD+P vs. Control			
Gene	**Coverage**	**n SNP**	**n Sig**	**n Ind**	**Emp P**	**Ass. SNP**	**n Sig**	**n Ind**	**Emp P**	**Ass. SNP**
ASAP1	0.97	86	1	1	0.062	rs11777289	0	0	1	n/a
KCNK17	**0.69**	**23**	**1**	**1**	**0.0004**	**rs11756091**	**1**	**1**	**0.04**	**rs11756091**
MCPH1	**0.9**	**166**	**1**	**1**	**0.022**	**rs6559175**	0	0	1	n/a
NAP5	0.37	68	1	1	0.1	rs1430170	0	0	1	n/a
RORA	**0.71**	**318**	**1**	**1**	**0.02**	**rs17237486**	**1**	**1**	**0.0001**	**rs8038077**
SLC9A9	0.9	511	1	1	0.37	rs9289666	0	0	1	n/a

Table 5.3e. Results of the overlap analysis for the top 100 SNPs from both the LOAD+P vs. LOAD-P and LOAD+P vs. control analyses. Table shows SNP ID, chromosome, base position, Affymetrix proxy, proxy base position, r^2 and D' between original SNP and proxy, p-value and OR for LOAD+P vs. LOAD-P and LOAD+P vs. control analysis, and p-value and OR from the O'Donovan schizophrenia (O'Donovan et al. 2008) and WTCCC bipolar disorder (WTCCC 2007) studies.

	SNP	rs11122300	rs17443484
	Chr	1	3
	BP	228287788	163156164
	Proxy	rs11122275	rs17502679
	BP	226519268	163143417
	r^2	0.82	1
	D'	1	1
LOAD+P vs. LOAD-P	*P*	0.22	8.26×10^{-5}
	OR	1.15	0.6
LOAD+P vs. Control	*P*	1 x 10-4	0.28
	OR	1.34	0.9
schizophrenia	*P*	0.09	0.004
	OR	0.85	1.33
bipolar disorder	*P*	0.002	0.02
	OR	0.84	1.16

Section 2: Secondary Analyses

5.4. Introduction

It might be expected that disease modifying alleles, or alleles associated with an LOAD+P subtype may also contribute risk for idiopathic psychoses, such as schizophrenia. Variants Identified as novel LOAD+P susceptibility markers could be further explored across traditional psychiatric diagnostic boundaries, to determine if these causative pathways are common to a broad psychosis phenotype. Secondary analyses were undertaken using both LOAD+P GWAS datasets to determine whether there is an overlap between the identified susceptibility loci for LOAD+P, schizophrenia, and bipolar disorder. The first, a SNP based analysis, investigated (i) variants which have previously shown association to LOAD+P, (ii) interesting putative psychosis susceptibility variants investigated in chapter 3, (iii) variants investigated in chapter 4 from the O'Donovan et al. schizophrenia GWAS (O'Donovan et al. 2008), (iv) variants from the szgene database (Allen et al. 2008), (v) associated variants from a recently published schizophrenia meta-analysis (Stefansson et al. 2009), and (vi) associated variants from a bipolar disorder meta-analysis (Ferreira et al. 2008). The second analysis used PLINK (v1.05) (Purcell et al. 2007) to investigate candidate genes by a set based method. This analysis was used to determine if a SNP within a gene region (gene ± 20kb) is significantly associated with disease after correcting for the number of variants tested at that locus. This analysis was performed to investigate genes which have (i) previously shown published association to LOAD+P, (ii) the putative psychosis candidate genes investigated in chapter 3 (iii) oligodendrocyte/myelin related (OMR) genes known to interact with *OLIG2*, identified from the STRING database (v8.1) (http://string.embl.de/), (iv) genes identified by the O'Donovan schizophrenia GWAS investigated in chapter 4, (v) the genes identified as 'top results' by the szgene database (Allen et al. 2008), (vi) associated genes from a recently published schizophrenia meta-analysis (Stefansson et al. 2009), and (vii) associated genes from a bipolar disorder meta-analysis (Ferreira et al. 2008).

5.5 Materials and Methods

For the SNP based analysis, a list of 191 SNPs was compiled for investigation. The WGAViewer program (v1.25) (Ge et al. 2008) was used to search for the original SNP and proxies at $r^2 \geq 0.3$. The program identifies the r^2 between SNPs by accessing the HapMap database. Once the SNP of interest or a suitable proxy was identified, p-values and ORs were retrieved from the logistic regression file for each GWAS dataset. One hundred and forty six SNPs were successfully investigated. For the gene set based analysis PLINK (v1.05) (Purcell et al. 2007) was used to determine if a SNP within the gene region (gene ± 20kb) was significantly associated with disease after correcting for the number of variants tested at the locus. A list of 89 candidate genes was compiled and the WGAViewer program (Ge et al. 2008) used to identify genotyped SNPs within the locus of interest (the gene + 40 kb of flanking sequence). The SNP list was used in a set-based logistic regression analysis, using the 1 principal component (PC) included in the primary association analysis, for each of the two LOAD+P GWAS datasets. Parameters were set at maximum set = 1, and number of permutations = 10,000 (re-run at 1,000,000 for significant results). Gene coverage was determined using the phase 1 and 2 CEPH genotype data, for the gene locus of interest, from the HapMap database (The International HapMap Consortium 2007), this was loaded into the HAPLOVIEW (v4.0) program (Barrett et al. 2005), with SNPs genotyped at the locus force included into the tagger tab at MAF ≥ 0.05 and $r^2 \geq 0.8$.

5.6 Results

5.6.1 SNP Based Analyses

The SNP analysis was performed for 5 separate datasets. The first comprised 9 SNPs identified from the literature as previously associated with LOAD+P (Borroni et al. 2004; Borroni et al. 2006; Carson et al. 2008; Craig et al. 2004b; Go et al. 2005; Sweet et al. 1998; Wilkosz et al. 2007; Zdanys et al. 2007) and 4 SNPs identified as putative susceptibility variants in chapter 3. None of the tested SNPs showed an association to LOAD+P in either the 'disease modifier' or 'disease subtype' analysis (Table 5.6a), including the *OLIG2* variant rs762237.

The second analysis took the 126 SNPs which underwent meta-analysis to create the szgene 'top results' list. Eighty seven of these variants were successfully investigated, the remaining variants, or a suitable proxy, were not present on the Illumina 610-quad chip. Two SNPs showed association with LOAD+P under the 'disease subtype' model. Marker rs4733376 (P = 0.038; OR = 1.24) is located in intron 6 of the long transcript of neuregulin1. Marker rs1806201 (P = 0.046; OR = 1.15) is a synonymous variant in the glutamate receptor N-methyl-D-aspartate receptor subunit 2B (*GRIN2B*). Table 5.6b shows the 4 SNPs which show p-values < 0.1 in either the 'disease modifier' or 'disease subtype' analyses. No association survives a conservative Bonferroni correction for multiple testing. The full results are given in Appendix Table 5.6.

The third analysis investigated the markers tested in chapter 4. These variants were identified as being associated to schizophrenia in stage 1 of the recent O'Donovan GWAS (O'Donovan et al. 2008). One SNP, rs9870579, showed evidence for association at $P < 0.05$ in both the within case (P = 0.012; OR = 1.51) and case-control analysis (P = 0.03; OR = 1.23), see Table 5.6c. This marker showed evidence for association in the MRC genetic resource for LOAD sample tested in chapter 4 (allelic P = 0.02, OR = 1.61: allelic P = 0.004, OR = 1.48, in LOAD+P vs. LOAD-P and LOAD+P vs. control analysis, respectively). However, neither of the observed associations survived a conservative Bonferroni correction for multiple testing.

A recent meta-analysis of schizophrenia combined data from 1500 markers genotyped as part of three GWAS of schizophrenia (Purcell et al. 2009; Shi et al. 2009; Stefansson et al. 2009). Eighteen independent markers had p-values < 1×10^{-5} in the combined results and were followed up in additional samples (Stefansson et al. 2009). Analysis of these SNPs in the LOAD+P GWAS showed some evidence for association of two variants under the disease subtype model (P = 0.03; OR = 0.87 and P = 0.04; OR = 1.32, for rs1572299 and rs9960767, respectively), see Table 5.6d. Variant rs1572299 is an intergenic SNP on chromosome 9, while rs9960767 is an intronic SNP in the transcription factor 4 (*TCF4*) gene. The TCF4 protein is a basic helix-turn-helix transcription factor, defects in which are a cause of Pitt-Hopkins syndrome, symptoms of which include epilepsy. However, neither association would survive a Bonferroni correction for multiple testing.

Table 5.6a. SNP based analysis of previous LOAD+P associated variants and candidate SNPs from chapter 3. The first 4 markers were implicated in chapter 3, while the remaining 9 SNPs have previously shown association to LOAD+P. Table shows gene name, SNP ID, SNP proxy, relative D' and r^2 value, p-value and OR in within case, and case-control analysis

					LOAD+P vs. LOAD-P		LOAD+P vs. Control	
Gene	**SNP**	**Proxy**	**D'**	**r^2**	***P***	**OR**	***P***	**OR**
DTNBP1	rs12525702	n/a	n/a	n/a	0.74	1.05	0.86	0.98
GRM3	rs2228595	rs17608250	1	0.85	0.12	1.35	0.09	1.24
OLIG2	rs2834072	n/a	n/a	n/a	0.29	0.90	0.70	0.98
OLIG2	rs762237	rs2834077	1	1	0.16	0.87	0.86	0.99
APOE	rs7412	n/a	n/a	n/a	n/a	n/a	n/a	n/a
APOE	rs429358	n/a	n/a	n/a	n/a	n/a	n/a	n/a
CHRNA7	rs6494223	n/a	n/a	n/a	0.60	0.96	0.32	0.94
COMT	rs4680	n/a	n/a	n/a	0.93	0.99	0.38	1.06
DRD1	B2 (rs4532)	n/a	n/a	n/a	0.24	1.08	0.55	1.05
DRD3	*Bal1* (rs6280)	n/a	n/a	n/a	0.21	0.88	0.08	0.89
HTR2A	T102C (rs6313)	n/a	n/a	n/a	0.76	1.04	0.75	1.02
IL-1β	-511 (rs16944)	rs10169916	1	1	0.36	1.10	0.49	1.05
NRG1	rs392499	n/a	n/a	n/a	0.33	0.91	0.96	1.00

n/a = not applicable

Table 5.6b. Top 4 results of the SNP based analysis for the szgene markers. Table shows gene name, gene position in 'top result' table, SNP ID, relative D' and r^2 value, p-value and OR in within case analysis and case-control analysis, and szgene OR and 95% confidence interval.

						LOAD+P vs. LOAD-P		LOAD+P vs. Control		Szgene	
Gene	'top result'	SNP	Proxy	D'	r^2	*P*	OR	*P*	OR	OR	95% CI
NRG1	**19**	**rs4733376**	**rs3757934**	**1**	**1**	0.15	1.25	**0.038**	**1.24**	1.08	0.94<OR<1.24
GRIN2B	**7**	**rs1806201**	**n/a**	**n/a**	**n/a**	0.16	1.16	**0.046**	**1.15**	1.08	0.96<OR<1.22
TPH1	11	rs1800532	rs2670765	1	1	0.07	1.19	0.31	1.08	1.25	1.08<OR<1.44
1L1B	20	rs1143634	n/a	n/a	n/a	0.09	1.20	0.22	1.10	0.96	0.82<OR<1.11

Table 5.6c. Results of the SNP based analysis for the markers identified from the O'Donovan schizophrenia GWAS (O'Donovan et al. 2008). Table shows SNP ID, SNP proxy, relative D' and r^2 value, p-value and OR in within case, and case-control analysis, and published O'Donovan p-value and OR.

				LOAD+P vs. LOAD-P		LOAD+P vs. Control		O'Donovan et al. 2008	
SNP	Proxy	D'	r^2	*P*	OR	*P*	OR	*P*	OR
rs9870579	**rs9860322**	**1**	**1**	**0.012**	**1.51**	**0.03**	**1.23**	4.72×10^{-5}	0.56
rs12613195	rs7557843	1	1	0.11	0.85	0.12	0.88	2.1×10^{-5}	0.72
rs12629685	rs6809722	1	0.73	0.12	0.86	0.22	0.92	5.4×10^{-5}	1.33
rs7192086	rs7189560	1	0.96	0.16	0.87	0.91	1	1.15×10^{-6}	1.45
rs6490121	n/a	n/a	n/a	0.17	1.15	0.99	1	4.72×10^{-7}	1.43
rs1344706	rs1366840	1	0.69	0.23	1.12	0.91	1	1.47×10^{-6}	0.7
rs10103330	rs10503253	1	1	0.29	0.88	0.51	0.95	4.03×10^{-6}	1.47
rs2890738	n/a	n/a	n/a	0.36	0.91	0.95	1	1.73×10^{-8}	1.44
rs11162231	rs12082120	1	0.73	0.48	0.91	0.25	0.9	3.22×10^{-4}	0.71
rs3784397	rs1869901	1	0.94	0.49	0.94	0.1	0.89	2.1×10^{-5}	1.35
rs10869675	n/a	n/a	n/a	0.49	0.93	0.22	0.91	1.29×10^{-5}	1.38
rs16984718	rs1516922	1	0.66	0.53	1.16	0.75	1.06	5.94×10^{-4}	2.1
rs2210539	n/a	n/a	n/a	0.61	0.94	0.71	0.97	1.38×10^{-3}	0.73
rs1893146	rs10502389	1	0.78	0.7	1.06	0.53	1.07	6.37×10^{-7}	1.55
rs4238270	rs336230	1	0.74	0.71	0.96	0.99	1	2.67×10^{-5}	0.72
rs3016384	n/a	n/a	n/a	0.76	1.03	0.35	0.93	7.83×10^{-6}	0.73
rs9922369	rs2111119	1	0.25	0.78	0.96	0.85	0.98	2.59×10^{-7}	2.26
rs7546928	rs10889994	1	1	0.97	1	0.98	1	1.29×10^{-4}	0.76
rs1602565	rs7938219	1	1	0.99	1	0.45	1.09	9.04×10^{-6}	1.55
rs17101921	n/a	n/a	n/a	n/a	n/a	n/a	n/a	6×10^{-3}	1.73
rs10835482	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.83×10^{-5}	1.45

Table 5.6d. Results of the SNP based analysis for the markers identified from the Stefansson schizophrenia meta-analysis (Stefansson et al. 2009). Table shows SNP ID, p-value and OR in within case, and case-control analysis, and published Stefansson p-value and OR.

	LOAD+P vs. LOAD-P		LOAD+P vs. Control		Stefansson et al. 2009	
SNP	*P*	OR	*P*	OR	*P*	OR
rs1572299	0.1	0.86	**0.03**	**0.87**	5.2×10^{-6}	1.08
rs9960767	0.11	1.4	**0.04**	**1.32**	5.7×10^{-9}	1.23
rs7776351	0.92	1.01	0.11	1.12	1.3×10^{-6}	1.1
rs13219354	0.27	1.16	0.15	1.14	1.5×10^{-10}	1.2
rs12807809	0.09	1.24	0.18	1.12	2.3×10^{-9}	1.15
rs13211507	0.37	1.14	0.47	1.07	1.1×10^{-10}	1.24
rs3747600	0.11	0.85	0.53	0.96	1.1×10^{-4}	1.07
rs1487222	0.71	0.97	0.58	1.04	1.6×10^{-4}	1.07
rs1010471	0.06	0.84	0.6	0.97	1.9×10^{-5}	1.08
rs7289747	0.76	1.06	0.6	1.07	1.6×10^{-2}	1.09
rs6913660	0.46	1.09	0.61	1.04	2.5×10^{-9}	1.15
rs2312147	0.19	1.13	0.69	1.03	3.3×10^{-7}	1.09
rs6932590	0.8	1.03	0.76	1.02	5.9×10^{-12}	1.15
rs3131296	0.32	1.14	0.79	0.98	2.2×10^{-10}	1.19
rs1502844	0.84	0.98	0.83	0.99	1.2×10^{-6}	1.09
rs7863476	0.39	0.9	0.84	1.02	8.2×10^{-5}	1.09
rs10812518	0.57	0.94	0.84	1.02	1.9×10^{-4}	1.08
rs17594721	0.09	1.73	0.9	0.97	5.1×10^{-7}	1.28

A meta-analysis of three bipolar disorder GWAS (Ferreira et al. 2008; Sklar et al. 2008; WTCCC 2007), resulting in a sample of 4,387 cases and 6,209 controls, genotyped on 325,690 SNPs, identified 39 SNPs (21 independent) showing a $P < 10^{-6}$ (Ferreira et al. 2008). Investigation of the 21 independent SNPs in the LOAD+P GWAS datasets showed evidence for association of two variants (Table 5.6e). Marker rs2314398 (proxy rs6746896) showed evidence for association in the within case analysis at $P = 0.016$ (OR = 1.28), while marker rs12899449 (proxy rs12903120) showed evidence for association in both the within case and case-control analysis, $P = 0.025$ (OR = 0.8) and $P = 0.03$ (OR = 0.86), respectively. Both rs2314398 and rs12899449 are intergenic SNPs on chromosomes 2 and 15 respectively. Neither association survives Bonferroni correction for multiple testing.

5.6.2. Gene Based Analyses

Gene based analysis was performed for 9 different SNP sets. The majority of genes analysed by this methodology had good gene coverage. The exceptions are the *CNP* gene with 27% coverage, the *OLIG2* gene, which has previously shown association to LOAD+P, with 40% coverage, and the gene encoding the OLIG2 interactor NKX2-2 with 8% coverage. The coverage of the c16orf5, *HIST1H2BJ* and *HIST1H2BL* genes were not determined. The extensive size of these loci meant that the HAPLOVIEW program was unable to fully load the genotype data. The first SNP set investigated genes previously associated with LOAD+P (Borroni et al. 2004; Borroni et al. 2006; Carson et al. 2008; Craig et al. 2004b; Go et al. 2005; Sweet et al. 1998; Wilkosz et al. 2007; Zdanys et al. 2007), as well as genes implicated as possible LOAD+P susceptibility genes in chapter 3 of this thesis (Table 5.6f). As expected, the *APOE* locus showed association to LOAD+P when compared to controls, but this did not surpass the associations seen with LOAD alone. The *BDNF* gene is the only other gene to show gene-wide evidence for association to LOAD+P with an empirical p-value of 0.01 for marker rs2203877. Neither the *OLIG2* nor *OLIG1* loci show any evidence for association. However, the gene coverage of *OLIG2* is low at 40%.

Table 5.6e. Results of the SNP based analysis for the markers identified from the Ferreira bipolar disorder meta-analysis (Ferreira et al. 2008). Table shows SNP ID, SNP proxy, relative D' and r^2 value, p-value and OR in within case, and case-control analysis, and published Ferreira p-value and OR.

				LOAD+P vs. LOAD-P		LOAD+P vs. Control		Ferreira et al. 2008	
SNP	**Proxy**	**D'**	**r^2**	***P***	**OR**	***P***	**OR**	***P***	**OR**
rs2314398	**rs6746896**	**1**	**0.88**	**0.016**	**1.28**	0.52	1.05	2.8×10^{-6}	0.85
rs12899449	**rs12903120**	**1**	**0.95**	**0.025**	**0.80**	**0.03**	**0.86**	3.5×10^{-7}	0.84
rs216345	n/a	n/a	n/a	0.08	0.94	0.54	0.88	4.1×10^{-6}	1.15
rs7042161	n/a	n/a	n/a	0.11	0.85	0.31	0.93	5.8×10^{-6}	0.87
rs2278702	rs8041826	1	0.81	0.12	0.83	0.07	0.85	6.3×10^{-6}	0.83
rs4130590	n/a	n/a	n/a	0.13	0.87	0.09	0.90	3.1×10^{-6}	0.86
rs17082664	rs2092602	1	1	0.16	1.19	0.55	1.05	3.6×10^{-6}	1.22
rs11720452	rs13061121	0.93	0.86	0.17	0.88	0.64	0.96	5.3×10^{-6}	0.87
rs7226677	rs8096369	1	0.80	0.25	1.19	0.76	1.03	6.7×10^{-6}	1.24
rs12290811	rs1944449	1	1	0.27	1.15	0.38	1.08	3.6×10^{-6}	1.2
rs16966460	rs997406	1	0.93	0.38	0.88	0.57	0.94	3.7×10^{-6}	1.26
rs1948368	rs1777894	1	1	0.48	0.93	0.56	0.96	5.6×10^{-6}	0.87
rs8015959	rs7142052	1	1	0.50	1.21	0.24	1.31	4.7×10^{-6}	1.59
rs4380451	n/a	n/a	n/a	0.60	1.06	0.90	1.01	3.9×10^{-6}	0.85
rs544368	n/a	n/a	n/a	0.71	1.05	0.72	0.97	6×10^{-6}	1.22
rs1006737	rs10774035	1	1	0.71	1.04	0.66	0.97	7×10^{-8}	1.18
rs10994336	rs4948418	1	1	0.76	0.95	0.56	1.08	9.1×10^{-9}	1.45
rs3821396	n/a	n/a	n/a	n/a	n/a	n/a	n/a	4.6×10^{-6}	1.23
rs12436436	n/a	n/a	n/a	n/a	n/a	n/a	n/a	4.9×10^{-6}	1.3
rs1601875	n/a	n/a	n/a	n/a	n/a	n/a	n/a	6.9×10^{-6}	0.87
rs703965	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7.5×10^{-6}	0.87

Table 5.6f. Results of the gene based analysis for previously associated LOAD+P genes (top 6 genes) and the putative candidate genes investigated in chapter 3. Table shows gene name, gene coverage, number of genotyped SNPs, number of significant SNPs, number of independent SNPs, empirical p-value and associated SNP for both LOAD+P vs. LOAD-P and LOAD+P vs. control analysis.

			LOAD+P vs. LOAD-P				LOAD+P vs. Control			
Gene	**Coverage**	**n SNP**	**n Sig**	**n Ind**	**Emp P**	**Ass. SNP**	**n Sig**	**n Ind**	**Emp P**	**Ass. SNP**
APOE	**0.42**	**5**	**0**	**0**	**1**	**n/a**	**1**	**1**	**1 X 10^{-4}**	**rs2075650**
IL-1β	0.82	11	1	1	0.18	rs11680809	1	1	0.12	rs11680809
HTR2A	0.8	52	1	1	0.65	rs12584920	1	1	0.31	rs4942576
CHRNA7	0.81	28	1	1	0.38	rs11071515	0	0	1	n/a
DRD1	0.7	24	0	0	1	n/a	0	0	1	n/a
DRD3	0.94	289	1	1	0.77	rs1486009	0	0	1	n/a
BDNF	**0.73**	**10**	**1**	**1**	**0.01**	**rs2203877**	**0**	**0**	**1**	**n/a**
OLIG1	1	8	1	1	0.14	rs4817527	0	0	1	n/a
GRM3	0.85	37	1	1	0.31	rs10952890	0	0	1	n/a
DISC1	0.9	31	1	1	0.33	rs17804163	0	0	1	n/a
DAOA	0.71	58	1	1	0.46	rs1041247	0	0	1	n/a
DTNBP1	0.79	32	1	1	0.47	rs17391774	0	0	1	n/a
NRG1	0.9	284	1	1	0.83	rs12680514	0	0	1	n/a
GRIK2	0.89	161	0	0	1	n/a	1	1	0.81	rs1360790
OLIG2	0.4	13	0	0	1	n/a	0	0	1	n/a
CNP	0.27	4	0	0	1	n/a	0	0	1	n/a
COMT	0.8	27	0	0	1	n/a	0	0	1	n/a

The second gene based analysis investigated predicted OLIG2 protein functional partners; see Figure 5.6a, identified from the STRING - Known and Predicted Protein-Protein Interactions database (v8.1) (http://string.embl.de/). This approach was adopted as over 300 oligodendrocyte and myelination genes have been identified. Analysing all of these loci would be computationally challenging at this time. Nine genes were investigated in this analysis, none show evidence for association to LOAD+P in either the disease modifier or disease subtype model (Table 5.6g).

The third gene based analysis investigated the genes from the szgene 'top results' list (Table 5.6h). Twenty-seven genes were tested as 'top results' 5, 14 and 27 actually represent chromosomal regions 10q26.13, 11p14.1 and 16p13.12, respectively. These loci were not analysed as the specific region of interest could not be accurately determined. As expected, the *APOE* locus showed significant evidence for association in the case-control analysis (empirical $P = 1 \times 10^{-4}$). Two other genes, *TPH1* (tryptophan hydroxylase 1) and *OPCML* (opioid binding protein/cell adhesion molecule-like) show gene-wide evidence for association in the within case analysis (empirical $P = 8 \times 10^{-4}$ and 3×10^{-2}, respectively). The association with *TPH1*, but not *OPCML*, withstands Bonferroni correction for the 27 genes tested (corrected $P = 0.02$ and 0.81 respectively). The specific intronic *TPH1* variant (rs17794760) showing association to LOAD+P has not been tested for association with schizophrenia.

The 12 genes identified by stage 1 of the O'Donovan schizophrenia GWAS (O'Donovan et al. 2008) were investigated in the LOAD+P GWAS datasets. Three genes *CLSTN2* (calsyntenin 2), *CNTLN* (centlein, centrosomal protein) and *OPCML* (opioid binding protein/cell adhesion molecule-like) showed evidence for association (Table 5.6i). The *OPCML* gene has previously been discussed following the szgene analysis, Both *CLSTN2* and *CNTLN* show evidence at an empirical p-value of 0.02, *CLSTN2* in the case-control analysis and *CNTLN* in the within case analysis. Neither association survives Bonferroni correction for multiple testing (corrected $P = 0.22$).

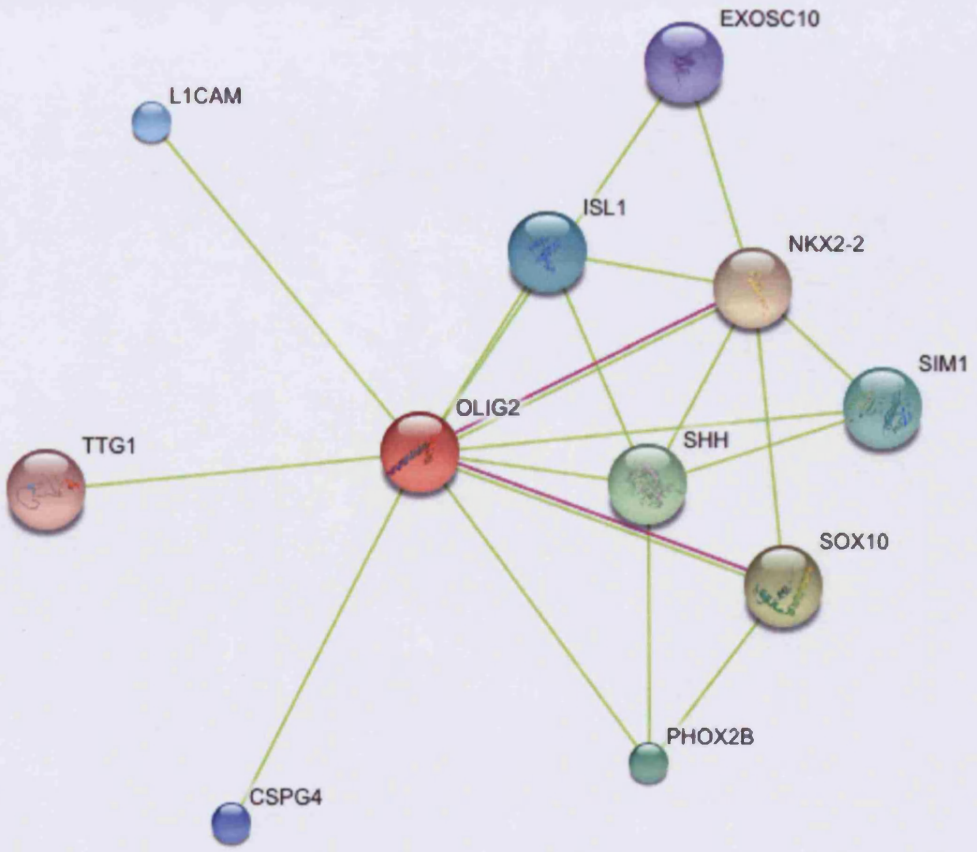


Figure 5.6a. Schematic of predicted OLIG2 functional partners. [Figure produced by STRING - Known and Predicted Protein-Protein Interactions database (v8.1) (http://string.embl.de/)].

Table 5.6g. Results of the gene based analysis for the genes predicted to be OLIG2 functional partners. Table shows gene name, gene coverage, number of genotyped SNPs, number of significant SNPs, number of independent SNPs, empirical p-value and associated SNP for both LOAD+P vs. LOAD-P and LOAD+P vs. control analysis.

			LOAD+P vs. LOAD-P				LOAD+P vs. Control			
Gene	Coverage	n SNP	n Sig	n Ind	Emp P	Ass. SNP	n Sig	n Ind	Emp P	Ass. SNP
C1ORF27	0.81	289	1	1	0.57	rs1474864	0	0	1	n/a
CSPG4	0.72	275	1	1	0.13	rs4886490	0	0	1	n/a
EXOSC10	0.82	10	0	0	1	n/a	0	0	1	n/a
ISL1	0.8	10	0	0	1	n/a	0	0	1	n/a
NKX2-2	0.08	102	1	1	0.56	rs10225087	0	0	1	n/a
PHOX2B	0.87	69	0	0	1	n/a	0	0	1	n/a
SHH	0.65	102	1	1	0.56	rs10225087	0	0	1	n/a
SIM1	0.94	50	1	1	0.44	rs1506084	0	0	1	n/a
SOX10	0.88	69	0	0	1	n/a	0	0	1	n/a

Table 5.6h. Results of the gene based analysis for the szgene 'top results'. Table shows gene name, gene coverage, number of genotyped SNPs, number of significant SNPs, number of independent SNPs, empirical p-value and associated SNP for both LOAD+P vs. LOAD-P and LOAD+P vs. control analysis. [NB: szgene 'top results' numbers 5, 14 and 27 are chromosomal regions].

				LOAD+P vs. LOAD-P				LOAD+P vs. Control			
'top result'	Gene	Coverage	n SNP	n Sig	n Ind	Emp P	Ass. SNP	n Sig	n Ind	Emp P	Assoc SNP
1	DISC1	0.9	31	1	1	0.33	rs17804163	0	0	1	n/a
2	SLC18A1	0.78	28	1	1	0.40	rs4921691	0	0	1	n/a
3	GABRB2	0.77	55	1	1	0.25	rs664806	1	1	0.64	rs9313879
4	DRD2	0.85	28	1	1	0.19	rs4938021	0	0	1	n/a
6	AKT1	0.66	4	0	0	1	n/a	0	0	1	n/a
7	GRIN2B	0.87	184	1	1	0.78	rs2300267	1	1	0.06	rs1861786
8	DGCR2	0.94	24	0	0	1	n/a	0	0	1	n/a
9	PLXNA2	0.8	12	0	0	1	n/a	0	0	1	n/a
10	RPGRIP1L	0.9	15	0	0	1	n/a	0	0	1	n/a
11	**TPH1**	**0.87**	**8**	**1**	**1**	**0.0008**	**rs17794760**	0	0	1	n/a
12	DRD4	0.53	6	0	0	1	n/a	0	0	1	n/a
13	DAOA	0.71	58	1	1	0.46	rs1041247	0	0	1	n/a
15	DRD1	0.7	24	0	0	1	n/a	0	0	1	n/a
16	HTR2A	0.8	52	1	1	0.65	rs12584920	1	1	0.31	rs4942576
17	RELN	0.87	189	1	1	0.55	rs4729939	1	1	0.54	rs7794418
18	**APOE**	**0.42**	**5**	0	0	1	n/a	**1**	**1**	**0.0001**	**rs2075650**
19	NRG1	0.9	284	1	1	0.83	rs12680514	0	0	1	n/a
20	IL1B	0.82	11	1	1	0.18	rs11680809	1	1	0.12	rs11680809
21	MTHFR	0.66	16	0	0	1	n/a	0	0	1	n/a
22	COMT	0.8	27	0	0	1	n/a	0	0	1	n/a
23	HP	0.78	10	0	0	1	n/a	1	1	0.27	rs1424241
24	DAO	0.79	15	1	1	0.23	rs10746134	0	0	1	n/a
25	TP53	0.68	8	0	0	1	n/a	0	0	1	n/a
26	ZNF804A	0.95	35	1	1	0.29	rs6745533	1	1	0.10	rs1427150
28	DTNBP1	0.79	32	1	1	0.47	rs17391774	0	0	1	n/a
29	**OPCML**	**0.88**	**419**	**1**	**1**	**0.03**	**rs750270**	1	1	0.13	rs10894589
30	RGS4	0.77	7	0	0	1	n/a	0	0	1	n/a

Table 5.6i. Results of the gene based analysis for the genes identified by the O'Donovan et al. GWAS of schizophrenia (O'Donovan et al. 2008). Table shows gene name, gene coverage, number of genotyped SNPs, number of significant SNPs, number of independent SNPs, empirical p-value and associated SNP for both LOAD+P vs. LOAD-P and LOAD+P vs. control analysis.

			LOAD+P vs. LOAD-P				LOAD+P vs. Control			
Gene	Coverage	n SNP	N Sig	n Ind	Emp P	Ass. SNP	n Sig	n Ind	Emp P	Ass. SNP
AGBL4	0.92	158	1	1	0.47	rs6701767	1	1	0.20	rs590503
CLSTN2	**0.87**	**191**	1	1	0.16	rs6806737	**1**	**1**	**0.02**	**rs6806737**
CNTLN	**0.98**	**85**	**1**	**1**	**0.02**	**rs3780256**	0	0	1	n/a
CSMD1	0.7	317	1	1	0.25	rs2627283	1	1	0.70	rs2627283
FGFR2	0.8	38	1	1	0.14	rs1649204	1	1	0.68	rs3135766
IRS2	0.87	15	0	0	1	n/a	0	0	1	n/a
NOS1	0.91	53	1	1	0.12	rs11068458	0	0	1	n/a
PCSK5	0.67	170	1	1	0.67	rs13288756	1	1	0.94	rs12000040
PLCB2	0.57	13	0	0	1	n/a	0	0	1	n/a
ST6GALNAC5	0.74	26	0	0	1	n/a	1	1	0.18	rs12124645
TRIM71	0.66	16	0	0	1	n/a	0	0	1	n/a
OPCML	**0.88**	**419**	**1**	**1**	**0.03**	**rs750270**	1	1	0.13	rs10894589

Fourteen genes were identified by the recent meta-analysis of 3 schizophrenia GWAS (Purcell et al. 2009; Shi et al. 2009; Stefansson et al. 2009), conducted by Stefansson and colleagues (Stefansson et al. 2009), as showing evidence for association at $P < 1 \times 10^{-5}$ (Stefansson et al. 2009). These were investigated in the LOAD+P GWAS datasets. Only neurogranin (*NRGN*) showed evidence for association (Table 5.6j), at an empirical p-value of 0.03 in the within case analysis. This association does not survive Bonferroni correction for multiple testing (corrected $P = 0.42$).

A meta-analysis of three bipolar disorder GWAS (Ferreira et al. 2008; Sklar et al. 2008; WTCCC 2007), identified 20 genes showing evidence for association to bipolar disorder at a $P < 10^{-6}$. These genes were investigated by set-based analysis using both the LOAD+P datasets. No gene showed any evidence for association to LOAD+P at a p-value < 0.05 in either the LOAD+P vs. LOAD-P or LOAD+P vs. control analysis (Table 5.6k).

Table 5.6j. Results of the gene based analysis for the genes identified by the Stefansson et al. GWAS of schizophrenia (Stefansson et al. 2009). Table shows gene name, gene coverage, number of genotyped SNPs, number of significant SNPs, number of independent SNPs, empirical p-value and associated SNP for both LOAD+P vs. LOAD-P and LOAD+P vs. control analysis.

			LOAD+P vs. LOAD-P				LOAD+P vs. Control			
Gene	Coverage	n SNP	n Sig	n Ind	Emp P	Ass. SNP	n Sig	n Ind	Emp P	Ass, SNP
c16orf5	n/a	4	0	0	1	n/a	0	0	1	n/a
FXR1	0.9	9	1	1	0.15	rs1805589	0	0	1	n/a
HIST1H2BJ	n/a	6	0	0	1	n/a	0	0	1	n/a
HIST1H2BL	n/a	8	0	0	1	n/a	0	0	1	n/a
IFT74	0.72	17	1	1	0.06	rs16910953	0	0	1	n/a
NOTCH4	0.91	78	1	1	0.75	rs3830041	1	1	0.45	rs204987
NRGN	**0.95**	**15**	**1**	**1**	**0.03**	**rs3830078**	1	1	0.08	rs1076095
PGBD1	0.82	12	0	0	1	n/a	1	1	0.27	rs1778507
PLAA	0.72	10	0	0	1	n/a	0	0	1	n/a
PRSS16	0.72	9	0	0	1	n/a	1	1	0.27	rs3800312
SLCO6A1	0.89	29	0	0	1	n/a	0	0	1	n/a
TCF4	0.88	74	1	1	0.21	rs1631486	1	1	0.19	rs17089789
TXNRD2	0.78	36	1	1	0.32	rs17210001	1	1	0.62	rs17210001
VRK2	0.93	21	0	0	1	n/a	0	0	1	n/a

Table 5.6k. Results of the gene based analysis for the genes identified by the Ferreira et al. GWAS of bipolar disorder (Ferreira et al. 2008). Table shows gene name, gene coverage, number of genotyped SNPs, number of significant SNPs, number of independent SNPs, empirical p-value and associated SNP for both LOAD+P vs. LOAD-P and LOAD+P vs. control analysis.

			LOAD+P vs. LOAD-P				LOAD+P vs. Control			
Gene	Coverage	n SNP	n Sig	n Ind	Emp P	Ass. SNP	n Sig	n Ind	Emp P	Ass. SNP
ANK3	0.87	187	1	1	0.10	rs1471247	1	1	0.07	rs1890951
ARNT2	0.79	370	1	1	0.32	rs16972744	0	0	1	n/a
C14ORF165	0.71	235	1	1	0.17	rs12589666	0	0	1	n/a
C15ORF53	0.69	471	1	1	0.82	rs2643208	0	0	1	n/a
CACNA1C	0.9	190	1	1	0.17	rs4765687	1	1	0.97	rs10774043
CMTM8	0.72	77	1	1	0.48	rs2053280	0	0	1	n/a
GARNL3	0.81	176	1	1	0.34	rs4350073	0	0	1	n/a
LMAN2L	1	43	1	1	0.05	rs968470	0	0	1	n/a
LRRN1	0.77	27	0	0	1	n/a	0	0	1	n/a
NPAS3	0.82	588	1	1	0.39	rs1958018	0	0	1	n/a
ODZ4	0.87	347	1	1	0.43	rs565758	0	0	1	n/a
PRSS3	0.48	14	0	0	1	n/a	0	0	1	n/a
S1PR1	0.52	77	1	1	0.76	rs6577244	0	0	1	n/a
SIAE	0.69	450	1	1	0.47	rs3830078	0	0	1	n/a
SPRED1	0.89	500	1	1	0.08	rs954432	0	0	1	n/a
SVEP1	0.84	324	1	1	0.56	rs10980573	0	0	1	n/a
SYNE1	0.85	175	1	1	0.84	rs214989	0	0	1	n/a
THOC1	0.88	23	0	0	1	n/a	0	0	1	n/a
ZMIZ1	0.67	117	1	1	0.46	rs3740259	0	0	1	n/a
ZNF385D	0.89	183	1	1	0.51	rs13340131	0	0	1	n/a

5.7. Discussion

This is the first LOAD GWAS to condition on the presence or absence of psychotic symptoms. The study draws upon 1,671 LOAD cases from the UK with comprehensive data on psychotic symptoms and 5,167 controls (before quality control). The 'disease modifier' hypothesis has been tested by comparison of LOAD cases with and without psychotic symptoms. A 'disease subtype' model has also been tested by comparison of LOAD+P cases to a large control population. Aside from the *APOE* locus, genome wide significant evidence of association with LOAD+P, when compared to healthy controls or LOAD cases without psychotic symptoms, was not observed. However, large odds ratios (1.5-1.6) were seen for numerous loci, particularly in the disease modifier analysis, indicating a large effect of these loci in the modification of psychosis in LOAD if the loci prove to be true susceptibility loci. The power of the present study to detect loci with effect sizes of the magnitude commonly observed in complex traits at a significance level of 0.05 is modest (e.g. 0.47 for LOAD+P vs. LOAD-P and 0.78 for LOAD+P vs. control for a risk allele with a frequency of 0.3 and an odds ratio of 1.3). For rarer variants (MAF < 5%), the power to detect true effects reduces considerably to less than 1%. In this study, at a genome-wide significance level of 9.5×10^{-8}, the power to detect common variants decreases considerably to < 0.001 and 0.007 under the disease modifier and disease subtype model, respectively. Despite this many SNPs which, while failing to reach the stringent genome-wide level of statistical significance, were identified and remain interesting as they could reflect true disease loci. Aside from SNPs at the *APOE* locus, the most significant association was observed at the RAR-related orphan receptor *A (RORA)* gene, which showed evidence of association in disease subtype and disease modifier analyses. The protein encoded by the *RORA* gene is a member of the NR1 subfamily of nuclear hormone receptors. It can bind to hormone response elements upstream of several genes to enhance the expression of those genes. The specific functions of this protein are unknown, but it has been shown to interact with proteins involved in organogenesis and differentiation. *RORA* has been highlighted as a candidate gene for bipolar disorder through a genetic and functional genomics analysis ($P = 1.9 \times 10^{-4}$) (Le-

Niculescu et al. 2008). In the disease modifier analysis SNP rs2034130 close to the microcephalin (*MCPH1*) gene showed the most evidence of association. *MCPH1* has been implicated in chromosome condensation and DNA damage induced cellular responses. It is thought to play a role in neurogenesis and regulation of the size of the cerebral cortex. Variant rs2034140 does not lie in a conserved region, and is not predicted to lie within a transcription factor binding site (TFBS). However, duplication of a region of chromosome 8, which includes the *MCPH1* gene, has been associated with autism (Glancy et al. 2009). Perhaps of more interest is marker rs11756091 ($P = 8.06 \times 10^{-6}$; OR = 1.53), a non-synonymous variant within the potassium channel, subfamily k, member 16 (*KCNK16*) gene on chromosome 6. The rs11756091 variant converts amino acid 301 from a proline to a histidine which may have an effect on structure and charge of the protein. This SNP is also in high linkage disequilibrium (LD) (D' = 1, $r^2 = 0.967$) with a synonymous variant rs3734618 predicted to be an exon splicing enhancer. Ion channel dysfunctions are well-recognized causes of episodic central nervous system disease including epilepsies, ataxias and migraine (Gargus 2006); a recent meta-analysis suggests they may also be involved in the pathogenesis of bipolar disorder (Ferreira et al. 2008). Other SNPs of interest highlighted by the LOAD+P versus control analysis include rs1430170 ($P = 9.29 \times 10^{-6}$; OR = 0.71). This is an intronic SNP within the Nck associated protein 5 (*NAP5*) gene on chromosome 2. The *NAP5* gene is expressed in adult brain and has recently shown highly suggestive association ($P=9.8 \times 10^{-6}$) to bipolar disorder in a European American GWAS (Smith et al. 2009). Polymorphism rs9289666 ($P = 9.29 \times 10^{-6}$; OR = 1.49) is also of interest as it resides within an intron of the solute carrier family 9 (*SLC9A9*) gene, which is a sodium/hydrogen exchanger expressed in the brain. Multiple studies have shown association of this gene to attention deficit disorder (ADHD) (de Silva et al. 2003; Lasky-Su et al. 2008). Also of relevance within the top 100 hits are SNPs within the opioid-binding protein/cell adhesion molecule-like (*OPCML*) gene and the tryptophan hydroxylase1 (*TPH1*) gene which are both well regarded candidate genes for susceptibility to schizophrenia. Also, the calsyntenin 2 (*CLSTN2*), which has shown some evidence for association to schizophrenia in a UK GWAS (O'Donovan et al. 2008), and the glutamate receptor, ionotropic, kainate 2 (*GRIK2*) gene, which is a strong candidate gene for susceptibility to psychosis.

The *GRIK2* gene is located on chromosome 6q16.3 in a region which shows significant linkage to schizophrenia (Cao et al. 1997; Levinson et al. 2000; Martinez et al. 1999) and bipolar disorder (Dick et al. 2003; Lambert et al. 2005; Pato et al. 2004), and was identified by two AD+P linkage screens (Bacanu et al. 2002; Hollingworth et al. 2007). Excluding SNPs at the *APOE* locus, none of the top 20 SNPs implicated in the LOAD+P versus LOAD-P or LOAD+P versus control analyses have been previously associated with LOAD, or LOAD+P. Two SNPs showed overlap between the LOAD+P GWAS and previous schizophrenia (O'Donovan et al. 2008) and bipolar disorder (WTCCC 2007) GWAS. Polymorphism rs17443484 is an intergenic marker on chromosome 3. Variant rs11122300 is an intronic SNP in the polypeptide N-acetylgalactosaminyltransferase 2 (*GALNT2*) gene on chromosome 1. The protein encoded by the *GALNT2* gene functions in the first step of oligosaccharide biosynthesis. While the identification of SNP overlap in LOAD+P, schizophrenia and bipolar disorder is interesting, the evidence for association of these variants in schizophrenia and bipolar disorder is not striking. Further work is required to determine is this degree of overlap could be expected by chance.

Two forms of secondary analysis were undertaken; the first was a SNP based analysis investigating SNPs of interest or suitable proxies for these in both the LOAD+P GWAS datasets. The second analysis was a gene based/SNP set analysis investigating gene regions of interest. The SNP based analysis identified 7 SNPs of 146 which show evidence for association with LOAD+P; an intronic variant in the neuregulin1 (*NRG1*) gene, a synonymous SNP in the glutamate receptor N-methyl-D-aspartate receptor subunit 2B (*GRIN2B*), an intergenic SNP on chromosome 9, an intronic SNP in the putative schizophrenia susceptibility gene transcription factor 4 (*TCF4*), intergenic SNPs on chromosomes 2 and 15 and rs9870579 an intronic SNP within the *CLSTN2* gene. No marker survived Bonferroni correction for multiple testing. Marker rs9870579 has shown evidence for association to LOAD+P in the MRC genetic resource for LOAD sample. This GWAS sample includes a large proportion of the MRC genetic resource for LOAD sample. Therefore it is not surprising that rs9870579 shows some evidence for association in this study. However, neither association survived Bonferroni correction for multiple testing. The *OLIG2* SNP rs762237 which has previously

shown association to LOAD+P was tested in this analysis. No evidence for association was found, suggesting that the original finding may be a false positive, or that the relaxed phenotype definition utilised in this study maybe too broad to identify an association at the *OLIG2* locus. The set based analysis identified 6 genes which show evidence for association with LOAD+P; brain-derived neurotrophic factor (*BDNF*), tryptophan hydroxylase 1 (*TPH1*), opioid binding protein/cell adhesion molecule-like (*OPCML*), calsyntenin 2 (*CLSTN2*), centlein, centrosomal protein (*CNTLN*) and neurogranin (*NRGN*). Only the association with *TPH1* withstands Bonferroni correction for multiple testing. *TPH1* encodes an aromatic amino acid hydroxylase which catalyzes the first and rate limiting step in the biosynthesis of serotonin, an important hormone and neurotransmitter. Mutations in this gene have been associated with an elevated risk for a variety of diseases and disorders, including schizophrenia and bipolar disorder. *TPH1* is associated with LOAD+P in the within case analysis, suggesting that this gene acts as a psychosis modifier rather than as a susceptibility gene for LOAD+P as a subtype of AD. The *OLIG2* gene shows no evidence for association to LOAD+P via set based analysis. However, the gene coverage is poor at only 40%.

The future prospects for this dataset are expansive. Imputation may highlight additional 'top hits' (Hao et al. 2009) and gene ontology (GO) analysis may identify interesting gene networks which should be prioritised for investigation (Harris et al. 2004). Independent replication of the identified loci in well characterised LOAD samples is essential. Fine mapping of the genes of interest may then be undertaken in an attempt to identify functional variants. Gene screening and sequencing in a limited number of LOAD cases may identify novel common variants, while novel rare variants maybe identified by deep sequencing of the loci of interest (Mardis 2008). As identified variants may not affect protein structure, allelic expression assays may be used to decipher if *cis*-acting variants are present at a specific locus (Bray et al. 2003b). The Illumina HumanHap beadchips have proven success in copy number variation (CNV) identification (Lin et al. 2009), making this analysis a must in our dataset.

In conclusion, this study does not identify any SNPs which meet strict criteria for genome wide significant association with LOAD+P, when compared to controls or LOAD cases without psychotic symptoms. However a number of sub

threshold associations were observed that a) show patterns of effect that are stronger than those generally observed in LOAD GWAS studies. b) are interesting biological and positional candidates for LOAD+P and c) show some overlap with others psychiatric disorders with psychotic features. It is interesting that some overlap between schizophrenia, bipolar disorder and psychosis in LOAD was observed. There is increased evidence that there is genetic overlap between the major psychiatric disorders (Lichtenstein et al. 2009; Purcell et al. 2009). It is plausible that this overlap will extend to other disease phenotypes with prominent psychotic symptoms. However, the specific SNPs associated with disease in the LOAD+P analyses differ from those associated with schizophrenia and bipolar disorder. The association of varying genetic polymorphisms within the same gene to overlapping phenotypes is not unusual in complex genetics (O'Donovan et al. 2009), and perhaps, implies that SNPs associated with schizophrenia and bipolar disorder are not associated with LOAD+P. Alternatively the LOAD+P sample is not adequately powered to detect these associations. Numerous genes have shown association to more than one disease/disorder. For example, the Lamin A/C (*LMNA*) gene, which encodes the nuclear envelope proteins lamin A and lamin C, has been found to be the causative gene for Autosomal dominant Emery–Dreifuss muscular dystrophy (EDMD-AD), in addition to a further eight clearly defined phenotypes via differing genetic variations (Rankin and Ellard 2006). A number of the loci identified by this study are good functional candidate genes for psychosis and warrant further investigation in larger, appropriately powered samples in which the presence of psychotic symptoms in LOAD is well characterised. For example, the RAR-related orphan receptor A *(RORA)* gene which is involved in the enhancement of gene expression, the potassium channel, subfamily k, member 16 (*KCNK16*) gene and the tryptophan hydroxylase 1 (*TPH1*) gene involved in the biosynthesis of serotonin.

6. Final Discussion

The aim of the research presented within this thesis was to detect a novel locus or loci conferring susceptibility to the psychosis observed among LOAD sufferers. LOAD is a clinically heterogeneous illness and increasing attention is now being paid to utilizing defined subgroups in the hope of unpicking the complex aetiology of the illness (Olson et al. 2001; Pericak-Vance et al. 2000; Sweet et al. 2003). Currently little is known about the underlying causes of the clinical differences observed in LOAD. Gaining a more comprehensive understanding of LOAD+P may aid both the study of this form of clinical heterogeneity and LOAD as a whole, providing a strong platform for the development of future preventative and therapeutic strategies.

This thesis is based on the evidence that psychosis in AD is a distinct form of AD with heritability estimates of 30-61% (Bacanu et al. 2005). The phenotype shows evidence of linkage to specific chromosomal regions (Avramopoulos et al. 2005; Bacanu et al. 2002; Hollingworth et al. 2007) and evidence for association with specific genetic variants (Borroni et al. 2004; Borroni et al. 2006; Borroni et al. 2007; Go et al. 2005; Holmes et al. 2001; Sweet et al. 1998; Sweet et al. 2005. Thus, psychosis in AD is an excellent candidate for gene mapping efforts. The published literature highlights an overlap in linkage regions and associated loci of neurodevelopmental psychoses and AD+P (Bacanu et al. 2002; Cao et al. 1997; Dick et al. 2003; Hollingworth et al. 2007; Lambert et al. 2005; Levinson et al. 2000; Martinez et al. 1999; Pato et al. 2004), supporting the theory that psychosis modifier genes act across neurodevelopmental and neurodegenerative disease. Under such a 'disease modifier' model, genetic variation would not increase the risk of developing AD, but could influence the presentation of psychosis in the presence of neurodegeneration. Alternatively, psychosis could identify a distinct 'sub-phenotype', or more homogeneous form of the disease. Under such a model, genetic variation would increase disease risk but only within those sufferers experiencing AD with psychosis. In this thesis both theories as to the development of psychosis in LOAD were explored.

6.1. Thesis Findings

Initial work focused on exploration of the current literature to determine criteria to classify psychosis presence or absence in LOAD. The Neuropsychiatric Inventory (NPI) (Cummings 1997) was used to assess the prevalence and severity of behavioural and psychological symptoms in all participants. Samples were classified based on the scores of the NPI, rated to reflect the worst episode of each symptom over the lifetime of the illness. Individuals classified as having late-onset Alzheimer's disease with psychosis (LOAD+P) had to meet a frequency threshold for presence of symptom(s) ≥ 2, this minimum criterion aimed to exclude those patients experiencing very few psychotic episodes which maybe attributable to another cause. For individuals to be classified as LOAD+P they also had to experience both hallucinations and delusions or experience either hallucinations or delusions with a NPI domain score > 3. These criteria were based on similar classification definitions of AD+P from previous studies being implicated in AD (Cummings et al. 2005). Of the 1,205 MRC genetic resource for LOAD patients, 379 individuals were categorised as LOAD+P (78% females, mean AAO = 76.07 years). LOAD cases who had not displayed any hallucinations or delusions were coded as LOAD-P. As prevalence and severity of psychosis in LOAD has been shown to increase with advancing disease progression (Paulsen et al. 2000b; Ropacki and Jeste 2005), individuals with mild-moderate LOAD (GDS < 5) were considered at unknown risk of developing psychosis and excluded from the LOAD-P subgroup. Two hundred and sixty nine individuals were categorised as LOAD-P (70% females, mean AAO = 75.97 years). These strict classification criteria were employed to isolate a more homogenous phenotype suitable for genetic exploration studies. One thousand three hundred and five elderly screened controls were also available for genotyping as part of this study.

A 2 stage approach to identifying genes implicated in the aetiology of LOAD+P was undertaken. In chapter 3 and 4 of this thesis specific genes and genetic polymorphisms which had previously shown evidence of association to neurodevelopmental psychoses, specifically schizophrenia, were tested for association to LOAD+P. In chapter three 73 variants from 11 psychosis candidate genes were investigated. The aim of this study was to elucidate if any 'historic'

putative psychosis candidate gene or variant contributes to the aetiology of LOAD+P. Six of the genes were investigated via a tagging approach to elicit if any variation within the locus was directly or indirectly associated with disease. Specific putative susceptibility variants at the remaining 5 loci were directly investigated for association with disease. Four polymorphisms at the *OLIG2* locus showed significant association with psychosis in LOAD under a disease modifier model. The strongest evidence for association was with marker rs762237 (P = 0.002, OR = 1.42), an observation that remains significant after correction for multiple testing (P = 0.019). This variant is located 6.7 kb from the 3' end of *OLIG2*. Polymorphism rs2834072 (P = 0.004, OR = 1.41) also shows significant evidence for association with LOAD+P and withstands correction for multiple testing (P = 0.05). This variant is located 8.9 kb 3' of the *OLIG2* gene. Markers rs13046814 (P = 0.03, OR = 1.30) and F16GA (P = 0.04, OR = 1.41) show nominal association with LOAD+P, but these do not withstand correction for multiple testing (P = 0.3 and P = 0.39 respectively). Variant rs13046814 is located within the 3' UTR of *OLIG2* and polymorphism F16GA is located 1 kb 3' of the gene. None of the associated variants have an obvious functional relevance and no marker in LD with an associated variant resides in a promoter region, a region of high conservation (>80%) or a predicted transcription factor binding site. The markers which associate with LOAD+P differ to those associated with schizophrenia (Georgieva et al. 2006). Implying that genetic variation in *OLIG2* modifies psychosis in LOAD by a varying mechanism to that seen in schizophrenia, and suggests a possible role for white matter abnormalities in the aetiology of a psychosis subtype of LOAD.

Numerous genes show association to more than one disorder. For example, the Tripartite motif-containing 32 *(TRIM32)* gene on chromosome 9q33.1 is hypothesised to be an E3-ubiquitin-ligase gene (Frosk et al. 2002). The single *TRIM32* missense variant D487N is associated with autosomal recessive Limb-girdle muscular dystrophy (LGMD) (Cossée et al. 2009), while the conserved homozygous missense mutation P130S is associated with Bardet–Biedl syndrome (BBS) (Chiang et al. 2006). The differing aetiology of psychosis in neurodevelopmental and neurodegenerative disease may explain the differing presentation of psychosis in these disorders. Psychosis in AD develops within a few years of dementia onset and usually persists only a matter of months before ceasing (Chen et al. 1991; Paulsen et al. 2000b; Ropacki and Jeste 2005).

Psychotic symptoms in neurodevelopmental conditions have a common clinical presentation. Hallucinations are the most common psychotic symptom which are predominantly auditory in nature (Böcker et al. 2000). Delusions of persecution, grandiosity and paranoid or bizarre delusions are common (Corner 2004; Tasman 1997). Despite the differing presentation, the core symptoms of psychosis, hallucinations and delusions are common to all phenotypes implicating the same loci in psychosis presentation.

In chapter four 21 'novel' variants identified by a recent genome-wide association study of schizophrenia (O'Donovan et al. 2008) were investigated for association with LOAD+P. No marker showed a significant association, in either disease modifier or disease subtype analysis, which survived correction for multiple testing. The most significant association was seen with variant rs9870579 in the LOAD+P versus control analysis (allelic $P = 0.004$; OR = 1.48). Marker rs9870579 is located in intron 1 of the calsyntenin 2 gene. Calsyntenin 2 (*CLSTN2*) is located on chromosome 3q23 and is expressed exclusively in brain. The calsyntenin family of proteins are predominantly postsynaptic membrane proteins of excitatory CNS synapses with putative calcium binding capacity (Hintsch et al. 2002). However, the highest levels of CLSTN2 protein are found in GABAergic neurons (Hintsch et al. 2002). Disrupted GABAergic signalling has been implicated in numerous and varied neurological and psychiatric pathologies including schizophrenia. The 3q23 region has previously shown linkage to AD and the *CLSTN2* gene has shown significant association to cognitive function (Liu et al. 2007). Thus, *CLSTN2* can be considered a putative functional and positional candidate gene for psychosis in LOAD. Marker rs9870579 does not appear to have a functional role. However, it is in high LD with polymorphism rs11927384, which does lie in a conserved region. Conservation suggests that this variant or the region in which it lies may have a functional role. For example, the conserved region may act as a binding site for transcription factors involved in regulation of gene expression. In the schizophrenia discovery sample rs9870579 had a significant p-value of 4.72×10^{-5} (O'Donovan et al. 2008). This association was the result of an over-representation of the major T allele. However, the associated allele in LOAD+P is the minor C allele. Therefore, the association seen with psychosis in LOAD is in the opposite direction to that seen in schizophrenia. A possible explanation for this finding could be that rs9870579 (or a variant in LD with rs9870579) influences susceptibility to psychosis

in neurodevelopmental and neurodegenerative disease by varying mechanisms. Alternatively, at least one of the observed rs9870579 associations could be a false positive finding.

In chapter 5 a hypothesis free analysis of genome-wide association data for LOAD conditioning on the presence or absence of psychosis was described. The analysis studied 526,554 markers in 645 LOAD+P cases, 505 LOAD-P cases and 5167 controls, including 1,135 elderly controls matched for age, sex and ethnicity. No genome-wide significant evidence for association was observed when LOAD+P cases where compared to LOAD-P. In the LOAD+P versus control analysis genome-wide significance was observed but only with the *APOE* locus which shows a greater association to LOAD alone. Large odds ratios (1.5-1.6) were seen for numerous loci, particularly in the disease modifier analysis, indicating a large effect of these loci in the modification of psychosis in AD. Therefore, despite not observing any SNPs that meet stringent levels of genome-wide significance, many SNPs were identified which remain interesting and could reflect true disease loci. Aside from SNPs at the *APOE* locus, the most significant association was observed at the RAR-related orphan receptor A *(RORA)* gene, which showed evidence for association in disease subtype and disease modifier analyses. The protein encoded by the *RORA* gene is a member of the NR1 subfamily of nuclear hormone receptors. It can bind to hormone response elements upstream of several genes to enhance the expression of those genes. The specific functions of this protein are unknown; it has been shown to interact with proteins involved in organogenesis and differentiation. *RORA* has been highlighted as a candidate gene for bipolar disorder through a genetic and functional genomics analysis (Le-Niculescu et al. 2008). In the disease modifier analysis SNP rs2034130 close to the microcephalin (*MCPH1*) gene showed the most evidence of association. *MCPH1* has been implicated in chromosome condensation and DNA damage induced cellular responses. It is thought to play a role in neurogenesis and regulation of the size of the cerebral cortex. Perhaps of more interest is marker rs11756091 ($P = 8.06 \times 10^{-6}$; OR = 1.53), a non-synonymous variant within the potassium channel, subfamily k, member 16 (*KCNK16*) gene on chromosome 6 which may affect protein charge and function. Ion channel dysfunctions are well-recognized causes of episodic central nervous system disease including epilepsies, ataxias and migraine (Gargus 2006); a recent meta-analysis suggests they may also be

involved in the pathogenesis of bipolar disorder (Ferreira et al. 2008). Secondary analyses of the disease modifier dataset highlights the tryptophan hydroxylase1 gene (*TPH1*).*TPH1* encodes an aromatic amino acid hydroxylase which catalyzes the first and rate limiting step in the biosynthesis of serotonin, an important hormone and neurotransmitter. Mutations in this gene have been associated with an elevated risk for a variety of diseases and disorders, including schizophrenia (Saetre et al. 2009). These GWAS analyses provide no evidence to support the previously identified association with the *OLIG2* gene. Suggesting that the original association of *OLIG2* with LOAD+P could be a false positive finding. However, the genetic coverage of the *OLIG2* gene is poor at only 40%. This is the first genome-wide association study of LOAD+P. Despite the lack of genome-wide significant findings some interesting candidate genes have been prioritised for further investigations.

6.2. Thesis Limitations

An understandable limit of the work presented in this thesis is power. This limitation was partially addressed in chapter 5 where the sample was developed to provide a 13% and 21% increase in power for the disease modifier and disease subtype analysis respectively. Power calculations show that to achieve a significant association with an effect size of 1.2 in a complex genetic disorder a minimum sample size of 1300 cases and 1300 controls is required (Faul et al. 2007). Due to this a true association may be missed if the effect size is modest, as is often seen in studies of complex genetics. A larger sample-set would greatly increase the study power. However, this would require recruitment of around 5000 LOAD patients with comprehensive data on occurrence of psychotic symptoms to produce a dataset with suitable power to confidently access genetic association of moderate to strong effect at a given locus. Power to detect smaller effect sizes which are proving to be common in complex disease would require a much larger data set of 10,000s of samples. However, the sample utilised in this thesis is the largest LOAD+P sample of any published study, and is substantial in comparison to the datasets studied for genetic association to LOAD+P (Wilkosz et al. 2007).

Phenotypic classification of LOAD+P is a limitation of any LOAD+P study. Research into risk factors for LOAD+P has been plagued with inconsistent results, owing largely to methodological variations between studies. There is no generalised classification system to assess psychosis in LOAD. The NPI is the most commonly used system with established reliability and validity in clinical and research settings (Cummings 1997). Despite the NPI being used as the 'common' classification system, there is no consensus criterion in place to diagnose the presence or absence of psychosis in AD. Thus, genetic association studies of LOAD+P are often based on varying phenotypic classification making comparisons between studies difficult and emphasizing the need for a universal phenotype definition. Also, the search for risk factors for neurodegenerative disease is fraught with numerous difficulties. For example, endophenotypic information may be systematically biased between cases and controls. On the whole data regarding dementia sufferers generally comes from an informant, or proxy. It is possible that the proxy of an dementia case may recall previous medical history differently to the proxy of a control, or the control themselves (Launer et al. 1999).

Another limitation of this study is the methodology of marker selection, or gene coverage used in chapters 3 and 4. Ideally each candidate gene would have been 'tagged' with a minimum r^2 of 0.8 and MAF of 0.05 to extract at least 80% of the common genetic information from the locus and identify an association signal either directly (genotyping the functional variant) or indirectly (genotyping a variant in LD with the true susceptibility marker). However, as this study took a novel approach to investigate psychosis susceptibility genes it was logical to investigate as many of these genes as possible. The only practical way to do this was to genotype polymorphisms with either a functional role e.g. reducing gene expression, or which had previously shown association to at least one form of psychosis. Using this methodology positive association(s) with alternate polymorphisms at the loci of interest would be missed. This limitation was partially addressed in chapter 5 where a genome-wide approach was utilised. The Illumina HumanHap 550k beadchip which was one of the Illumina platforms used in this study has been shown to have average coverage of approximately 90% for common SNPs with MAF > 5% (Mägi et al. 2007). This data is based on the SNPs included in phase 1 of the HapMap project (The International HapMap Consortium 2003). However, the distribution of markers across the genome is not even. The

'lumpiness' of marker distribution means that some loci have good coverage while others have very poor. For example, the *OLIG2* locus, which is of particular interest to this study, has poor coverage of 40%. Thus, potential positive associations may be missed if they reside at these poorly represented loci. Imputation, which is discussed below, may partially resolve this issue by providing genotype data at untyped loci.

Also of note is that this study is only designed to identify common variation of large to moderate effect size. The genetic risk for LOAD+P may be due to a combination of common SNPs of various effect sizes, rare SNPs and copy number variants, other unsuspected genomic mechanisms, gene-gene or gene-environment interactions, and epigenetic effects.

6.3. Future Work

There is a long way to go to fully characterize the genetic basis of LOAD+P. The analysis of genome-wide association data in this thesis suggests that there are no loci of large effect contributing to the aetiology of psychosis in AD. However, the power of these analyses are inadequate and some loci have not been sufficiently interrogated. Further work on this dataset may involve imputation, haplotype analysis, gene ontology analysis and copy number variation analysis. The genome-wide imputation of genotypes has recently attracted significant attention given its broad applicability in the era of GWAS (Hao et al. 2009). Current algorithms can accurately impute genotypes for untyped markers, which enables researchers to identify evidence for association at these markers. Imputation allows the pooling of data between studies conducted using different SNP sets and may facilitate the interpretation of results through localisation of causal polymorphisms. Imputation algorithms are based on the high correlation of linkage disequilibrium (LD) between the nearby markers.C onsequently, testing assayed SNPs for association to traits of interest will also have some power to capture signals for untyped causal SNPs (Hao et al. 2009). Further, if the assayed SNPs are strategically distributed across the genome (e.g. tag SNPs), maximal genetic coverage can be achieved (Barrett and Cardon 2006). The LD in a reference population, such as the HapMap CEPH

population, which is genotyped for both the typed and untyped markers in a study, is used to determine the LD structure in the study population. From this the genotypes of unobserved SNPs can be imputed (i.e. predicted) based on nearby markers and then directly tested for association with phenotypes of interest (Marchini et al. 2007). Imputing genotypes is surprisingly accurate. However, GWAS incorporating imputed genotypes may only have a slight increase in power on top of assayed SNPs. The reason for this modest increase in power is likely because, adding more markers via imputation only results in modest gain in genetic coverage, but worsens the multiple testing penalties (Hao et al. 2009).

Genome-wide association methods based on haplotypes comprising multiple SNPs on the same inherited chromosome may provide additional power for mapping disease genes and identify associations not seen with single SNPs (Zhao et al. 2003). In particular if the common disease-common variant theory applies. According to this, the genetic variants related to a complex disease are old mutations and are common in the population (minor allele frequencies > 5%). Subsequent mutations and recombinations in the ancestral haplotype at which the disease mutation occurred shortened the haplotypes that descended from this ancestor (Nolte et al. 2007). However, in the current generation the haplotypes will still share a fragment around the disease locus (Nolte et al. 2007). Over recent years novel methods of haplotype analysis have been implicated or are under development, which are fast enough and hence practical to use even for marker densities of 500,000 SNPs/genome (Nolte et al. 2007).

Gene Ontology (GO) is a method to describe gene products in terms of their associated biological processes, cellular components and molecular functions in a species-independent manner (Harris et al. 2004). Numerous methods, such as Association LIst Go AnnoTatOR (ALIGATOR) (Holmans et al. 2009b), and AmiGO (Carbon et al. 2009), have been developed to identify GO categories that are enriched for association signals in GWAS. ALIGATOR was designed in Cardiff by Professor Peter Holmans. The program converts a list of significant SNPs into a list of significant genes, applying corrections for gene size and non-independent categories, and is not influenced by LD (Holmans et al. 2009b). GO analyses identify overrepresented GO categories, and help prioritise genes for investigation.

Future meta-analysis of GWAS data may prove fruitful in identifying common variation for LOAD+P. This will require collaboration between groups with large LOAD sample-sets which have comprehensive phenotype data for behavioural symptomology. Newer methodologies also include, but are not limited to, study of copy number variation (CNV) and deep sequencing of candidate loci with the goal of identifying rare risk-influencing variants. We can reasonably hope that as this technology is applied to LOAD+P, more small-to-medium-effect risk loci will be identified. Copy number variation results from deletions, inversions, insertions and duplications. CNVs are inherited but can also arise during development. CNVs are usually defined as a DNA segment that is 1 kb or larger and present at variable copy number in comparison with a reference genome (Feuk et al. 2006). A CNV can be simple in structure, such as tandem duplication, or may involve complex gains or losses of homologous sequences at multiple sites in the genome. It is estimated that approximately 0.4% of the genomes of unrelated people typically differ with respect to copy number (Redon et al. 2006). Multiple complementary technologies have been developed to identify and annotate CNVs, which include but are not limited to; SNP genotyping arrays, and clone-based comparative genomic hybridization. The Illumina HumanHap beadchips have proven success in CNV identification (Lin et al. 2009), making this analysis a must in our dataset.

Several new sequencing instruments (so-called 'next generation' or 'deep sequencing') are now available and are already transforming the field of genetics. When coupled with the appropriate computational algorithms, our ability to answer questions about an organism's mutational spectrum, from single base to large copy number polymorphisms, on a genome-wide scale, is likely to radically alter our understanding of human genetics (Mardis 2008). Dideoxynucleotide sequencing of DNA has been a staple part of molecular genetics since its first description in 1977 (Sanger et al. 1977), and has undergone a steady metamorphosis from a cottage industry into a large-scale production enterprise (Mardis 2008). Dideoxynucleotide approaches to focused mutation discovery have used directed PCR to amplify selected genomic regions from individual samples, followed by capillary sequencing, alignment of the resulting sequence traces and algorithmic detection of sequence variants (Nickerson et al. 2001; Wilson et al. 2003). What sets next-

generation sequencing apart from conventional capillary-based sequencing is the ability to process millions of sequence reads in parallel rather than 96 at a time (Mardis 2008). Also, next generation sequence reads are produced from fragment 'libraries' that have not been subject to the conventional vector-based cloning and amplification stages used in capillary sequencing. As such, some of the cloning bias issues that impact genome representation in sequencing projects may be avoided, although each sequencing platform may have its own associated biases (Mardis 2008). Next generation sequencing, as is the case with most new technologies, is still relatively expensive. Sequencing platforms which prioritise genome-wide exon sequencing or 'exome' sequencing are now readily available to allow sequencing of coding regions at workable financial cost.

6.4. Conclusions

Late-onset Alzheimer's disease is a major cause of dementia and is becoming increasingly important in aging populations. The presence of psychotic symptoms in LOAD is associated with many serious consequences and may be a marker for a more severe form of the disease (Weamer et al. 2009). An obstacle in the search for risk factors for LOAD+P is the acquisition of adequately sized and phenotypically defined samples. However, previous studies have found that AD+P is genetically determined, with the phenotype shown to associate with specific chromosomal locations and loci (Avramopoulos et al. 2005; Bacanu et al. 2002; Bacanu et al. 2005; Borroni et al. 2004; Borroni et al. 2006; Borroni et al. 2007; Go et al. 2005; Hollingworth et al. 2007; Holmes et al. 2001; Sweet et al. 1998; Sweet et al. 2005). This study utilised a novel approach of using schizophrenia putative susceptibility genes and GWAS results to interrogate psychosis susceptibility variants in LOAD with psychosis. This is a conservative hypothesis being tested. However, it is a logical approach to undertake in one of the early genetic association studies of psychosis in LOAD, drawing vital information from the established field of schizophrenia genetics to inform the choice of variants to test. This analysis proved fruitful with the identification of the *OLIG2* and *CLSTN2* loci

which associate with LOAD+P. This study presents the results of the first genome-wide association study of LOAD to condition on the presence or absence of psychosis. Despite the lack of genome-wide significant findings some interesting candidate genes have been prioritised for further investigations which a) show patterns of effect that are stronger than those generally observed in LOAD GWAS studies, b) are interesting biological and positional candidates for LOAD+P, and c) show some overlap with other disorders with psychotic features. The future prospects for this dataset are expansive. Imputation may highlight additional 'top hits' and gene ontology (GO) analysis may identify interesting gene networks which should be prioritised for investigation. This dataset may also provide a useful tool for future meta-analyses and gene exploration. The potential of this dataset may be maximised by the use of complimentary methodologies. For example, cross disciplinary analyses including data from gene expression arrays and inclusion of data from comparative genomics which can highlight regions of biologically relevant conservation. The hunt for functional susceptibility variants for LOAD+P will undoubtedly include the methods discussed and presented in this thesis. Fine mapping and the identification of novel susceptibility variants, in conjunction with assays to determine the functional relevance of a marker e.g. if the variant is related to protein function or gene expression, will be of great clinical importance. Psychotic symptoms are associated with many serious consequences in LOAD and the identification of these genes would increase our understanding of the mechanisms that can cause psychiatric complications in some individuals with AD; providing targets for therapy and a platform for future treatment development.

Appendices

Chapter 3 Appendix

SNP	Forward PCR	Reverse PCR	Extension
rs3738401	ACGTTGGATGAAAGCTGCCAGCTTGGACG	ACGTTGGATGAGACCCGTGTAGCCAAGAGA	CGACCCGCGATGTCTCTCTC
rs6675281	ACGTTGGATGACAACGTGCTGTAGGAAACC	ACGTTGGATGGCCCTTCTCTCTCTGATGTT	TCTGGACGGCTAAAGAC
rs821616	ACGTTGGATGTCTTCTACAGACAGGCTTCC	ACGTTGGATGGAAGCTGACTTGGAAGCTTG	TCCTGGAGCTGTAGGC
ex12, 4 bp del	ACGTTGGATGACTTTGCCTTGCTTCATCTG	ACGTTGGATGCTTCACACAGCAATCCACAG	GCTGCAAGATGAAAAGGATATGA

Table 3.2. MAF and genotype counts of tested *DISC1* SNPs through female samples. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs3738401	A	0.33	0.3	0.32	0.32	31/118/122	13/72/79	69/303/321	79/287/330
rs6675281	T	0.16	0.12	0.15	0.14	5/76/190	3/33/128	16/174/503	13/164/519
rs821616	T	0.27	0.31	0.29	0.28	18/110/143	16/71/77	52/294/347	56/282/358

Table 3.3. Individual genotyping of *DISC1* SNPs through female LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	X^2	*P*	OR	X^2	*p*	X^2	*p*	OR	X^2	*p*	X^2	*p*	OR	X^2	*P*
rs3738401	1	0.3	1.2 (0.87-1.57)	1.47	0.48	0.28	0.6	1.06 (0.86-1.31)	0.49	0.78	0	0.9	1 (0.86-1.18)	1.23	0.54
rs6675281	2.6	0.1	1.4 (0.93-2.10)	3.44	0.18	1.57	0.21	1.19 (0.9-1.57)	2.11	0.35	0.8	0.4	1.1 (0.89-1.37)	0.85	0.65
rs821616	2	0.2	1.2 (0.92-1.68)	2.13	0.34	0.36	0.55	1.07 (0.86-1.34)	0.57	0.75	0.1	0.8	1 (0.87-1.20)	0.56	0.75

Table 3.4. MAF and genotype counts of tested *DISC1* SNPs through male samples. Table shows; SNP ID, minor allele, MAF and genotype counts in LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts			
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD	Control
rs3738401	A	0.28	0.3	0.29	0.34	8/25/41	6/28/33	20/122/140	46/198/179
rs6675281	T	0.14	0.18	0.14	0.14	0/21/53	3/18/46	5/71/206	5/106/312
rs821616	T	0.26	0.26	0.25	0.28	5/28/41	5/25/37	17/109/156	32/176/215

Table 3.5. Individual genotyping of *DISC1* SNPs through male LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. control samples.

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					LOAD vs. Control				
	Alleles			Genotype		Alleles			Genotype		Alleles			Genotype	
SNP ID	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*P*
rs3738401	0.2	0.7	1.1 (0.66-1.86)	0.98	0.61	2.45	0.12	1.36 (0.92-2.0)	4.82	0.09	4.8	0	1.3 (1.03-1.63)	5.06	0.08
rs6675281	0.7	0.4	1.3 (0.7-2.5)	4.96	0.08	0.02	0.88	1.04 (0.63-1.72)	1.93	0.38	0.1	0.7	1.1 (0.78-1.43)	0.43	0.81
rs821616	0	0.9	1 (0.6-1.74)	0.03	0.99	0.45	0.5	1.15 (0.77-1.71)	0.53	0.77	1.6	0.2	1.2 (0.92-1.48)	1.6	0.45

Table 3.6. Relative PCR and extension primers for each *DTNBP1* marker tested.

NP	Forward PCR	Reverse PCR	Extension
470454	ACGTTGGATGCCAAAACAAGAGGAGGCAAG	ACGTTGGATGAGTCCAAATCAAGGTGTGGG	GGAGGACAGGTACTGGATTCAG
19539	ACGTTGGATGTCTTTGAAGACTTCCTTTCG	ACGTTGGATGCTACCACTAACAACCAAAAAG	GACATGGTCTTAAAATGTATAAAA
525702	ACGTTGGATGAATCTCTACTGAGTAGAGGG	ACGTTGGATGAGTGAAACCAGGTTTTAGGC	TGTTAAAGATAACATTAAATCTCATTAC
13207	ACGTTGGATGTCTTTGAAGACTTCCTTTCG	ACGTTGGATGCTACCACTAACAACCAAAAAG	TCCTTTCGTAAAGCCA
19538	ACGTTGGATGTCACTGTTTTCATTGCTGGG	ACGTTGGATGAGTGAGGTAAGTAGCACAAG	GACAGAGCAGTTTACATC

Table 3.7. Results of haplotype analysis at the *DTNBP1* locus. Table shows analysis dataset, number of markers analysed, chi square statistic and p-value.

Dataset	Number of Markers	χ2	Global *P*
LOAD+P v LOAD-P	5	0.09	0.77
LOAD+P v Control	5	1.88	0.17
LOAD v Control	5	1.17	0.28

Table 3.8. Relative primers for the *GRIK2* variants. The TAA repeat was genotyped by genescan fluorescent PCR. SNP rs6922753 was genotyped by Amplifluor Uniprimer chemistry.

Variant	Forward	Reverse	Label
TAA repeat	CAACACCTTTTCTCTAACCCC	CTCGGCCAGTTTTTACAACTTG	Hex

Variant	Allele 1	Allele 2	Reverse
rs6922753	GAAGGTCGGAGTCAACGGATTCAAGGCAGATGTTCTTCTCCTG	GAAGGTGACCAAGTTCATGCTGACAAGGCAGATGTTCTTCTCCTA	GGACCCGCTTTATGAGTCTAA

Table 3.9. Relative PCR and extension primers for each *GRM3* marker tested.

SNP	Forward PCR	Reverse PCR	Extension
rs187993	ACGTTGGATGGTTAATCTCACCTCTAATAC	ACGTTGGATGCCCTGCTTGATTATCACCAC	AAACTTTATATGTTCTACAGCA
rs13242038	ACGTTGGATGTGAGGATGGAAGTCATGCTG	ACGTTGGATGGTTGCTAGGTTCAATTTCTC	AGTCATGCTGCAAATATGAG
rs917071	ACGTTGGATGTAAAGGGCTCCTAGGAAAAC	ACGTTGGATGTACAGCTTCCAAAAGGCCTG	GTTGAGACAACAAGACACATA
rs6465084	ACGTTGGATGTGACACAAAGTTCTCTTTCC	ACGTTGGATGCCGCTGCTCTTTCCATATTG	GCTCTTTCCAAATTACCATTAAAT
rs2228595	ACGTTGGATGACGACTCGCGGGAGCTCATT	ACGTTGGATGCTTGATGATGCTCTCCTGCG	CTAGCCGCCAGCCGCGCCAATGC
rs1468412	ACGTTGGATGTGTTCCTTCAGCTTGCAATG	ACGTTGGATGAACTGGAGACTGGTTTTGCC	GAGCTTGCAATGTTATAGGCAGTA
rs2282966	ACGTTGGATGTTTCCTTGGGAGGTTGAGAC	ACGTTGGATGTTAATCCTTCAAGGGCCCAG	GGGCTCCTAGGAAAACAACTCT

Table 3.10. Results of haplotype analysis at the *GRM3* locus. Table shows analysis dataset, number of markers analysed, chi square statistic and p-value.

Dataset	Number of Markers Analysed	χ2	Global *P*
LOAD+P v LOAD-P	7	0.007	0.93
LOAD+P v Control	7	1.66	0.2
LOAD v Control	7	4.75	0.03

Table 3.11. Relative PCR and Extension primers for each *NRG1* marker tested.

Variant	Forward PCR	Reverse PCR	Extension
rs12681411	TCTTTGCACTAGCAGGCTCA	TGCAGCCTATTGACTTGGTG	TCAAGGTACAGCTCAGGTTTT
rs3924999	ACGTTGGATGTTGCCTGGTGATCAGAGTTG	ACGTTGGATGCCGATTCCTGGCTTTTCATC	GTTAGCCTTGCCTCCCC
SNP8NRG221533	TCAATTTAAGGCATCAGTTTTCAA	TCTGTGGTTGTCTAAATCCAGAA	TAAAAAAGAGATATATGATATTTGG
478B14-848	CCACATGTCCAACTGAAGAGG	TCTCCATGTGTAAAACAATACATATCA	n/a
420M19-1395	CTTTTAATCATGAAAGAATAGCAAAAA	TGTTGTTGTATATTTCAGAATTTCCTT	n/a

Table 3.12. Relative PCR and extension primers for the *BDNF* val/met marker.

SNP	Forward PCR	Reverse PCR	Extension
rs6265	ACGTTGGATGTTTTCTTCATTGGGCCGAAC	ACGTTGGATGGCTTGACATCATTGGCTGAC	ATCCAACAGCTCTTCTATCA

Table 3.13. MAF and genotype counts of tested *BDNF* SNP through female samples. Table shows; SNP ID, minor allel
LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts		
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD
rs6265	T	0.17	0.2	0.19	0.17	8/82/196	9/54/117	35/241/54

Table 3.14. Individual genotyping of *BDNF* SNP through female LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs

	LOAD+P vs. LOAD-P					LOAD+P vs. Control						
	Alleles			Genotype		Alleles			Genotype			
SNP ID	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*	OR	χ^2	*p*	χ^2	*p*
rs6265	1.22	0.27	1.21 (0.86-1.69)	1.74	0.42	0.02	0.88	1.02 (0.79-1.31)	0.53	0.77	2.5	0.1

Table 3.15. MAF and genotype counts of tested *BDNF* SNP through male samples. Table shows; SNP ID, minor allele, M
LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Counts		
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOAD
rs6265	T	0.23	0.2	0.21	0.17	4/29/46	5/20/50	19/106/224

Table 3.16. Individual genotyping of *BDNF* SNP through male LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD vs. c

	LOAD+P vs. LOAD-P					LOAD+P vs. Control						
	Alleles			Genotype		Alleles			Genotype			
SNP ID	X^2	*p*	OR	X^2	*P*	X^2	*p*	OR	X^2	*p*	X^2	*p*
rs6265	0.53	0.47	1.22 (0.71-2.11)	1.83	0.4	4.31	0.04	1.53 (1.02-2.29)	4.34	0.11	4.37	0.04

Table 3.17. Relative PCR and extension primers for each *DAOA* marker tested.

SNP	Forward PCR	Reverse PCR
3'UTR	ACGTTGGATGAAGACTGACCTTCCAGATCC	ACGTTGGATGCAGGCCTTTATGTCAGAAGC
rs1341402	ACGTTGGATGCACACACATCTTCAACATTTG	ACGTTGGATGGGAAATGTTAACTAACATTA
rs1421292	ACGTTGGATGATGTACTTCCCAGTCCTTGC	ACGTTGGATGTTAAAGGCACTGAGAGGAGC
rs2391191	ACGTTGGATGAGATCCAGATATACATTGGG	ACGTTGGATGGAGTTTTCAGATTTGCTCAG
rs3916965	ACGTTGGATGGGTTTTCTTTTGAAGGACAG	ACGTTGGATGGGAAAAAAGTGATGACATTGC
rs3918342	ACGTTGGATGCCTTAGGAGATGGATATAGG	ACGTTGGATGGTGCAGTCTCATTCACTATC
rs778293	ACGTTGGATGCATAGAAGAAAAGCTTGTG	ACGTTGGATGCTAGTTGCCCCCCAAAATTG
rs778294	ACGTTGGATGCGGTGATGAGGTTACCTTTC	ACGTTGGATGGCTGCTTACTTCTTCAAGCC
rs954581	ACGTTGGATGCTATTAGCCTTTACTTTTCCC	ACGTTGGATGTTGACGTATCTCTTCTCAGG

Table 3.19. Relative PCR and extension primers for each *CNP* marker tested.

SNP	Forward PCR	Reverse PCR
3235AG	ACGTTGGATGAGATCATGCCACTGCACTCC	ACGTTGGATGGGAGTGACTACTGCTCAAAG
rs35967904 (7284GA)	ACGTTGGATGAACCTGGAAGTAGGGCTCG	ACGTTGGATGCACCATTCAGGAACCTGGAC
rs10540926	ACGTTGGATGTTGGAAGAGCATCGTGGAAG	ACGTTGGATGACTTCTTGTTCCCTGTAGCG
rs11079028	ACGTTGGATGAATAACTGACCCTCCCTTCC	ACGTTGGATGTTGGTCCAGGTTCCTGAATG
rs11296	ACGTTGGATGTAGTCTGTGTGCTGTGGTTC	ACGTTGGATGGTGACAACCCGATTAGCAAG
rs12602950	ACGTTGGATGTGGAGAGGTGATGCTTAGTG	ACGTTGGATGATACCAACATCCGCCACCAG
rs12952915	ACGTTGGATGAAGACAGGATCCGACTTTGC	ACGTTGGATGCTTTTTCTGCGTGTTGAGGG
rs2070106	ACGTTGGATGTGGGCACAGGTTTGCCTTTC	ACGTTGGATGTGGCCAAGAACATGGAGGTC
rs4796750	ACGTTGGATGAGAGGAACAGGGGTGTTAAG	ACGTTGGATGTTGAAAGAATGCCTGGCCTG
rs4796751	ACGTTGGATGTTCAGTAAGACCGGAAGCTC	ACGTTGGATGTCCCTGGTGCTTGAAGTTTC

Table 3.21. Relative PCR and extension primers for each *OLIG2* marker tested.

SNP	Forward PCR	Reverse PCR
F16GA	ACGTTGGATGGTCTAAGCAAAGGGTGCCTG	ACGTTGGATGGTGCAGAAGATTTCCCCAG
rs1005573	ACGTTGGATGTAAGGTGGATCCGTTTGAGG	ACGTTGGATGGAAGTGAGGGCCTTTCTTTG
rs1059004	ACGTTGGATGTCCTCACTAGAACTCATCCG	ACGTTGGATGCGCTCTCAGGGAAAGAAGTT
rs11701698	ACGTTGGATGTATTGGGATAGGACTGAGGG	ACGTTGGATGTGCTGTCAAGATTGGCTGAG
rs11701762	ACGTTGGATGAAACTGTGCACCCAGCTGGA	ACGTTGGATGACTGTGTGCCAGTGTCTTCC
rs13046814	ACGTTGGATGTAAAACAGATGATACACTC	ACGTTGGATGAAACTGGGCTTTGTAGCGTC
rs2834070	ACGTTGGATGGACATCTGTCCAGACACATC	ACGTTGGATGAGAGAAAGCACACCAAAGCC
rs2834072	ACGTTGGATGATGGTTCAGACAAACCCCTC	ACGTTGGATGTTATGTAGGGAAGGTACCAA
rs762178	ACGTTGGATGCTTCTTGTCCTTCTTGGTGG	ACGTTGGATGACAAGCTAGGAGGCAGTGG
rs762237	ACGTTGGATGGCATGATGCACGGTTAGTTC	ACGTTGGATGTTTCCATGTAGAGCATGGGC
rs881666	ACGTTGGATGGTCCCGTCTTACAAGTAAGG	ACGTTGGATGCCACCTTCATTTACTGGCTG

Table 3.23. Relative PCR and extension primers for each *OLIG1* marker tested.

SNP	Forward PCR	Reverse PCR
rs17632819	ACGTTGGATGTCTCTGAATGACTCCCAGCC	ACGTTGGATGTATTCCATGTTAGCCTACGG
rs11088236	ACGTTGGATGTACCCTTGCCCCTCTCTAAC	ACGTTGGATGTAGGCTAGAGGGAAAAATGG
rs2834076	ACGTTGGATGTTAAGAGACAATGCACTCTG	ACGTTGGATGGTAAGAGTGCAATCCATGTG
rs10483016	ACGTTGGATGGCTAAGCATTGTTGCAGGTG	ACGTTGGATGATGTCTGTGCTTCCGATTCC
rs2834079	ACGTTGGATGGCCTCAGCATCAGGGATATT	ACGTTGGATGACACTGCTCCCTGTTCTTAC
rs2834083	ACGTTGGATGTTCACTCCTTTCTGAAAACC	ACGTTGGATGAAACAGGCCGATGTGTTTGG
rs928736	ACGTTGGATGAGGCAGCATGTGTGTGTTTC	ACGTTGGATGACTTGTACGCTCCCCATTTC
rs7278735	ACGTTGGATGCCCTGGAAAATGGGTTAAGC	ACGTTGGATGGCTTCTGTCCAAGGATATAG
rs11088237	ACGTTGGATGGTAATAACAGGACCCACCTC	ACGTTGGATGACAAGAAAAGCATTGGCAGG
rs8131457	ACGTTGGATGAACACACTCCGGAGTCTTTC	ACGTTGGATGTTGGGAGATGGTTCACCAAG
rs7276171	ACGTTGGATGTAGTTCAGGCATGGCCTATC	ACGTTGGATGCTATTTCCTGGCATCTGCTC
rs4817527	ACGTTGGATGCTCAGCCTCAGATACATTTC	ACGTTGGATGCAGCCAGAGGATTGTGACTT
rs2834086	ACGTTGGATGTGGTGTCTGTGTTGATTCCC	ACGTTGGATGGAAAGTTCTGTCTGGTCAAC
rs2834087	ACGTTGGATGATGCAGTGAAATTCAGCGTC	ACGTTGGATGGTTGGTGCGTTTCCTGAGTG
rs12481815	ACGTTGGATGAGACCCTGGCATGGATACCG	ACGTTGGATGAGCAAAGCCCTGGTCATGG

Table 3.25. Relative PCR and extension primers for each *COMT* marker tested.

SNP	Forward PCR	Reverse PCR
rs165599	ACGTTGGATGGGCTGACTCCTCTTCGTTTC	ACGTTGGATGACAGTGGTGCAGAGGTCAG
rs2020917	ACGTTGGATGATGATGATATCCCACCTCCC	ACGTTGGATGCTAACCTCTAGAGTCTAGGG
rs4680	ACGTTGGATGTTTTCCAGGTCTGACAACGG	ACGTTGGATGACCCAGCGGATGGTGGATTT
rs737865	ACGTTGGATGTCCTACGGTCCCTCAGGCTT	ACGTTGGATGCTAACAGACCTGCTTTTTGG

Table 3.26. MAF and genotype counts of tested *COMT* SNPs through female samples. Table shows; SNP ID, minor alle
LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Co		
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	L
rs2020917	T	0.29	0.28	0.29	0.28	20/107/127	17/56/87	54/2
rs737865	C	0.29	0.29	0.29	0.28	21/106/127	18/56/86	56/2
rs4680	G	0.48	0.48	0.48	0.5	59/124/71	39/74/47	165/3
rs165599	G	0.3	0.29	0.3	0.3	20/113/121	10/74/76	55/2

Table 3.27. Individual genotyping of *COMT* SNPs through female LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					
	Alleles			Genotype		Alleles			Genotype		
SNP ID	χ^2	*p*	OR	χ^2	*P*	χ^2	*p*	OR	χ^2	*P*	χ^2
rs2020917	0.06	0.8	1.04 (0.76-1.42)	2.46	0.29	0.36	0.55	1.07 (0.85-1.35)	0.99	0.61	0.35
rs737865	0.01	0.91	1.02 (0.75-1.39)	2.33	0.31	0.31	0.58	1.07 (0.85-1.34)	0.66	0.72	0.35
rs4680	0.001	0.97	1.01 (0.76-1.33)	0.26	0.88	0.82	0.37	1.1 (0.9-1.35)	0.8	0.67	0.94
rs165599	0.05	0.82	1.04 (0.76-1.41)	0.43	0.81	0	0.97	1 (0.8-1.26)	1.92	0.38	0

Table 3.28. MAF and genotype counts of tested *COMT* SNPs through male samples. Table shows; SNP ID, minor allele
LOAD+P, LOAD-P, LOAD and control samples.

		MAF				Genotype Count		
SNP ID	MA	LOAD+P	LOAD-P	LOAD	Control	LOAD+P	LOAD-P	LOA
rs2020917	T	0.26	0.32	0.29	0.28	2/ 18/ 39	9/ 25/ 33	31/97/
rs737865	C	0.27	0.32	0.29	0.28	9/ 17/ 39	9/ 25/ 33	32/96/
rs4680	G	0.48	0.43	0.47	0.44	19/ 24/ 22	12/ 34/ 21	62/12
rs165599	G	0.28	0.29	0.31	0.28	7/ 23/ 35	3/ 33/ 31	27/112

Table 3.29. Individual genotyping of *COMT* SNPs through male LOAD+P vs. LOAD-P, LOAD+P vs. control and LOAD v

	LOAD+P vs. LOAD-P					LOAD+P vs. Control					
	Alleles			Genotype		Alleles			Genotype		
SNP ID	χ^2	*p*	OR	χ^2	*P*	χ^2	*p*	OR	χ^2	*P*	χ^2
rs2020917	1.13	0.29	1.33 (0.78-2.27)	1.67	0.43	0.13	0.72	1.08 (0.71-1.64)	2.9	0.23	0.41
rs737865	0.85	0.36	1.28 (0.75-2.18)	2	0.37	0.04	0.84	1.04 (0.69-1.58)	4.35	0.11	0.44
rs4680	0.52	0.47	1.19 (0.74-1.94)	3.3	0.19	0.47	0.49	1.14 (0.79-1.65)	4.46	0.11	0.55
rs165599	0.01	0.91	1.03 (0.61-1.76)	3.6	0.17	0.01	0.94	1.02 (0.67-1.53)	0.49	0.78	0.91

Table 3.31. Results of haplotype analysis at the *COMT* locus in males only. Table shows analysis dataset, number of markers analysed, chi square statistic and p-value.

Dataset	Number of Markers Analysed	χ2	Global *P*
LOAD+P v LOAD-P	4	0.23	0.63
LOAD+P v Control	4	0.530	0.47
LOAD v Control	4	1.49	0.22

Table 3.32. Results of haplotype analysis at the *COMT* locus in females only. Table shows analysis dataset, number of markers analysed, chi square statistic and p-value.

Dataset	Number of Markers Analysed	$\chi 2$	Global *P*
LOAD+P v LOAD-P	4	0.004	0.95
LOAD+P v Control	4	0.3	0.58
LOAD v Control	4	0.26	0.61

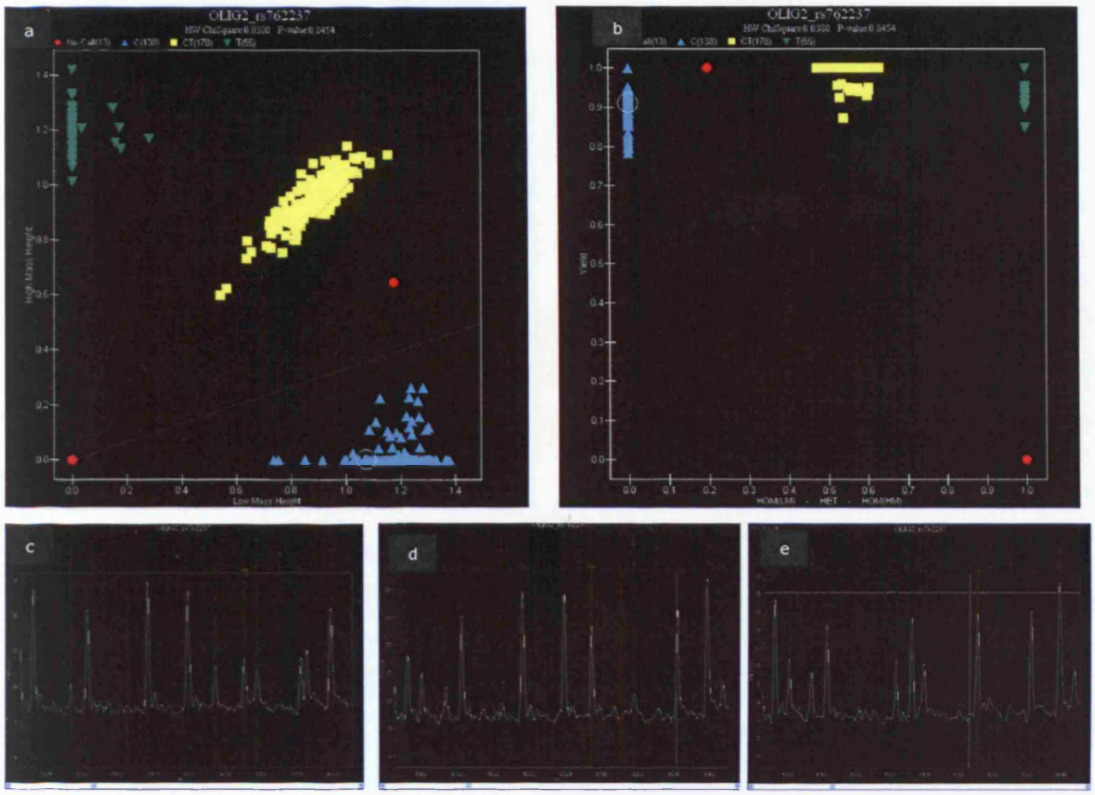


Figure 3.1. *Example of Sequenom TyperAnalyser genotyping suite for OLIG2 variant rs762237.* a. Is a log height cluster diagram through 384 samples. Red cluster indicates blank/failed samples; green cluster indicates T homozygotes (see e); yellow cluster indicates heterozygotes (see c); and blue cluster indicates C homozygotes (see d). b. Is an alternate yield v skew cluster view of the same samples. Clusters are as described for a. c. shows an example of heterozygote spectra. Remaining extension primer peak is indicated by red dotted line. Peak for the C allele is indicated by the left yellow dotted line. Peak for the T allele is indicated by the right yellow dotted line. d. Is an example of C allele homozygote spectra. Remaining extension primer peak is indicated by left red dotted line. Expected peak for the T allele is indicated by the right red dotted line. Peak for the C allele is indicated by the yellow dotted line. e. Is spectra for a T allele homozygote. Remaining extension primer peak is indicated by left red dotted line. Expected peak for the C allele is indicated by the right red dotted line. Peak for the T allele is indicated by the yellow dotted line. a and b are used to identify suspicious genotype calls, specified by the TyperAnalyser algorithm, and the spectra output (c,d or e) is utilised by the user to manually allocate a genotype. [Figure produced using Sequenom TyperAnalyser software.]

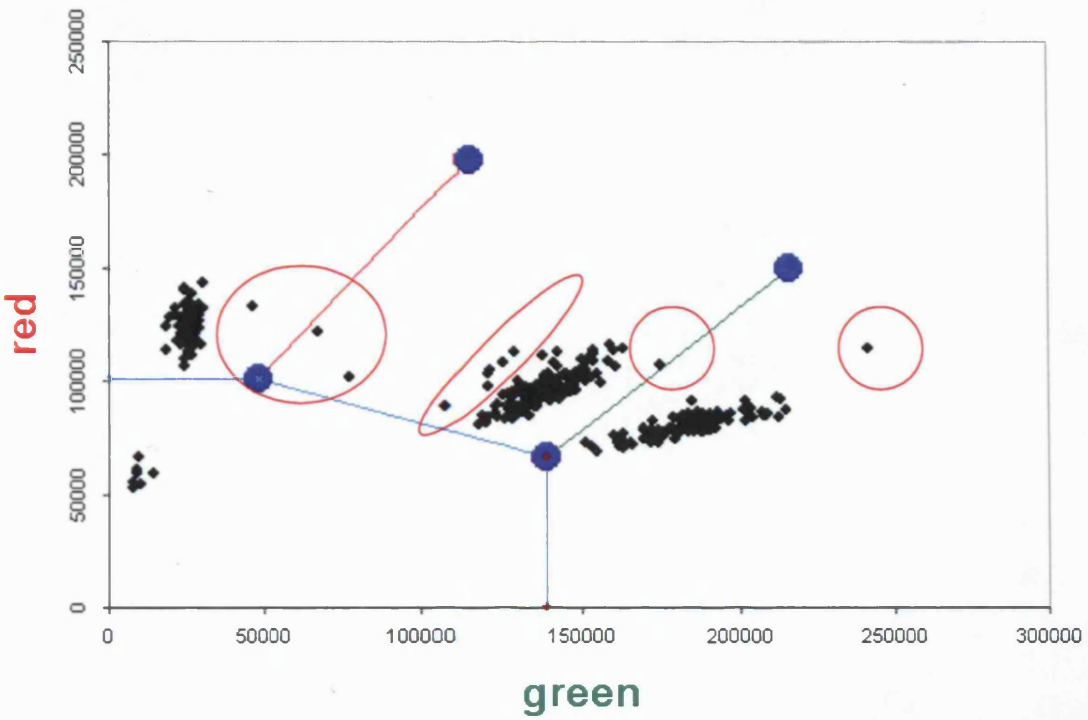


Figure 3.2. *Example of Amplifluor genotyping cluster through 384 samples for OLIG1 variant rs11088236.* Schematic is a plot of red allele fluorescence against green allele fluorescence as labelled. Each black spot represents a sample genotype. Cluster below the turquoise lines are the blank or failed samples. Cluster above the turquoise line and to the left of the red line are the red allele homozygotes. Cluster above the turquoise line, to the right of the red line and the left of the green line are the heterozygotes. Cluster above the turquoise line and to the right of the green line are the green allele homozygotes. Genotypes circled in red are genotypes which were removed before statistical analysis. [Figure produced using Microsoft Excel Macro].

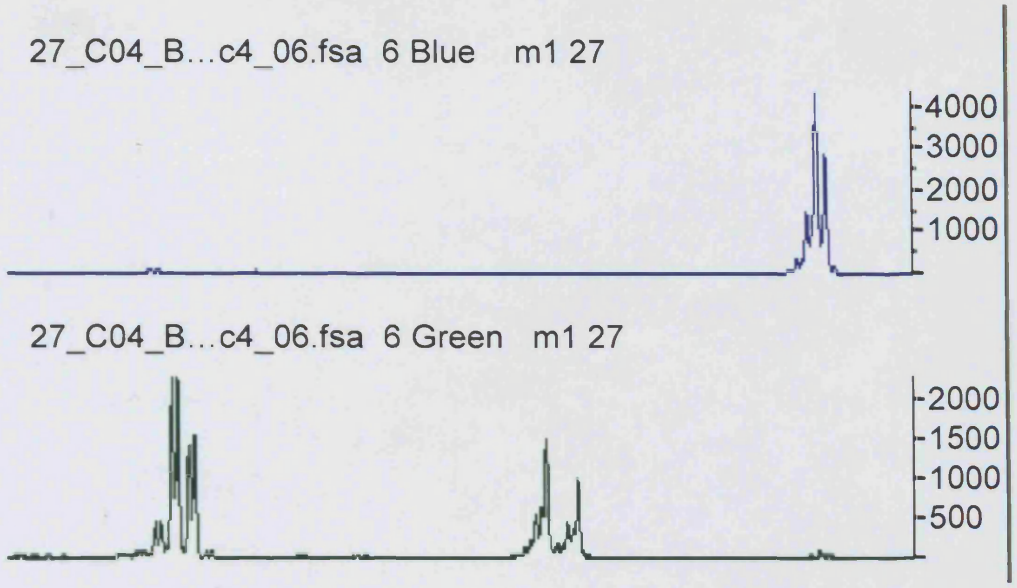


Figure 3.3. *Example of genescan fluorescent PCR plot, for the GRIK2 TAA repeat and NRG1 microsatellites 420M19-1395 and 478B14-848, from genotyper 2.5 software.*

Figure shows genotypes for one sample. The top blue line represents the Fam fluorescent dye and shows a homozygote genotype for the *NRG1* microsatellite 420M19-1395. The bottom green line represents the Hex fluorescent dye and shows heterozygous genotypes for both the *GRIK2* TAA repeat (left), and the *NRG1* microsatellite 478B14-848 (right). The exact genotypes are determined by analysis of the amplimer sizes in comparison to the Genescan™ -500 ROX™ STANDARD, which is run in unison with the sample.

Chapter 4 Appendix

Table 4.1. Chromosome, gene, base position and flanking sequence of each SNP variant tested.

Chr	SNP	Gene	BP	Flanking Sequer
1	rs7546928	AGBL4	73023563	TGTCCTTACTTTGTCTCCAAATGCTC[A/G]CA
1	rs11162231	ST6GALNAC5	77040318	CAACTGAAACTTGCAACTCTCAAGGC[G/T]CA
2	rs16984718	intergenic	18430882	TATGTAGTTGCTAACTTTTCATCCTT[G/A]AA
2	rs2890738	intergenic	144273491	TTGTAGAAAGACACAGAAGTTCCTCA[C/T]CC
2	rs12613195	ZNF804a	185197466	AGTTCTATATTACATTTTTCAGAGCA[C/G]TA
2	rs1344706	ZNF804a	185486673	TTTGTGACTTTGTTTCTTAGTTTTTG[C/A]TA
3	rs12629685	TRIM71	32855082	ATGCAGTGGAGAAGTGAAGTCCTAAC[G/A]CA
3	rs9870579	CLSTN2	141162306	AGCCAGGGGCTGACTCAGCTTCATAC[C/T]TG
8	rs10103330	CSMD1	4167498	AGGCCCTGAAGAGAGTAAGTAAGATT[A/T]TG
9	rs2210539	c9orf39	17158867	TGATGAATTGTGAAGTTTTGTGTTGG[G/A]TC
9	rs10869675	PCSK5	75819589	GCCTCTTACTTAAAAAGAGAAAGCGC[G/A]AA
10	rs17101921	FGFR2	123143285	TTTTCTCTTCTGCGATACGCTGATAA[A/G]AA
11	rs10835482	intergenic	29079278	AGTGGGGCATATTCATCAAATGATGA[T/C]GT
11	rs1602565	intergenic	29118712	TTCCATCTTTGTGCTGTAAAGTAGTA[T/C]GG
11	rs3016384	OPCML	132078600	CTGTCAGATATGTCTGGCCTTCTCTA[T/C]AC
12	rs6490121	NOS1	116192578	AGACTACTCTTTGCAACTGAAATGTA[A/G]AC
13	rs4238270	IRS2	109268028	GGAAGGGGCTCCTGAGAGATCCAGAG[G/T]TA
15	rs3784397	PLCB2	38384136	CCCTTCCTGAAATGTGCTACAGTAAC[T/C]GG
16	rs7192086	intergenic	12969112	CCTCTCCTAAGTGTAGCTCCTAAAGA[T/A]AT
16	rs9922369	RPGRIP1L	52205983	CAGGAGCTAGTGAACACTTTTGGGTT[G/A]AA
18	rs1893146	intergenic	8977427	ACACAAGGGAACAGTTCTCCTGACTC[G/A]AT

Table 4.2. Nucleotide change, forward primer, reverse primer and extension primer used for genotyping of each variant forward and reverse primer are specific to the Sequenom™ assay which has been described elsewhere.

SNP	N/C	Forward Primer	Reverse Primer
rs7546928	A/G	ACGTTGGATGCAAGCCCTGTCCTTACTTTG	ACGTTGGATGGTTCACTCCCTTTGCTGTAG
rs11162231	G/T	ACGTTGGATGCCTCCTTTTCTTGGCAACTG	ACGTTGGATGCACCTAGGAATAAGCCACAG
rs16984718	G/A	ACGTTGGATGCTTCGTTCCATCATCCAACC	ACGTTGGATGTGTGCAAGGCTGCAAGTTTC
rs2890738	C/T	ACGTTGGATGCCTCTAAAAACTTTGAGATTC	ACGTTGGATGGGGATCGTTTCCAATAACATC
rs12613195	C/G	ACGTTGGATGGTGAGGGATGTGGTAAGTTC	ACGTTGGATGCTTGGCACATATGCTCAGTC
rs1344706	C/A	ACGTTGGATGCCAGATAGATATCCAAGAAG	ACGTTGGATGCAAAGCCTTATCTCTTCACAG
rs12629685	G/A	ACGTTGGATGAGCTCCCCTATATCAACTGC	ACGTTGGATGCATGCAGTGGAGAAGTGAAG
rs9870579	C/T	ACGTTGGATGTGACCATGATAGAAGCCAGG	ACGTTGGATGCCCTTATCTCAAGAGTCTCC
rs10103330	A/T	ACGTTGGATGTAGGCCCTGAAGAGAGTAAG	ACGTTGGATGGTCCAGCATTTTTCTGCTTC
rs2210539	G/A	ACGTTGGATGTGTTGCTGACCTGGTCTTTC	ACGTTGGATGGGGTTATAGTAGAGTAGGTG
rs10869675	G/A	ACGTTGGATGTGAGCCTGTAAGTTCAGAGC	ACGTTGGATGCCCAGAGTATTGAGAATGCC
rs17101921	A/G	ACGTTGGATGGTTTTCTCTTCTGCGATACG	ACGTTGGATGCCCTGTGCTAGCTATTTTAC
rs10835482	T/C	ACGTTGGATGGAATGCTTCCTTGAAAACCAC	ACGTTGGATGTTCAAATGTTTAAGTGGGGC
rs1602565	T/C	ACGTTGGATGGGGTGTTGAAACTATTTTGC	ACGTTGGATGAAGCTTCCATCTTTGTGCTG
rs3016384	T/C	ACGTTGGATGATGACTTCCCTGGCAAAGAC	ACGTTGGATGTTTGCAATCCTTGTTGCCTG
rs6490121	A/G	ACGTTGGATGACCCAGAGACTACTCTTTGC	ACGTTGGATGCTAATGCCTCAGCCTCAAAG
rs4238270	G/T	ACGTTGGATGCTTGTTCCTTTGGTTCGGC	ACGTTGGATGTGAAGTGTGGACTCCTGCTG
rs3784397	T/C	ACGTTGGATGGGATTTCCTAGGTGGGCTTG	ACGTTGGATGCCCTTCCTGAAATGTGCTAC
rs7192086	T/A	ACGTTGGATGTTTGCATGTCTCTCTGTGCC	ACGTTGGATGGAGCTCCAAAGCATCGATTC
rs9922369	G/A	ACGTTGGATGAATCAGAGGCAGCCAAAGTG	ACGTTGGATGGGGATCAGGAGCTAGTGAAC
rs1893146	G/A	ACGTTGGATGGCTTTGTGAGCAATGGTGTG	ACGTTGGATGCACACAAGGGAACAGTTCTC

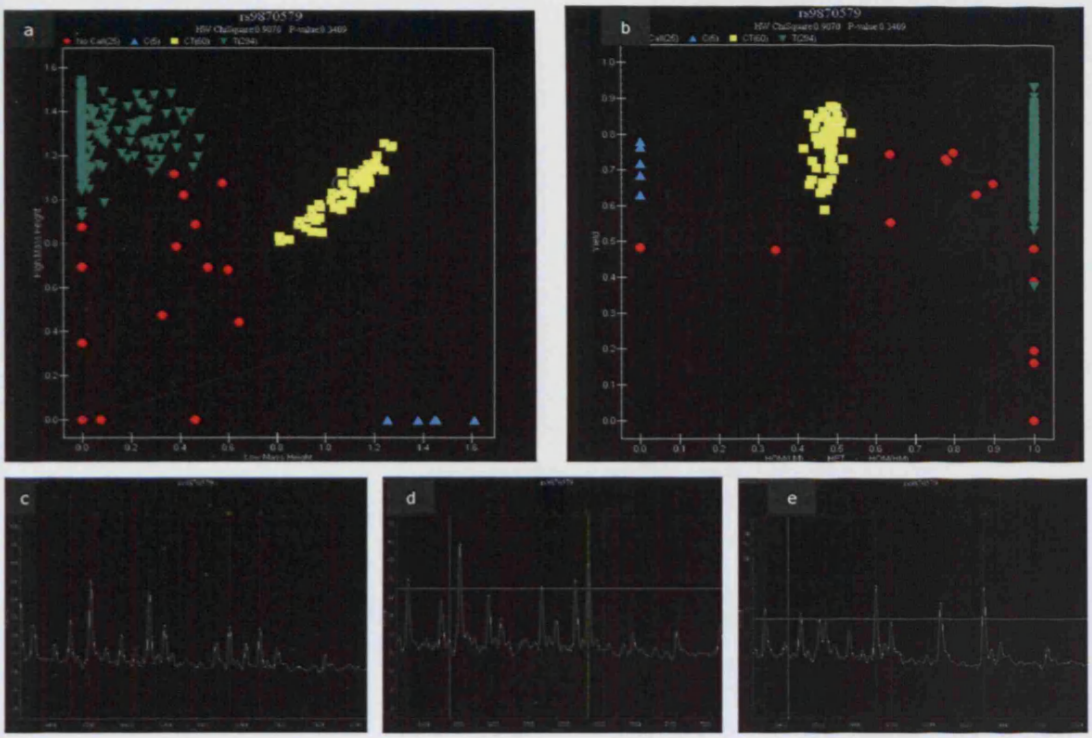


Figure 4.1. *Example of Sequenom TyperAnalyser genotyping suite for variant rs9870579.*
a. Is a log height cluster diagram through 384 samples. Red cluster indicates blank/failed samples; green cluster indicates T homozygotes (see e); yellow cluster indicates heterozygotes (see c); and blue cluster indicates C homozygotes (see d). b. Is an alternate yield v skew cluster view of the same samples. Clusters are as described for a. c. shows an example of heterozygote spectra. Remaining extension primer peak is indicated by red dotted line. Peak for the C allele is indicated by the left yellow dotted line. Peak for the T allele is indicated by the right yellow dotted line. d. Is an example of C allele homozygote spectra. Remaining extension primer peak is indicated by left red dotted line. Expected peak for the T allele is indicated by the right red dotted line. Peak for the C allele is indicated by the yellow dotted line. e. Is spectra for a T allele homozygote. Remaining extension primer peak is indicated by left red dotted line. Expected peak for the C allele is indicated by the right red dotted line. Peak for the T allele is indicated by the yellow dotted line. a and b are used to identify suspicious genotype calls, specified by the TyperAnalyser algorithm, and the spectra output (c,d or e) is utilised by the user to manually allocate a genotype. [Figure produced using Sequenom TyperAnalyser software.]

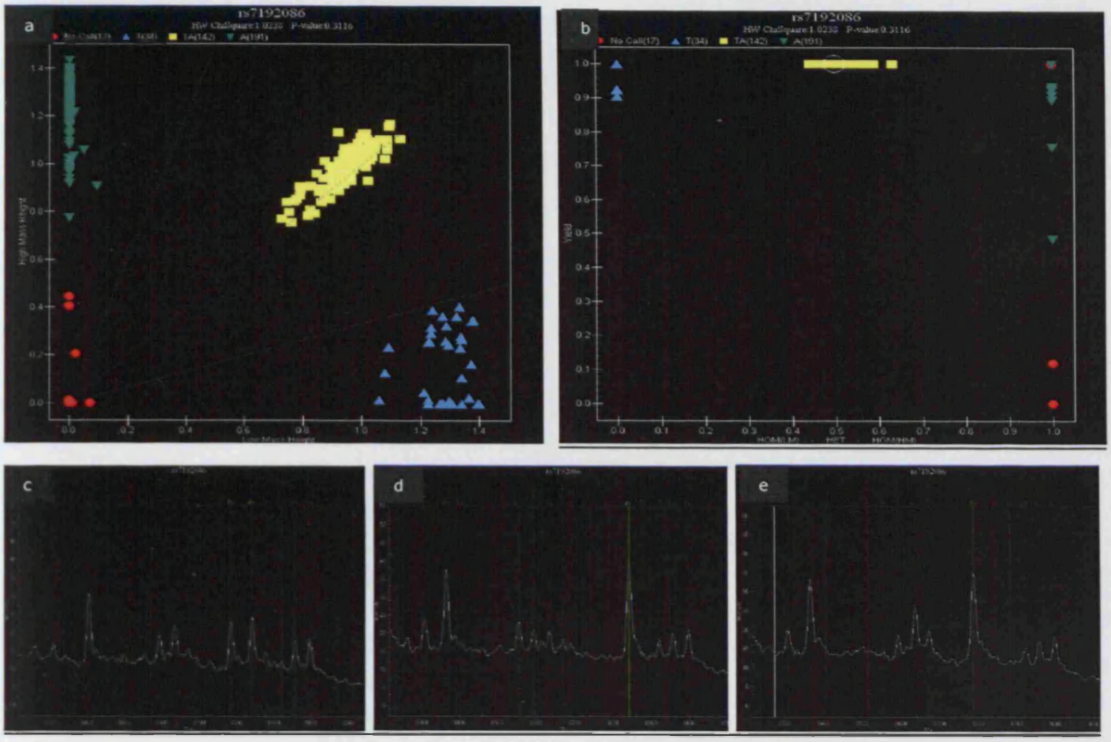


Figure 4.2. *Example of Sequenom TyperAnalyser genotyping suite for variant rs7192086.* a. Is a log height cluster diagram through 384 samples. Red cluster indicates blank/failed samples; green cluster indicates A homozygotes (see d); yellow cluster indicates heterozygotes (see c); and blue cluster indicates T homozygotes (see e). b. Is an alternate yield v skew cluster view of the same samples. Clusters are as described for a. c. shows an example of heterozygote spectra. Remaining extension primer peak is indicated by red dotted line. Peak for the T allele is indicated by the left yellow dotted line. Peak for the A allele is indicated by the right yellow dotted line. d. Is an example of A allele homozygote spectra. Remaining extension primer peak is indicated by left red dotted line. Expected peak for the T allele is indicated by the right red dotted line. Peak for the A allele is indicated by the yellow dotted line. e. Is spectra for a T allele homozygote. Remaining extension primer peak is indicated by left red dotted line. Expected peak for the A allele is indicated by the right red dotted line. Peak for the T allele is indicated by the yellow dotted line. a and b are used to identify suspicious genotype calls, specified by the TyperAnalyser algorithm, and the spectra output (c,d or e) is utilised by the user to manually allocate a genotype. [Figure produced using Sequenom TyperAnalyser software.]

Chapter 5 Appendix

Mayo / ALS (US)	10.0	n/a	5.0	n/a
Coriell / Mayo+ALS (US)	25.0	n/a	n/a	n/a
610 (US) / Mayo+ALS (US)	25.0	n/a	14.0	n/a
610 (US) / Coriell	20.0	18.5	n/a	n/a

n/a = not applicable.

Post QC					
n*	999	229	136	48	1412
LOAD+P (%)	374 (37.4)	93 (40.6)	61 (44.9)	15 (31.3)	543 (38.5)
LOAD-P (%)	343 (34.3)	52 (22.7)	12 (8.8)	10 (20.8)	417 (29.5)
Possible LOAD+P# (%)	138 (13.8)	60 (26.2)	49 (36.0)	10 (20.8)	257 (18.2)
LOAD-P, mild LOAD+ (%)	144 (14.4)	24 (10.5)	14 (10.3)	13 (27.1)	195 (13.8)

* Total number of LOAD cases with available Neuropsychiatric Inventory and genotype data.

Mild psychotic symptoms present but do not meet criteria for LOAD+P.

+ No psychotic symptoms present but have not past psychosis risk stages of LOAD.

Table 5.3. LOAD+P versus LOAD-P. Results for SNPs with p-values ≤ $1x10^{-4}$. Table shows SNP rank, chromosome, ba
associated allele, p-value and OR in LOAD+P vs. LOAD-P and LOAD+P vs. control analyses.

Rank	Chr	SNP	BP	Gene	Type	Allele	*P*
1	8	rs2034140	6224172	MCPH1	INTERGENIC	G	7.62E-06
2	6	rs11756091	39390784	KCNK16	NON-SYN	T	8.06E-06
3	8	rs10956535	131623943	ASAP1	INTERGENIC	C	8.79E-06
4	4	rs753129	56363188	AC110611.3-1	DOWNSTREAM	G	9.98E-06
5	9	rs960644	105060805	RP11-341A22.1	INTERGENIC	G	1.04E-05
6	12	rs2682778	117544535	SUDS3	INTERGENIC	T	1.45E-05
7	10	rs1444400	52904161	PRKG1	INTRONIC	G	1.48E-05
8	10	rs1444401	52904934	PRKG1	INTRONIC	A	1.50E-05
9	10	rs6480276	52907458	PRKG1	INTRONIC	G	1.59E-05
10	2	rs11680774	158954231	CCDC148	INTRONIC	C	2.00E-05
11	2	rs2883854	158947298	CCDC148	INTRONIC	C	2.07E-05
12	6	rs3734618	39392162	KCNK16	SYNONYMOUS	C	2.09E-05
13	1	rs12045777	29301591	EPB41	INTRONIC	G	2.18E-05
14	12	rs1520780	117524108	SUDS3	INTERGENIC	A	2.75E-05
15	4	rs2695234	24413336	SOD3	DOWNSTREAM	T	3.39E-05
16	4	rs12648438	188865648	AC115540.3	INTERGENIC	A	3.40E-05
17	6	rs13201744	6071844	F13A1	INTERGENIC	T	4.31E-05
18	4	rs2100889	88917369	IBSP	INTERGENIC	A	4.51E-05
19	2	rs3771599	159105674	PK4P;PKP4	INTRONIC	G	4.88E-05
20	2	rs11674284	159081135	PK4P;PKP4	INTRONIC	C	4.90E-05
21	2	rs6711582	159036181	PK4P;PKP4	INTRONIC	T	5.28E-05
22	2	rs2356186	159072718	PK4P;PKP4	INTRONIC	T	5.86E-05
23	1	rs2640467	29428745	MECR	INTRONIC	C	5.91E-05

24	6	rs9404549	104959117	RP11-427E4.1	INTERGENIC	T	5.91E-05
25	7	rs10235799	149431398	AC006008.2	INTERGENIC	C	6.04E-05
26	12	rs11535963	18245787	AC087240.17	INTERGENIC	T	6.48E-05
27	12	rs11044223	18675030	PIK3C2G	INTRONIC	C	6.65E-05
28	1	rs3929673	14225658	C1orf196	INTRONIC	T	6.88E-05
29	6	rs7756959	104974286	RP11-427E4.1	INTERGENIC	C	6.94E-05
30	4	rs6828769	188885970	AC115540.3	INTERGENIC	G	7.01E-05
31	2	rs3764835	159227614	AC005042.2	INTRONIC	T	7.13E-05
32	16	rs17720179	27096354	JMJD5	INTERGENIC	T	7.15E-05
33	2	rs3771635	159158409	PK4P;PKP4	INTRONIC	G	7.44E-05
34	4	rs1989924	113564134	ALPK1	INTRONIC	A	7.49E-05
35	14	rs10143543	23265976	AL160237.4-1	INTERGENIC	A	7.53E-05
36	6	rs7740010	104962863	RP11-427E4.1	INTERGENIC	A	7.54E-05
37	6	rs156235	104978232	RP11-427E4.1	INTERGENIC	C	7.54E-05
38	16	rs8061432	25762112	HS3ST4	INTRONIC	T	7.64E-05
39	2	rs3755365	70798073	ADD2	INTRONIC	C	7.74E-05
40	16	rs17674628	8799972	PMM2	INTRONIC	A	7.87E-05
41	10	rs11252926	556379	DIP2C	INTRONIC	A	8.02E-05
42	7	rs17156246	81872957	CACNA2D1	INTRONIC	T	8.06E-05
43	3	rs9867560	163153274	AC104471.6	INTERGENIC	A	8.26E-05
44	3	rs17443484	163156164	AC104471.6	INTERGENIC	T	8.26E-05
45	10	rs11252923	556195	DIP2C	INTRONIC	T	8.35E-05
46	1	rs1146413	81371728	AL158218.11	INTERGENIC	C	8.58E-05
47	3	rs9865618	163165408	AC104471.6	INTERGENIC	G	8.87E-05
48	2	rs2080394	70862957	FIGLA	INTRONIC	C	8.89E-05
49	15	rs17237486	58991573	RORA	INTRONIC	G	9.03E-05
50	10	rs7089944	13017856	CCDC3	INTRONIC	C	9.08E-05

51	2	rs3771408	70834654	ADD2	INTRONIC	A	9.24E-05
52	3	rs2139266	142009170	TRIM42	INTERGENIC	C	9.32E-05
53	13	rs9512519	26522181	USP12	INTERGENIC	C	9.42E-05
54	1	rs1768404	81361200	AL158218.11	INTERGENIC	A	9.70E-05
55	8	rs7015252	131624931	ASAP1	INTERGENIC	A	9.75E-05
56	2	rs2681031	23084067	AC104807.5-2	INTERGENIC	G	9.98E-05
57	2	rs7597989	187935145	CALCRL	INTRONIC	A	0.0001
58	4	rs6826655	188845047	AC097521.2-2	INTERGENIC	A	0.0001
59	14	rs399223	58431254	AL049873.3-2	INTERGENIC	C	0.0001
60	13	rs9284246	108125789	MYO16	INTRONIC	G	0.0001
61	6	rs9377643	105041172	RP11-427E4.1	INTERGENIC	C	0.0001
62	2	rs17808559	158880898	CCDC148	INTRONIC	T	0.0001
63	2	rs4853462	191832049	MYO1B	INTRONIC	C	0.0001
64	14	rs177726	58447157	AL049873.3-2	INTERGENIC	T	0.0001
65	14	rs442555	58365937	AL049873.3-2	UPSTREAM	C	0.0001
66	10	rs9664256	83767735	NRG3	INTRONIC	G	0.0001
67	13	rs12560710	30053442	AK122825	INTERGENIC	A	0.0001
68	5	rs683004	29539692	AC010374.6	INTERGENIC	A	0.0001
69	10	rs3903435	83766437	NRG3	INTRONIC	G	0.0001
70	10	rs4881399	557325	DIP2C	INTRONIC	T	0.0001
71	3	rs13070407	25468182	RARB	INTRONIC	G	0.0001
72	14	rs6573225	58354640	AL049873.3-2	INTERGENIC	C	0.0001
73	6	rs267339	104985242	RP11-427E4.1	INTERGENIC	A	0.0001
74	11	rs750270	132591508	OPCML	INTRONIC	A	0.0001
75	5	rs620508	29543052	AC010374.6	INTERGENIC	G	0.0001
76	8	rs7842680	56093552	XKR4	INTERGENIC	C	0.0001
77	3	rs925665	5101391	AC090955.3	DOWNSTREAM	A	0.0001

78	11	rs598829	131247810	HNT;NTM	INTRONIC	A	0.0001
79	4	rs2303513	119946397	SEC24D	INTRONIC	A	0.0001
80	11	rs17794760	18012496	TPH1	INTRONIC	A	0.0001
81	3	rs4613440	14530815	GRIP2	SYNONYMOUS	T	0.0001
82	6	rs775258	101840784	GRIK2	INTERGENIC	G	0.0001
83	2	rs3755361	70798759	ADD2	INTRONIC	T	0.0002
84	19	rs4805441	34780133	POP4	INTERGENIC	C	0.0002
85	9	rs902143	108221732	AL512649.6	INTERGENIC	A	0.0002
86	3	rs7614031	118057332	AC069504.7	INTERGENIC	A	0.0002
87	12	rs2285831	46411969	RAPGEF3	INTERGENIC	A	0.0002
88	7	rs12669501	93832944	COL1A2	INTERGENIC	A	0.0002
89	8	rs11781115	26737367	ADRA1A	INTRONIC	G	0.0002
90	16	rs4548875	25818713	HS3ST4	INTRONIC	A	0.0002
91	19	rs3810342	43599425	RASGRP4	INTRONIC	G	0.0002
92	2	rs1918901	187851922	AC074020.4	INTERGENIC	C	0.0002
93	5	rs4527553	65899491	AC092373.2-2	INTRONIC	T	0.0002
94	16	rs2304469	8803595	PMM2	INTRONIC	C	0.0002
95	5	rs286969	153301961	FAM114A2	INTERGENIC	T	0.0002
96	14	rs11851391	93287901	PRIMA1	INTRONIC	A	0.0002
97	5	rs1550825	14961578	AC016575.7	UPSTREAM	A	0.0002
98	18	rs7231736	831321	YES1	INTERGENIC	G	0.0002
99	8	rs6470563	128777752	MYC	INTERGENIC	C	0.0002
100	14	rs983449	58481542	AL049873.3-2	INTERGENIC	T	0.0002
101	19	rs6509701	58075997	ZNF320	SYNONYMOUS	C	0.0002
102	1	rs1894654	7274021	CAMTA1	INTRONIC	G	0.0002
103	2	rs7563401	69833697	ANXA4	INTRONIC	A	0.0002
104	1	rs12038526	47955182	AL691459.25	INTERGENIC	G	0.0002

105	9	rs16922740	105116091	RP11-341A22.1	INTERGENIC	C	0.0002
106	10	rs10823083	52925664	PRKG1	INTRONIC	A	0.0002
107	9	rs1329771	72964676	TRPM3	INTRONIC	G	0.0002
108	9	rs7852394	119611072	TLR4	INTERGENIC	C	0.0002
109	19	rs6509698	58050701	ZNF468	INTRONIC	T	0.0002
110	7	rs4729127	93846471	COL1A2	INTERGENIC	T	0.0002
111	10	rs7079742	19016665	ARL5B	INTERGENIC	C	0.0002
112	5	rs607077	29531321	AC010374.6	INTERGENIC	G	0.0002
113	11	rs2468782	18092218	SAA3P	INTRONIC	G	0.0002
114	3	rs295464	140789265	NMNAT3	INTRONIC	G	0.0002
115	16	rs4362392	25818949	HS3ST4	INTRONIC	C	0.0002
116	2	rs3771644	159175252	PK4P;PKP4	INTRONIC	A	0.0002
117	13	rs4343137	111113593	RP11-65D24.2	INTRONIC	T	0.0002
118	3	rs9823958	141037367	CLSTN2	INTERGENIC	A	0.0002
119	2	rs7574570	191806761	MYO1B	INTERGENIC	C	0.0002
120	9	rs10739508	119636032	TLR4	INTERGENIC	C	0.0002
121	2	rs7567400	69834799	ANXA4	INTRONIC	A	0.0002
122	5	rs905858	29506179	AC010374.6	INTERGENIC	G	0.0002
123	13	rs9513354	97550726	FARP1	INTERGENIC	A	0.0002
124	18	rs8099057	34021742	n/a	INTERGENIC	A	0.0002
125	2	rs10445783	191809743	MYO1B	INTERGENIC	G	0.0002
126	2	rs12470671	8420670	AC011747.5	INTERGENIC	A	0.0002
127	4	rs7687115	188845851	AC097521.2-2	INTERGENIC	G	0.0002
128	19	rs3764640	1158238	STK11	INTRONIC	A	0.0002
129	9	rs7854254	119624938	TLR4	INTERGENIC	G	0.0002
130	7	rs11767477	77802603	MAGI2	INTRONIC	C	0.0002
131	3	rs188423	140773159	NMNAT3	INTRONIC	T	0.0002

132	6	rs6931820	93963094	EPHA7	INTERGENIC	T	0.0003
133	13	rs1517897	36015431	RP11-56M2.1	INTERGENIC	T	0.0003
134	16	rs12049	8797129	TMEM186;C16orf51	3 PRIME UTR	T	0.0003
135	8	rs10099199	18422501	PSD3	INTERGENIC	G	0.0003
136	9	rs10810562	16387155	BNC2	INTERGENIC	A	0.0003
137	3	rs6800179	141050356	CLSTN2	INTERGENIC	C	0.0003
138	3	rs6441421	163152503	AC104471.6	INTERGENIC	T	0.0003
139	7	rs10954664	81613491	CACNA2D1	INTRONIC	G	0.0003
140	3	rs1376967	83584638	AC129415.1-2	INTERGENIC	G	0.0003
141	8	rs6559175	6521408	MCPH1	INTERGENIC	C	0.0003
142	13	rs11617079	42495865	DNAJC15	NON-SYN	G	0.0003
143	13	rs2281782	42496191	DNAJC15	INTRONIC	C	0.0003
144	8	rs1441249	87683359	CNGB3	INTRONIC	T	0.0003
145	6	rs855250	9716873	OFCC1	INTERGENIC	C	0.0003
146	4	rs4699587	95834792	PDLIM5	INTERGENIC	C	0.0003
147	12	rs678941	112573616	RBM19	INTERGENIC	T	0.0003
148	3	rs7637623	152812216	AC069067.17	INTERGENIC	G	0.0003
149	1	rs7556195	230799588	SIPA1L2	INTERGENIC	G	0.0003
150	13	rs1927554	35965699	RP11-56M2.1	UPSTREAM	C	0.0003
151	13	rs4271475	103916726	RP11-217F17.1	INTERGENIC	T	0.0003
152	11	rs4758480	3175285	MRGPRG	INTERGENIC	T	0.0003
153	10	rs10827186	33413756	RPL7AP53	INTERGENIC	G	0.0003
154	1	rs655146	60383617	RP11-575B7.2	INTERGENIC	G	0.0003
155	1	rs1041705	80236125	RP11-339A11.1	INTERGENIC	C	0.0003
156	3	rs11706561	25430847	RARB	INTRONIC	T	0.0003
157	2	rs1517443	221268006	AC019051.8	INTERGENIC	G	0.0003
158	12	rs1663588	67563432	CPM	INTRONIC	A	0.0003

159	4	rs16890321	13979845	AC093777.4	INTERGENIC	T	0.0003
160	14	rs6573334	23273635	AL160237.4-1	INTERGENIC	T	0.0003
161	11	rs10431058	106986728	ELMOD1	INTERGENIC	T	0.0003
162	15	rs954432	36753947	C15orf53	INTERGENIC	C	0.0003
163	1	rs7515917	14311629	C1orf196	INTRONIC	G	0.0003
164	15	rs2711644	51939427	AC066611.6	INTERGENIC	T	0.0004
165	7	rs16885938	77798165	MAGI2	INTRONIC	T	0.0004
166	11	rs604967	112191202	AP003100.3	INTERGENIC	T	0.0004
167	5	rs2074344	136989299	KLHL3	INTRONIC	G	0.0004
168	10	rs1930457	59419237	RP11-448K10.1	INTERGENIC	A	0.0004
169	6	rs10945617	159872422	RP3-393E18.1	UPSTREAM	A	0.0004
170	4	rs4082297	54303520	LNX1	INTERGENIC	C	0.0004
171	9	rs10963919	19021231	FAM154A	INTRONIC	G	0.0004
172	2	rs7593799	187791545	AC074020.4	INTERGENIC	T	0.0004
173	7	rs700316	147002743	AC006992.2	INTRONIC	A	0.0004
174	10	rs1125478	59289172	RP11-448K10.1	INTERGENIC	T	0.0004
175	20	rs2427460	61061227	SLC17A9	INTRONIC	C	0.0004
176	16	rs11150535	81644467	CDH13	INTRONIC	T	0.0004
177	12	rs621042	18680274	PIK3C2G	INTRONIC	A	0.0004
178	2	rs2312548	69809264	ANXA4	INTERGENIC	A	0.0004
179	3	rs1447721	141038576	CLSTN2	INTERGENIC	C	0.0004
180	11	rs868344	117510329	SCN4B	3 PRIME UTR	T	0.0004
181	8	rs10088108	20107092	ATP6V1B2	INTRONIC	G	0.0004
182	6	rs1544050	39387109	KCNK17	INTRONIC	C	0.0004
183	1	rs2990678	13709800	LRRC38	INTRONIC	A	0.0004
184	1	rs2365716	154881818	BCAN	INTRONIC	C	0.0004
185	5	rs329320	133936260	PHF15	INTRONIC	G	0.0004

186	5	rs2042243	135611811	TRPC7	INTRONIC	A	0.0004
187	9	rs4877108	90573556	NXNL2	INTERGENIC	C	0.0004
188	14	rs7160035	23270407	AL160237.4-1	INTERGENIC	T	0.0004
189	4	rs9996873	170793421	CLCN3	INTRONIC	G	0.0004
190	14	rs17121169	85038905	AL049775.2	INTERGENIC	C	0.0004
191	2	rs7564839	158936837	CCDC148	INTRONIC	G	0.0004
192	10	rs7094118	11296448	CUGBP2	INTRONIC	C	0.0004
193	1	rs10493201	55537080	GOT2L1	INTERGENIC	C	0.0004
194	2	rs1583631	211771056	ERBB4	INTERGENIC	T	0.0004
195	4	rs4861491	182864573	AC108142.1	INTERGENIC	T	0.0004
196	5	rs12189367	9040361	SEMA5A	INTERGENIC	A	0.0004
197	2	rs1569135	187823643	AC074020.4	INTERGENIC	G	0.0004
198	20	rs1016404	50336317	ZFP64	INTERGENIC	A	0.0004
199	11	rs643122	112192679	AP003100.3	INTERGENIC	C	0.0004
200	13	rs2897319	46665008	RP11-94N9.1	INTERGENIC	C	0.0004
201	2	rs3771084	187951878	CALCRL	INTRONIC	A	0.0004
202	14	rs10484053	95081398	GLRX5	DOWNSTREAM	T	0.0004
203	12	rs2130635	82372409	AC090680.11	INTERGENIC	C	0.0004
204	16	rs1532472	84743139	AC040170.9	INTERGENIC	T	0.0005
205	9	rs7036118	19023376	FAM154A	UPSTREAM	T	0.0005
206	11	rs4565912	123409605	OR10G8	DOWNSTREAM	C	0.0005
207	1	rs1426786	81313972	RP11-2B19.1	INTERGENIC	A	0.0005
208	7	rs2520570	112063917	AC005192.1	INTERGENIC	G	0.0005
209	10	rs1999505	59413258	RP11-448K10.1	INTERGENIC	C	0.0005
210	9	rs7047010	15077382	RP11-54D18.4	INTERGENIC	C	0.0005
211	2	rs11690896	118507870	AC009303.3-1	INTERGENIC	C	0.0005
212	18	rs1544241	845264	YES1	INTERGENIC	T	0.0005

213	16	rs1864	8797034	TMEM186;C16orf51	3 PRIME UTR	G	0.0005
214	14	rs1263805	21052472	METTL3	UPSTREAM	C	0.0005
215	7	rs2693727	129204966	MIRN183	UPSTREAM	G	0.0005
216	2	rs4851161	98823293	C2orf55	INTRONIC	C	0.0005
217	10	rs1414395	13374142	PHYH	INTRONIC	T	0.0005
218	19	rs8102902	3720049	RAX2	INTRONIC	G	0.0005
219	14	rs10149208	58313091	AL121819.6	INTERGENIC	A	0.0005
220	14	rs7155550	58304017	AL121819.6	DOWNSTREAM	C	0.0005
221	1	rs12035356	153020091	KCNN3	INTRONIC	C	0.0005
222	6	rs911182	137076157	MAP3K5	INTRONIC	G	0.0005
223	11	rs2445290	4976992	OR51L1	SYNONYMOUS	A	0.0005
224	18	rs7236716	54318116	ALPK2	INTRONIC	T	0.0005
225	11	rs687572	128144543	FLI1	INTRONIC	C	0.0005
226	3	rs11712892	142016513	TRIM42	INTERGENIC	A	0.0005
227	13	rs9519340	103919432	RP11-217F17.1	INTERGENIC	A	0.0005
228	2	rs7596564	70859754	FIGLA	INTRONIC	A	0.0005
229	2	rs7597155	69838939	ANXA4	INTRONIC	T	0.0005
230	3	rs10937074	183120027	AC007547.26-2	INTERGENIC	A	0.0005
231	15	rs779885	60549664	AC032011.15	INTERGENIC	G	0.0005
232	10	rs7913161	125554890	CPXM2	INTRONIC	G	0.0005
233	2	rs12622388	69834530	ANXA4	INTRONIC	C	0.0005
234	21	rs12482075	37881096	KCNJ6	INTERGENIC	T	0.0005
235	1	rs2481665	62367265	INADL	INTRONIC	C	0.0005
236	11	rs11030026	27467406	LIN7C	INTERGENIC	A	0.0005
237	3	rs12638515	164473177	AC104637.5	INTERGENIC	G	0.0005
238	1	rs13353041	55546344	GOT2L1	INTERGENIC	A	0.0005
239	6	rs12207681	9728632	OFCC1	INTERGENIC	C	0.0005

240	2	rs6705773	221286642	AC019051.8	INTERGENIC	G	0.0005
241	5	rs255240	123187997	AC008580.7	INTERGENIC	C	0.0005
242	3	rs9868790	117961048	BZW1L1	INTERGENIC	C	0.0005
243	4	rs1872270	88828705	DMP1	INTERGENIC	A	0.0005
244	4	rs7438821	188887227	AC115540.3	INTERGENIC	T	0.0005
245	16	rs246174	14287432	AC040173.5	INTERGENIC	A	0.0005
246	18	rs12232768	23033599	CHST9	INTERGENIC	C	0.0005
247	10	rs16911207	59352070	RP11-448K10.1	INTERGENIC	C	0.0005
248	11	rs7939810	27589985	AC104563.14	INTERGENIC	G	0.0005
249	3	rs9831813	129961232	RAB7A	INTRONIC	A	0.0005
250	3	rs4234525	14522806	GRIP2	INTRONIC	T	0.0005
251	9	rs10125646	19035502	RRAGA	UPSTREAM	G	0.0005
252	1	rs7511712	118081431	AL390877.1	INTERGENIC	G	0.0005
253	5	rs6555968	171045081	C5orf50	INTERGENIC	A	0.0005
254	8	rs10097956	24150656	ADAM28	INTERGENIC	G	0.0005
255	16	rs11863067	17701614	XYLT1	INTERGENIC	T	0.0005
256	14	rs12889310	82462910	AL163171.4	INTERGENIC	C	0.0005
257	6	rs267333	104963333	RP11-427E4.1	INTERGENIC	A	0.0005
258	1	rs12569332	209952869	LPGAT1	INTERGENIC	C	0.0005
259	10	rs17684713	127942864	ADAM12	INTRONIC	A	0.0005
260	1	rs126013	29092857	EPB41	5 PRIME UTR	A	0.0005
261	14	rs8005907	95857500	ATG2B	INTRONIC	C	0.0005
262	2	rs13407117	158889282	CCDC148	INTRONIC	A	0.0005
263	4	rs10516985	97049034	AC093828.3	INTERGENIC	C	0.0005
264	5	rs6875311	123207148	AC008580.7	INTERGENIC	C	0.0005
265	8	rs2912004	6495247	MCPH1	DOWNSTREAM	A	0.0005
266	8	rs11774901	131550501	ASAP1	INTERGENIC	T	0.0005

267	8	rs7835207	143205285	TSNARE1	INTERGENIC	G	0.0005
268	2	rs3771449	70796926	ADD2	INTRONIC	G	0.0005
269	10	rs7067598	23257705	ARMC3	INTRONIC	G	0.0006
270	6	rs12213004	9762524	OFCC1	INTERGENIC	T	0.0006
271	2	rs6707940	70851745	FIGLA	INTRONIC	G	0.0006
272	14	rs1112627	82465636	AL163171.4	INTERGENIC	T	0.0006
273	13	rs912521	28059336	RP11-57H24.1	INTERGENIC	A	0.0006
274	18	rs17805412	9874503	TXNDC2	UPSTREAM	A	0.0006
275	14	rs754131	58291699	AL121819.6	INTERGENIC	G	0.0006
276	2	rs934287	203416552	ICA1L	INTRONIC	T	0.0006
277	6	rs10806716	159872442	RP3-393E18.1	UPSTREAM	A	0.0006
278	18	rs9807134	33939251	n/a	INTERGENIC	A	0.0006
279	14	rs1547350	103716079	KIF26A	3 PRIME UTR	T	0.0006
280	1	rs2999865	13713882	LRRC38	UPSTREAM	A	0.0006
281	6	rs7748110	108025075	SOBP	INTRONIC	A	0.0006
282	10	rs7897633	52627727	PRKG1	INTRONIC	G	0.0006
283	5	rs6873055	153253361	GRIA1	INTERGENIC	A	0.0006
284	16	rs896401	8394656	C16orf68	INTERGENIC	G	0.0006
285	2	rs3771669	159217221	PK4P;PKP4	INTRONIC	G	0.0006
286	16	rs1079348	8780957	ABAT	INTRONIC	C	0.0006
287	6	rs4707016	84591076	C6orf59;RIPPLY2	INTERGENIC	A	0.0006
288	13	rs1360974	101794714	FGF14	INTRONIC	T	0.0006
289	1	rs2152391	80281442	RP11-339A11.1	INTERGENIC	A	0.0006
290	1	rs10493658	80286294	RP11-339A11.1	INTERGENIC	T	0.0006
291	4	rs17615522	38204296	AC096739.3	INTERGENIC	C	0.0006
292	2	rs3771604	159123225	PK4P;PKP4	INTRONIC	T	0.0006
293	11	rs11234095	83990352	DLG2	INTRONIC	G	0.0006

294	11	rs10898274	83990370	DLG2	INTRONIC	T	0.0006
295	11	rs4579932	131403489	HNT;NTM	INTRONIC	C	0.0006
296	3	rs6764249	38705121	SCN10A	INTERGENIC	G	0.0006
297	1	rs7556538	90666105	AC098656.2	INTERGENIC	C	0.0006
298	10	rs4748737	21258985	NEBL	INTRONIC	G	0.0006
299	19	rs718133	40563547	AC002511.1-2	UPSTREAM	T	0.0006
300	14	rs7149631	58303507	AL121819.6	DOWNSTREAM	T	0.0006
301	20	rs11574739	42476364	HNF4A	INTRONIC	C	0.0006
302	15	rs8039372	60409849	AC032011.15	INTERGENIC	C	0.0006
303	4	rs9307041	88862789	AC093895.3	INTERGENIC	A	0.0006
304	3	rs1499508	65667103	AC121493.1	INTRONIC	C	0.0006
305	11	rs532859	106984862	ELMOD1	INTERGENIC	A	0.0006
306	17	rs481648	15672648	AC015922.5	UPSTREAM	C	0.0006
307	9	rs1124647	105085893	RP11-341A22.1	INTERGENIC	G	0.0006
308	9	rs10811094	18999041	FAM154A	INTRONIC	A	0.0006
309	10	rs4880336	133783495	JAKMIP3	INTRONIC	T	0.0007
310	5	rs10055973	180052524	AC113427.2	INTERGENIC	A	0.0007
311	5	rs11738161	180052616	AC113427.2	INTERGENIC	A	0.0007
312	5	rs13188129	180052650	AC113427.2	INTERGENIC	T	0.0007
313	5	rs11738216	180052755	AC113427.2	INTERGENIC	C	0.0007
314	10	rs7908946	127903558	ADAM12	INTRONIC	A	0.0007
315	4	rs12509170	99765951	TSPAN5	INTRONIC	G	0.0007
316	4	rs7688470	21781096	AC093735.2	INTERGENIC	T	0.0007
317	10	rs7094054	83769819	NRG3	INTRONIC	T	0.0007
318	4	rs7655220	21746131	KCNIP4	INTERGENIC	G	0.0007
319	3	rs6783373	184440267	MCF2L2	INTRONIC	A	0.0007
320	10	rs7079003	1967135	ADARB2	INTERGENIC	T	0.0007

321	21	rs4818184	41124301	DSCAM	INTRONIC	A	0.0007
322	2	rs3771450	70796646	ADD2	INTRONIC	C	0.0007
323	2	rs7577964	24451783	AC009228.4-2	INTERGENIC	A	0.0007
324	17	rs6503608	33835225	SOCS7	INTERGENIC	T	0.0007
325	7	rs2693737	129218230	MIRN183	INTERGENIC	C	0.0007
326	13	rs9300636	100362458	NALCN;VGCNL1	INTERGENIC	C	0.0007
327	20	rs6067566	48888318	BCAS4	INTRONIC	T	0.0007
328	13	rs7327207	19392558	ZMYM2	INTERGENIC	C	0.0007
329	12	rs1351681	33589991	AC024153.22	INTERGENIC	T	0.0007
330	1	rs11240240	202883128	LRRN2	INTRONIC	C	0.0007
331	3	rs9853945	130011320	RAB7A	INTRONIC	G	0.0007
332	9	rs3780256	17476802	CNTLN	INTRONIC	T	0.0007
333	15	rs11635381	99488806	CHSY1	INTERGENIC	A	0.0007
334	9	rs10867685	82864268	RP11-553J6.1	INTERGENIC	G	0.0007
335	6	rs16888746	162010971	PARK2	INTRONIC	T	0.0007
336	1	rs4653186	36635516	LSM10	INTRONIC	T	0.0007
337	2	rs35907069	69794284	ANXA4	INTERGENIC	T	0.0007
338	2	rs12612409	69794791	ANXA4	INTERGENIC	A	0.0007
339	18	rs1789597	52003253	AC009271.7	INTRONIC	G	0.0007
340	9	rs11142705	72955297	TRPM3	INTRONIC	A	0.0007
341	17	rs16968987	52365061	COIL	INTERGENIC	T	0.0007
342	14	rs9972193	58488475	AL049873.3-2	INTERGENIC	C	0.0007
343	4	rs2869703	88845302	AC093895.3	INTERGENIC	C	0.0007
344	18	rs11081236	6433601	AP005202.3	INTERGENIC	T	0.0007
345	15	rs12443269	51914772	AC066611.6	INTERGENIC	C	0.0007
346	10	rs7083429	68973427	CTNNA3	INTRONIC	C	0.0007
347	2	rs1157699	187967149	CALCRL	INTRONIC	T	0.0007

348	13	rs9575351	83049998	AL512782.6-2	INTERGENIC	C	0.0008
349	16	rs7188565	6748158	AC125796.2	INTERGENIC	A	0.0008
350	13	rs1333464	92629476	GPC6	INTERGENIC	T	0.0008
351	18	rs9956147	33926617	n/a	INTERGENIC	C	0.0008
352	2	rs7603997	24378462	ITSN2	NON-SYN	T	0.0008
353	15	rs4778147	25426340	GABRG3	INTRONIC	C	0.0008
354	1	rs1752221	81368322	AL158218.11	INTERGENIC	T	0.0008
355	3	rs1353021	172573441	TNIK	INTRONIC	A	0.0008
356	16	rs17671037	6740651	AC125796.2	INTERGENIC	C	0.0008
357	16	rs860380	72116684	C16orf47	INTERGENIC	C	0.0008
358	13	rs1517881	36027895	RP11-56M2.1	INTERGENIC	T	0.0008
359	4	rs9999510	146857486	ZNF827	INTERGENIC	C	0.0008
360	1	rs2003046	10955414	C1orf127	INTERGENIC	A	0.0008
361	14	rs4569181	75883430	ESRRB	INTERGENIC	A	0.0008
362	4	rs17211183	92428986	TMSL3	INTERGENIC	A	0.0008
363	11	rs871437	132309082	OPCML	INTRONIC	C	0.0008
364	8	rs2249963	11512635	C8orf14	INTERGENIC	C	0.0008
365	4	rs7697101	21783869	AC093735.2	INTERGENIC	C	0.0008
366	5	rs7707671	74323498	AC116337.2	UPSTREAM	C	0.0008
367	10	rs7912903	1974760	ADARB2	INTERGENIC	T	0.0008
368	1	rs12039988	29298420	EPB41	INTRONIC	A	0.0008
369	13	rs1927522	26230836	GPR12	3 PRIME UTR	T	0.0008
370	5	rs17113869	152355671	GRIA1	INTERGENIC	C	0.0008
371	15	rs723988	99496248	CHSY1	INTERGENIC	T	0.0008
372	3	rs13096015	183100621	AC007547.26-2	INTERGENIC	G	0.0008
373	3	rs6794105	22939325	AC099544.2	INTERGENIC	A	0.0008
374	15	rs13380359	24884848	GABRG3	INTRONIC	G	0.0008

375	2	rs7599725	221292815	AC019051.8	INTERGENIC	T	0.0008
376	4	rs17539365	22323903	GBA3	INTRONIC	A	0.0008
377	2	rs2702089	24496795	AC009228.4-2	INTERGENIC	A	0.0008
378	1	rs12741645	44335934	RP5-1198O20.5	INTERGENIC	T	0.0008
379	3	rs2090702	14518673	GRIP2	INTRONIC	T	0.0008
380	22	rs2235171	30980200	SLC5A4	NON-SYN	T	0.0008
381	3	rs1580800	20914295	AC104441.2	INTERGENIC	A	0.0008
382	5	rs7718958	134121831	DDX46	UPSTREAM	C	0.0008
383	5	rs13189947	153050585	GRIA1	INTRONIC	A	0.0008
384	11	rs12224791	123424338	AP001884.4	INTERGENIC	G	0.0008
385	5	rs1461243	153263155	FAM114A2	INTERGENIC	C	0.0008
386	1	rs7513428	165781896	CREG1	INTRONIC	A	0.0008
387	9	rs10964182	19484098	AL158206.8-2	UPSTREAM	T	0.0008
388	17	rs759974	347709	VPS53	INTERGENIC	T	0.0008
389	10	rs1243194	22020520	MLLT10	INTRONIC	T	0.0008
390	9	rs16937267	19001300	FAM154A	INTRONIC	C	0.0008
391	4	rs6829972	14252310	AC093777.4	INTERGENIC	G	0.0008
392	7	rs42523	93878935	COL1A2	INTRONIC	C	0.0008
393	10	rs6482189	21929144	MLLT10	INTRONIC	A	0.0008
394	6	rs9502396	5992248	NRN1	INTERGENIC	G	0.0008
395	6	rs1324103	93957737	EPHA7	INTERGENIC	C	0.0008
396	3	rs182568	4312312	SUMF1	INTRONIC	T	0.0009
397	11	rs4930103	1981120	H19	INTERGENIC	G	0.0009
398	7	rs4256491	15384424	TMEM195	INTRONIC	A	0.0009
399	6	rs9688888	169462608	XXyac-YX65C7_A.4	INTERGENIC	G	0.0009
400	12	rs2555319	118199160	CCDC60	INTERGENIC	C	0.0009
401	9	rs7041706	16382810	BNC2	INTERGENIC	C	0.0009

402	1	rs7520966	54417850	CYB5RL	INTRONIC	T	0.0009
403	4	rs13152723	21812805	AC093735.2	INTERGENIC	G	0.0009
404	2	rs6730730	228232354	SLC19A3	INTERGENIC	G	0.0009
405	8	rs2013265	24148445	ADAM28	INTERGENIC	A	0.0009
406	7	rs4615456	15440324	TMEM195	INTRONIC	G	0.0009
407	16	rs1369790	59149079	AC018554.7	INTERGENIC	G	0.0009
408	8	rs777801	116245300	TRPS1	INTERGENIC	T	0.0009
409	8	rs9642799	106326386	ZFPM2	INTERGENIC	A	0.0009
410	11	rs1400756	110585670	C11orf53	INTERGENIC	C	0.0009
411	7	rs17169762	34361776	AC005582.1	INTRONIC	C	0.0009
412	6	rs434310	95061282	RP3-399J4.2	INTERGENIC	A	0.0009
413	12	rs10844642	33621635	AC024153.22	DOWNSTREAM	C	0.0009
414	5	rs29645	123176335	AC008580.7	INTERGENIC	T	0.0009
415	6	rs718868	147892684	SAMD5	INTRONIC	T	0.0009
416	11	rs1124847	56712658	LRRC55	3 PRIME UTR	G	0.0009
417	3	rs9861350	141971631	TRIM42	INTERGENIC	A	0.0009
418	7	rs2392581	38539759	AMPH	INTRONIC	G	0.0009
419	6	rs7766161	170011280	C6orf70	INTERGENIC	G	0.0009
420	13	rs9319313	26241960	GPR12	INTERGENIC	G	0.0009
421	16	rs11644878	25820846	HS3ST4	INTRONIC	A	0.0009
422	10	rs11014358	18794501	CACNB2	INTRONIC	G	0.0009
423	9	rs1411355	109512117	AL389915.19	INTERGENIC	A	0.0009
424	9	rs3739482	19497991	SLC24A2	INTERGENIC	A	0.0009
425	14	rs1955429	84997096	AL049775.2	INTERGENIC	T	0.0009
426	13	rs1517896	36011534	RP11-56M2.1	INTERGENIC	A	0.0009
427	11	rs4756930	18076471	SAAL1	INTRONIC	T	0.0009
428	3	rs6551273	88258879	ZNF654	INTERGENIC	G	0.0009

429	18	rs1944351	52002040	AC009271.7	INTRONIC	A	0.0009
430	2	rs6718240	23081861	AC104807.5-2	INTERGENIC	G	0.0009
431	15	rs11634818	60143678	FAM148A	UPSTREAM	G	0.0009
432	4	rs11725282	187194106	AC104070.3	INTERGENIC	C	0.0009
433	1	rs1591785	155472908	ETV3	INTERGENIC	T	0.0009
434	6	rs421424	8967099	BX255934.7	INTERGENIC	A	0.0009
435	9	rs880242	131669653	USP20	INTRONIC	G	0.0009
436	5	rs12651858	68613223	CCDC125	3 PRIME UTR	C	0.0009
437	5	rs17704210	157961941	EBF1	INTERGENIC	G	0.0009
438	13	rs4771929	95650914	HS6ST3	INTRONIC	T	0.0009
439	11	rs287756	41688339	n/a	INTERGENIC	T	0.0009
440	3	rs9843001	120535281	AC092981.3-1	INTRONIC	T	0.0009

n/a = not applicable

Table 5.4. LOAD+P versus control. Results for SNPs with p-values ≤ $1x10^{-4}$. Table shows SNP rank, chromosome, base associated allele, p-value and OR in LOAD+P vs. Control, LOAD vs. control and LOAD+P vs. LOAD-P analyses.

Rank	Chr	SNP	BP	Gene	Type	Allele	*P*	OR	LOAD GWA *P*
1	19	rs2075650	50087459	TOMM40	INTRONIC	C	3.60E-34	2.47	1.753E-157
2	19	rs157580	50087106	TOMM40	INTRONIC	G	1.96E-11	0.62	9.62E-54
3	19	rs6859	50073874	PVRL2	3PRIME_UTR	T	3.54E-11	1.54	6.946E-41
4	19	rs439401	50106291	APOE;PKP2	UPSTREAM	T	5.94E-08	0.68	2.652E-23
5	19	rs8106922	50093506	TOMM40	INTRONIC	G	6.05E-08	0.69	5.373E-39
6	15	rs8038077	58791939	RORA	INTRONIC	G	1.56E-07	1.78	0.02102
7	19	rs405509	50100676	APOE;PKP2	UPSTREAM	A	1.20E-06	1.37	4.884E-37
8	7	rs868055	129222427	MIRN183	INTERGENIC	T	1.80E-06	1.62	0.0004112
9	12	rs1906950	25408885	AC092451.12-2	INTERGENIC	A	4.88E-06	1.35	0.0009067
10	8	rs4527852	125542605	TRMT12	INTERGENIC	T	5.56E-06	1.61	0.1853
11	2	rs1430170	133749869	AC010974.1	INTRONIC	G	9.29E-06	0.71	0.01094
12	8	rs10094093	4493322	AC019176.4-2	INTERGENIC	T	9.93E-06	0.68	0.06029
13	3	rs9289666	144727672	SLC9A9	INTRONIC	A	1.31E-05	1.49	0.02476
14	19	rs10402948	490266	CDC34	INTRONIC	T	1.35E-05	1.63	0.1486
15	2	rs4671328	58788786	IK	INTERGENIC	A	1.39E-05	0.75	0.004147
16	7	rs4726443	140618341	AC005692.1	INTERGENIC	T	1.39E-05	2.51	0.0168
17	8	rs16899656	125602072	TATDN1	INTRONIC	G	1.42E-05	1.58	0.0699
18	16	rs11648322	10082553	GRIN2A	INTRONIC	T	1.56E-05	1.33	0.02505
19	10	rs6482252	23397218	AL139815.12-3	INTERGENIC	G	1.58E-05	1.33	0.003285
20	10	rs7902903	23398083	AL139815.12-3	INTERGENIC	C	1.69E-05	1.33	0.008537
21	12	rs12372711	25418920	AC092451.12-2	INTERGENIC	G	1.73E-05	1.33	0.0004256
22	2	rs1861410	58787095	IK	INTERGENIC	C	1.85E-05	0.75	0.006573
23	2	rs1519473	17633576	VSNL1	INTRONIC	C	1.87E-05	1.60	0.1121

24	4	rs753129	56363188	AC110611.3-1	DOWNSTREAM	G	2.04E-05	1.36	0.03285
25	2	rs4038129	17638227	VSNL1	INTRONIC	C	2.10E-05	1.61	0.3779
26	15	rs8031076	48149784	ATP8B4	INTRONIC	A	2.17E-05	1.32	0.0204
27	2	rs1949510	17641610	VSNL1	INTRONIC	A	2.35E-05	1.60	0.149
28	16	rs11645153	81676584	CDH13	INTRONIC	C	2.63E-05	1.39	0.02804
29	19	rs4919850	507632	GZMM	INTERGENIC	T	3.05E-05	1.44	0.2901
30	5	rs11740920	132777389	FSTL4	INTRONIC	C	3.24E-05	1.36	0.02142
31	2	rs6741951	58812616	IK	INTERGENIC	T	3.25E-05	0.73	0.006944
32	17	rs9904097	73496579	TNRC6C	INTERGENIC	G	3.32E-05	1.32	0.006898
33	12	rs4759721	129589514	RIMBP2	INTERGENIC	C	3.39E-05	0.76	0.1954
34	7	rs1558542	55101766	EGFR	INTRONIC	T	3.48E-05	1.38	0.4633
35	16	rs922302	81367787	AC099506.3	INTERGENIC	G	3.50E-05	1.37	0.03484
36	8	rs12678035	15315148	AC091559.7	INTERGENIC	C	3.72E-05	0.30	0.001775
37	2	rs10176091	58839545	IK	INTERGENIC	T	3.82E-05	0.74	0.001166
38	6	rs9404549	104959117	RP11-427E4.1	INTERGENIC	T	4.04E-05	1.44	0.2545
39	5	rs6870951	133092859	FSTL4	INTERGENIC	A	4.08E-05	1.46	1.027
40	2	rs1978346	217269712	IGFBP5	UPSTREAM	G	4.11E-05	1.31	0.2391
41	11	rs2512152	117392967	AP002962.2	INTERGENIC	A	4.18E-05	0.73	0.137
42	19	rs379758	48990002	LYPD5	DOWNSTREAM	G	4.21E-05	1.46	0.1053
43	3	rs578641	147138054	AC055758.23	INTERGENIC	C	4.55E-05	0.71	0.394
44	15	rs10519257	48176213	ATP8B4	INTRONIC	G	4.63E-05	1.34	0.01263
45	10	rs7079742	19016665	ARL5B	INTERGENIC	T	4.64E-05	0.76	0.0334
46	10	rs941856	116160015	AFAP1L2	INTERGENIC	G	4.68E-05	1.59	0.02923
47	5	rs10474519	76929703	AC108173.1	INTERGENIC	T	4.75E-05	1.49	0.0000544
48	17	rs4789523	73524449	TNRC6C	INTERGENIC	A	4.78E-05	1.33	0.004594
49	16	rs7189966	81371504	AC099506.3	INTERGENIC	C	4.93E-05	1.36	0.04047
50	12	rs12366756	49689473	SLC11A2	INTRONIC	C	4.97E-05	0.67	0.005768

51	6	rs7756959	104974286	RP11-427E4.1	INTERGENIC	C	5.02E-05	1.43	0.05733
52	3	rs6800179	141050356	CLSTN2	INTERGENIC	C	5.53E-05	1.43	0.05311
53	11	rs1400756	110585670	C11orf53	INTERGENIC	C	5.61E-05	1.44	1.018
54	8	rs12543670	4498860	AC019176.4-2	INTERGENIC	G	5.67E-05	0.72	0.01861
55	20	rs747680	60977520	DIDO1	UPSTREAM	A	5.71E-05	1.30	0.00263
56	8	rs10956535	131623943	ASAP1	INTERGENIC	C	5.71E-05	1.30	1.007
57	9	rs9314866	79907769	RP11-336N8.1	INTERGENIC	A	5.79E-05	0.76	0.0004003
58	10	rs6585251	115641243	NHLRC2	INTRONIC	T	5.80E-05	0.74	0.1915
59	18	rs17240415	64801868	CCDC102B	INTRONIC	A	6.02E-05	0.74	0.2614
60	18	rs280989	4419639	n/a	INTERGENIC	G	6.05E-05	0.73	0.003578
61	10	rs2670106	79111719	KCNMA1	INTERGENIC	C	6.19E-05	0.63	0.0005924
62	12	rs11169654	49667102	SLC11A2	3PRIME_UTR	A	6.32E-05	0.67	0.008652
63	6	rs156235	104978232	RP11-427E4.1	INTERGENIC	C	6.48E-05	1.42	0.1691
64	3	rs7612414	89872049	AC026305.20-3	INTERGENIC	G	6.52E-05	1.61	0.2217
65	2	rs848607	36623696	AC007401.2	INTRONIC	A	6.61E-05	0.75	0.979
66	6	rs7740010	104962863	RP11-427E4.1	INTERGENIC	A	6.63E-05	1.42	0.2374
67	12	rs831429	102585961	STAB2	INTRONIC	C	7.06E-05	1.32	0.351
68	13	rs9512519	26522181	USP12	INTERGENIC	C	7.21E-05	0.49	0.002622
69	18	rs206749	64155406	TMX3	INTERGENIC	G	7.23E-05	1.29	0.007995
70	19	rs745952	55189335	VRK3	INTRONIC	T	7.39E-05	1.43	0.003302
71	7	rs2693737	129218230	MIRN183	INTERGENIC	C	7.51E-05	1.50	0.02569
72	18	rs4799872	32425512	FHOD3	INTRONIC	A	7.89E-05	1.30	0.0218
73	1	rs7520521	70020703	LRRC7	INTRONIC	G	8.07E-05	0.77	0.1674
74	15	rs8040030	48163879	ATP8B4	INTRONIC	A	8.18E-05	1.29	0.002706
75	16	rs9927466	59162687	AC018554.7	INTERGENIC	T	8.28E-05	1.30	1.015
76	6	rs7759504	132108194	ENPP3	INTRONIC	T	8.39E-05	0.44	0.4142
77	10	rs1576479	115669955	NHLRC2	INTERGENIC	A	8.43E-05	0.77	0.1237

78	7	rs219824	98483453	SMURF1	INTRONIC	A	8.44E-05	1.30	0.01067
79	10	rs7913176	115634030	NHLRC2	NON_SYN	T	8.56E-05	0.74	0.2138
80	9	rs1328533	79674282	GNAQ	INTRONIC	C	8.60E-05	0.76	0.0007377
81	10	rs10870210	134597903	C10orf93	INTRONIC	A	8.77E-05	1.70	0.1317
82	3	rs2171513	113667617	BTLA	3PRIME_UTR	T	8.79E-05	0.39	0.0003671
83	9	rs11145589	79662751	GNAQ	INTRONIC	T	8.86E-05	0.73	0.003245
84	4	rs10519397	137901725	AC006572.2	INTERGENIC	T	8.87E-05	0.59	0.2014
85	7	rs17155687	107831342	NRCAM	INTRONIC	T	9.00E-05	1.57	0.001551
86	16	rs1436046	59175549	AC018554.7	INTERGENIC	C	9.00E-05	1.29	0.3766
87	6	rs267339	104985242	RP11-427E4.1	INTERGENIC	A	9.05E-05	1.38	0.02812
88	19	rs17312204	55249688	AC010624.8-1	INTRONIC	G	9.33E-05	1.53	0.02061
89	6	rs9354570	67978123	AL591004.3	UPSTREAM	T	9.59E-05	1.43	0.3268
90	15	rs8041340	48148130	ATP8B4	INTRONIC	C	9.72E-05	0.77	0.0001887
91	1	rs6661589	70022134	LRRC7	INTRONIC	A	9.90E-05	0.77	0.149
92	12	rs10745978	102582910	STAB2	INTRONIC	C	0.0001007	1.29	0.01781
93	10	rs1107442	112293585	RP11-73H14.1	INTERGENIC	G	0.0001035	1.34	0.09801
94	10	rs1986577	20351249	PLXDC2	INTRONIC	T	0.0001047	1.30	0.1112
95	12	rs1031477	48504911	KIAA1602	INTRONIC	C	0.0001054	0.78	0.0541
96	18	rs4798993	73985341	AC139070.3	INTERGENIC	T	0.0001066	0.72	0.05808
97	12	rs7296829	102585727	STAB2	INTRONIC	C	0.0001103	1.28	0.08018
98	2	rs6716814	78229692	AC064872.3	INTERGENIC	A	0.0001138	1.32	0.09362
99	6	rs6939897	67969845	AL591004.3	INTERGENIC	C	0.0001149	1.43	0.2999
100	19	rs285684	38963499	CHST8	INTERGENIC	T	0.0001156	0.75	0.05875
101	15	rs12909131	48174970	ATP8B4	INTRONIC	T	0.0001157	1.32	0.02987
102	10	rs35084455	87559938	GRID1	INTRONIC	T	0.0001208	1.72	0.1177
103	3	rs6806737	141171105	CLSTN2	INTRONIC	A	0.0001211	1.40	0.08341
104	14	rs17558231	69406803	SMOC1	INTERGENIC	C	0.0001219	1.52	0.01306

105	2	rs6727330	217271690	IGFBP5	UPSTREAM	C	0.0001241	1.29	1.02
106	2	rs1991108	220611959	AC114765.6	INTERGENIC	A	0.0001283	1.37	0.005102
107	1	rs11122300	228287788	GALNT2	INTRONIC	T	0.000132	1.34	0.0007599
108	6	rs7756211	20661764	CDKAL1	INTRONIC	T	0.0001346	1.30	0.2429
109	12	rs17027488	96741146	RNU6-2	INTERGENIC	G	0.0001365	1.31	0.02805
110	2	rs12991296	78235370	AC064872.3	INTERGENIC	C	0.0001366	1.42	0.01626
111	12	rs755598	102583920	STAB2	INTRONIC	G	0.0001378	1.28	0.08816
112	2	rs885640	159783429	TANC1	INTRONIC	G	0.0001412	1.29	0.0006816
113	12	rs2398518	129600639	RIMBP2	INTERGENIC	A	0.0001446	1.28	0.1045
114	6	rs7773295	107744179	PDSS2	INTRONIC	A	0.0001448	1.34	0.1411
115	3	rs6808569	124456196	SEC22A	INTRONIC	T	0.0001493	0.70	0.3577
116	5	rs620508	29543052	AC010374.6	INTERGENIC	G	0.00015	0.76	0.3436
117	2	rs2861264	78220585	AC064872.3	INTERGENIC	A	0.0001553	1.42	0.03514
118	10	rs2926872	14058238	FRMD4A	INTRONIC	G	0.0001598	1.45	0.009523
119	3	rs1915728	89915500	AC026305.20-3	INTERGENIC	G	0.0001604	1.65	0.9926
120	3	rs3804592	123461919	CASR	INTRONIC	T	0.0001627	0.67	0.1962
121	6	rs7758851	20650200	CDKAL1	INTRONIC	T	0.0001642	1.29	0.2893
122	13	rs1951898	36404819	RP11-421P11.8	INTERGENIC	A	0.0001655	1.42	1.024
123	16	rs2549159	81421748	AC099506.3	INTERGENIC	C	0.0001663	1.27	0.04008
124	8	rs777801	116245300	ADD2	INTRONIC	T	0.0001671	0.53	0.4034
125	2	rs3755365	70798073	TRPS1	INTERGENIC	C	0.0001671	1.30	1.014
126	21	rs2096471	35045555	CLIC6	INTERGENIC	C	0.000171	1.33	0.1448
127	10	rs7908946	127903558	ADAM12	INTRONIC	A	0.0001737	0.73	1.006
128	9	rs1926381	7329425	RP11-87M1.1	INTERGENIC	T	0.0001752	0.72	0.005402
129	18	rs12454977	74657618	SALL3	INTERGENIC	A	0.0001767	0.70	0.9847
130	22	rs760656	31173938	BPIL2	INTRONIC	C	0.0001785	0.70	0.0001062
131	17	rs9909561	2687549	GARNL4	INTRONIC	A	0.0001795	0.78	0.1619

132	8	rs6578080	141291830	TRAPPC9	INTRONIC	G	0.0001801	1.28	1.017
133	5	rs1298248	103436968	AC091894.2	INTERGENIC	T	0.0001813	1.29	1.01
134	12	rs7979925	100866032	AC084398.25-2	INTERGENIC	C	0.0001817	0.78	0.03238
135	17	rs9889827	73538266	TNRC6C	INTERGENIC	C	0.0001841	1.28	0.02459
136	9	rs3808720	17654191	SH3GL2	INTRONIC	T	0.0001861	0.78	0.002869
137	4	rs2309472	182537727	n/a	INTERGENIC	A	0.0001912	0.77	0.3867
138	8	rs2034140	6224172	AC026305.20-3	INTERGENIC	G	0.0001927	0.46	0.1873
139	3	rs6788646	89915684	MCPH1	INTERGENIC	A	0.0001927	1.61	0.9917
140	4	rs13108814	81570062	C4orf22	INTRONIC	A	0.0001939	1.33	0.001548
141	11	rs7936316	81924308	FAM181B	INTERGENIC	A	0.0001966	0.45	0.002175
142	6	rs2745933	20858693	CDKAL1	INTRONIC	T	0.0001978	0.78	0.02156
143	2	rs2593708	134695885	MGAT5	INTRONIC	T	0.0002009	1.28	0.01839
144	3	rs1533393	124446769	SEC22A	INTRONIC	C	0.0002022	0.71	0.3762
145	6	rs1338715	87300547	AL391417.7-2	INTERGENIC	A	0.0002034	1.29	0.08087
146	4	rs12648767	17167201	LAP3	INTERGENIC	A	0.0002035	1.28	0.05669
147	8	rs2702910	6811301	DEFA10P	DOWNSTREAM	T	0.0002037	0.76	0.01027
148	11	rs7929650	12958762	RASSF10	INTERGENIC	G	0.0002062	1.48	0.01913
149	6	rs2674394	93563130	AL589947.3-2	INTERGENIC	A	0.0002064	1.37	0.3141
150	5	rs683004	29539692	AC010374.6	INTERGENIC	A	0.000207	0.76	0.277
151	16	rs11150535	81644467	CDH13	INTRONIC	T	0.000207	1.27	1.02
152	3	rs1520593	89927248	AC026305.20-3	INTERGENIC	C	0.0002079	1.61	0.9985
153	18	rs7229111	32432481	FHOD3	INTRONIC	A	0.000209	1.27	0.05808
154	6	rs2674371	93553719	AL589947.3-2	UPSTREAM	C	0.0002094	1.37	0.3855
155	9	rs3780232	17680180	SH3GL2	INTRONIC	C	0.0002097	0.78	0.001098
156	11	rs576825	131200086	HNT;NTM	INTRONIC	C	0.0002109	1.27	0.08473
157	10	rs4339947	6735491	PRKCQ	INTERGENIC	C	0.0002158	1.29	0.002172
158	3	rs7653338	90020778	AC095349.10	INTERGENIC	A	0.0002219	1.60	0.9985

159	17	rs1122634	45976364	EPN3	UPSTREAM	T	0.0002236	1.27	0.1095
160	3	rs7623722	28857984	AC097361.2	INTERGENIC	G	0.0002267	1.27	1.012
161	3	rs4580491	124454040	n/a	n/a	C	0.0002278	0.71	0.383
162	6	rs9342616	67942640	AL590874.6	INTERGENIC	T	0.0002292	0.79	0.04672
163	19	rs3764650	997520	ABCA7	INTRONIC	C	0.000233	1.47	0.00001556
164	9	rs10981195	113803202	AL138756.23-1	UPSTREAM	A	0.000236	1.30	0.4158
165	12	rs11047988	25432786	AC092451.12-2	INTERGENIC	G	0.0002379	1.27	0.001824
166	4	rs6834555	9671424	WDR1	INTERGENIC	C	0.0002448	0.73	0.04556
167	11	rs2085334	92820698	CCDC67	INTERGENIC	G	0.0002456	1.29	0.001077
168	4	rs35495915	54764155	PDGFRA	INTERGENIC	T	0.0002535	0.73	0.08204
169	2	rs12986742	58828647	IK	INTERGENIC	C	0.0002593	0.79	0.04324
170	19	rs10402271	50021054	BCAM	DOWNSTREAM	G	0.0002594	1.27	1.541E-26
171	2	rs13425498	78206058	AC064872.3	INTERGENIC	T	0.0002639	1.31	0.2859
172	5	rs3909548	128997514	ADAMTS19	INTRONIC	A	0.0002648	1.41	0.07702
173	7	rs2693727	129204966	MIRN183	UPSTREAM	G	0.0002665	1.46	0.0374
174	16	rs844395	10010407	GRIN2A	INTRONIC	A	0.0002678	0.78	0.03064
175	3	rs6770430	90106009	AC095349.10	INTERGENIC	A	0.0002685	1.62	1.008
176	19	rs377702	50054507	PVRL2	INTRONIC	T	0.0002693	1.27	8.375E-11
177	12	rs1871898	128415853	TMEM132D	INTRONIC	G	0.0002703	1.27	0.02216
178	18	rs11876226	20907560	ZNF521	INTRONIC	G	0.000271	1.34	0.006904
179	3	rs9876068	142901893	RNF7	INTERGENIC	C	0.0002728	0.43	0.00002937
180	22	rs5753436	29776921	AC005005.1-1	INTERGENIC	G	0.0002784	0.66	0.0361
181	11	rs3096716	86134830	PRSS23	INTERGENIC	T	0.000282	1.30	1.02
182	3	rs7648855	89993520	AC095349.10	INTERGENIC	G	0.0002892	1.59	0.9928
183	16	rs10492859	81419315	AC099506.3	INTERGENIC	G	0.0002893	0.74	0.1008
184	10	rs7089454	127894700	ADAM12	INTRONIC	G	0.0002897	0.75	0.9964
185	16	rs718510	59153108	AC018554.7	INTERGENIC	A	0.0002923	0.78	0.04267

186	10	rs821705	108073458	AL133395.21	INTERGENIC	T	0.0002926	1.27	0.007213
187	3	rs6766386	147136793	AC055758.23	INTERGENIC	T	0.0002984	0.71	0.9996
188	11	rs6592039	81828131	FAM181B	INTERGENIC	A	0.0003009	0.43	0.06322
189	19	rs17801681	56368461	SIGLECP3	3PRIME_UTR	A	0.0003016	1.56	0.158
190	1	rs1258033	46782614	KNCN	UPSTREAM	C	0.000304	0.76	0.006502
191	11	rs2433438	86132394	PRSS23	INTERGENIC	G	0.0003049	1.29	1.025
192	6	rs9473511	49299933	RP1-142O9.2	INTERGENIC	T	0.0003103	1.52	0.1599
193	6	rs9358383	21107366	CDKAL1	INTRONIC	G	0.0003109	1.29	0.09538
194	12	rs2301529	49676014	SLC11A2	INTRONIC	T	0.000312	0.73	0.06983
195	6	rs2767576	156921465	AL158033.18	INTERGENIC	C	0.0003122	1.43	0.00006301
196	8	rs3857946	125675435	MTSS1	INTRONIC	A	0.0003135	1.47	0.04825
197	16	rs837688	10007044	GRIN2A	INTRONIC	G	0.0003185	1.26	0.1657
198	10	rs10904321	4736345	AKR1CL2	INTERGENIC	C	0.0003196	1.27	0.001922
199	9	rs12343558	18807951	C9orf94;ADAMTSL1	INTRONIC	C	0.0003208	1.29	1.012
200	3	rs9869506	111458818	AC117430.3-2	INTERGENIC	G	0.0003234	0.64	0.00153
201	6	rs9377643	105041172	RP11-427E4.1	INTERGENIC	C	0.0003256	1.38	0.4068
202	18	rs8091319	9674723	AP000902.5	INTERGENIC	T	0.0003258	1.31	0.2576
203	10	rs528320	13326337	C10orf49	INTERGENIC	C	0.0003269	1.37	0.03679
204	5	rs7702830	111798779	EPB41L4A	INTERGENIC	C	0.000327	1.34	0.04778
205	3	rs966866	141164738	CLSTN2	INTRONIC	C	0.0003291	1.28	0.01939
206	11	rs1949707	81771147	FAM181B	INTERGENIC	C	0.000331	0.44	0.04758
207	10	rs7097356	127901574	ADAM12	INTRONIC	C	0.0003314	0.76	0.996
208	14	rs6575028	88503521	TTC8	INTERGENIC	T	0.0003345	1.33	0.002949
209	14	rs2369018	95082844	GLRX5	DOWNSTREAM	T	0.0003345	0.75	0.03062
210	11	rs10831608	11361390	GALNTL4	INTRONIC	T	0.0003357	1.26	0.0361
211	8	rs3824110	146142089	ZNF16	INTRONIC	A	0.0003371	0.72	0.004323
212	20	rs6097096	50772458	AL049736.10	INTERGENIC	A	0.0003384	1.38	0.001378

213	10	rs3936497	28619975	MPP7	INTERGENIC	C	0.0003385	1.51	0.01732
214	12	rs10860757	100544577	MYBPC1	INTRONIC	C	0.000339	0.67	0.07681
215	19	rs8103315	49946008	BCL3	INTRONIC	T	0.0003412	1.36	2.942E-07
216	10	rs10827932	20351525	PLXDC2	INTRONIC	T	0.0003414	1.27	0.002538
217	18	rs1893311	20881576	ZNF521	INTERGENIC	T	0.0003425	1.34	0.008352
218	16	rs12922279	59151989	AC018554.7	INTERGENIC	C	0.0003445	0.78	0.04185
219	8	rs10104503	6666441	XKR5	INTRONIC	T	0.0003454	1.28	0.1771
220	11	rs10837311	39938524	AL591004.3	INTERGENIC	T	0.0003466	0.73	0.004395
221	6	rs9294750	67960760	AC021749.6-3	INTERGENIC	C	0.0003466	0.79	0.2614
222	15	rs12903325	48140569	ATP8B4	INTRONIC	C	0.0003485	1.30	0.03243
223	12	rs12824958	49620342	AC008121.43-2	UPSTREAM	C	0.0003488	0.73	0.09133
224	18	rs4799869	32416808	FHOD3	INTRONIC	A	0.000353	1.27	0.05578
225	12	rs10745935	100913627	CCDC53	INTERGENIC	G	0.0003538	0.61	0.9973
226	9	rs504896	32586503	NDUFB6	INTERGENIC	A	0.0003548	1.49	0.08986
227	15	rs6603018	82512023	ADAMTSL3	INTERGENIC	T	0.0003548	1.36	1.009
228	9	rs10971038	32582503	NDUFB6	INTERGENIC	A	0.0003577	1.49	0.05517
229	12	rs11044223	18675030	PIK3C2G	INTRONIC	C	0.0003593	0.65	0.3888
230	2	rs1615070	61246679	C2orf74	DOWNSTREAM	C	0.0003728	1.26	0.3594
231	15	rs906194	82541642	AC103965.5	INTRONIC	A	0.0003748	1.36	1.021
232	14	rs17103033	34626231	C14orf10;PPP2R3C	INTRONIC	G	0.0003749	1.35	0.0002787
233	10	rs17684713	127942864	ADAM12	INTRONIC	A	0.0003754	0.73	0.9905
234	5	rs1345686	111835478	EPB41L4A	INTERGENIC	T	0.0003761	1.34	0.03761
235	1	rs34411680	54992999	PARS2	DOWNSTREAM	C	0.0003784	1.48	0.04713
236	2	rs10514647	204869721	PARD3B	INTERGENIC	G	0.0003786	1.46	0.1614
237	2	rs6436883	230213277	DNER	INTRONIC	T	0.0003815	1.26	0.0001091
238	2	rs17338519	2150112	MYT1L	INTRONIC	T	0.0003826	1.41	0.1575
239	22	rs80533	39415915	MCHR1	INTERGENIC	A	0.0003836	1.29	0.002373

240	9	rs7871498	37374977	ZCCHC7	INTERGENIC	A	0.0003895	1.31	0.1293
241	10	rs9888067	96638214	RP11-400G3.3	INTERGENIC	A	0.0003916	0.74	0.4248
242	8	rs1893884	99621703	STK3	INTRONIC	A	0.0003924	1.26	0.08192
243	4	rs6848311	187455812	F11	INTERGENIC	T	0.0003949	0.73	0.4312
244	2	rs744373	127611085	BIN1	INTERGENIC	G	0.0003959	1.28	0.00000320
245	14	rs11628156	55409820	AL355773.4	INTERGENIC	G	0.0004021	1.27	0.0004217
246	14	rs1885186	88499809	TTC8	INTERGENIC	T	0.0004042	1.32	0.008811
247	18	rs12454185	74667117	SALL3	INTERGENIC	G	0.0004094	0.72	0.9905
248	3	rs6790000	124374854	PDIA5	INTERGENIC	C	0.0004111	0.74	0.09121
249	6	rs17445948	54653025	RP3-334F4.1	INTERGENIC	A	0.0004122	1.47	0.2397
250	2	rs17338512	2152335	MYT1L	INTRONIC	A	0.0004128	1.41	0.1439
251	3	rs6788684	25937362	OXSM	INTERGENIC	A	0.0004194	1.35	0.3767
252	3	rs1705589	171408358	PRKCI	INTERGENIC	C	0.0004206	0.74	0.06422
253	11	rs598829	131247810	HNT;NTM	INTRONIC	A	0.0004219	1.27	1.006
254	1	rs11579916	119589065	AL359915.14	INTERGENIC	T	0.0004256	0.72	0.03498
255	8	rs13248917	39918113	AC019129.1	INTERGENIC	C	0.0004293	1.26	0.03945
256	2	rs7607908	44160954	IDO2	INTRONIC	A	0.0004293	1.36	1.015
257	9	rs10738482	17650410	SH3GL2	INTRONIC	A	0.0004318	0.79	0.005816
258	2	rs6753886	107992900	SLC5A7	INTRONIC	T	0.000434	0.78	0.2364
259	14	rs2401785	88487005	TTC8	INTERGENIC	A	0.0004351	1.32	0.002753
260	8	rs12541503	6662868	XKR5	INTRONIC	T	0.0004398	1.29	0.154
261	10	rs9663603	108129695	AL133395.21	INTERGENIC	C	0.0004424	1.29	0.02182
262	10	rs11006995	28625502	AC022021.10-3	INTERGENIC	A	0.0004453	1.55	0.001416
263	11	rs10899236	70129782	SHANK2	INTRONIC	G	0.0004483	0.79	0.04133
264	3	rs7616301	90102238	AC095349.10	INTERGENIC	C	0.0004511	1.56	0.9839
265	3	rs7651217	90099238	AC095349.10	INTERGENIC	C	0.0004511	1.56	0.9839
266	7	rs854568	94787737	PON1	INTRONIC	C	0.0004566	1.30	0.0008371

267	13	rs4942106	41776392	AKAP11	INTRONIC	A	0.0004571	1.29	1.024
268	11	rs7106873	70103491	SHANK2	INTRONIC	T	0.00046	1.25	0.08738
269	11	rs10894589	131998236	OPCML	INTRONIC	T	0.0004603	0.57	0.9747
270	3	rs9863121	173938537	ECT2	INTERGENIC	T	0.0004658	1.28	0.3032
271	11	rs1545527	59010971	AP003778.3-4	UPSTREAM	G	0.0004683	1.27	1.016
272	14	rs1958018	32646064	NPAS3	INTRONIC	G	0.0004684	1.29	1.015
273	16	rs4541063	83783372	FAM92B	INTERGENIC	C	0.0004731	0.78	0.1542
274	10	rs4748275	6750189	PRKCQ	INTERGENIC	G	0.0004801	1.28	0.03334
275	15	rs12442068	78463829	ARNT2	INTERGENIC	G	0.0004849	1.31	0.07071
276	18	rs12954185	73970814	AC139070.3	INTERGENIC	C	0.0004863	0.73	0.007873
277	18	rs4595890	60404482	AC008245.6	INTERGENIC	A	0.0004905	0.76	0.0202
278	7	rs17171118	148148237	MYT1L	INTRONIC	T	0.000493	1.36	0.0008541
279	2	rs12467137	2154578	EZH2	INTRONIC	T	0.000493	1.40	0.187
280	11	rs12800502	28260001	METT5D1	INTRONIC	G	0.0004939	0.72	0.0284
281	10	rs1576480	115669985	NHLRC2	INTERGENIC	C	0.0004968	1.25	0.03966
282	6	rs2206579	20733613	CDKAL1	INTRONIC	C	0.0004969	1.27	1.019
283	16	rs4889197	71952116	C16orf47	INTERGENIC	A	0.0005004	1.25	0.0787
284	12	rs1190655	91124864	AC025164.37-2	INTERGENIC	C	0.0005017	1.26	0.01157
285	8	rs17282526	131277125	ASAP1	INTRONIC	T	0.0005042	1.25	0.001061
286	1	rs12043001	176073958	SEC16B	INTERGENIC	G	0.0005045	0.70	0.002307
287	3	rs9289466	135520086	RPL39P5	INTERGENIC	C	0.000507	0.79	0.2435
288	4	rs17001970	77589062	SHROOM3	INTRONIC	T	0.0005096	1.53	0.1309
289	11	rs1048099	17453092	ABCC8	SYNONYMOUS	C	0.0005096	1.31	0.1337
290	6	rs4712540	20871150	STK32B	INTRONIC	C	0.0005098	1.25	0.01137
291	4	rs1374621	5176625	CDKAL1	INTRONIC	T	0.0005098	1.94	0.09377
292	14	rs2363506	76466445	AC007686.5	REGULATORY	A	0.0005112	0.80	0.004536
293	17	rs1052507	62497273	HELZ	3PRIME_UTR	G	0.0005151	1.42	0.05301

294	12	rs1861786	13891734	GRIN2B	INTRONIC	T	0.0005168	0.79	0.9887
295	4	rs6850106	151121994	AC096756.3-2	INTERGENIC	C	0.000518	2.01	0.05634
296	12	rs1356083	129575938	RIMBP2	INTERGENIC	G	0.000518	1.25	0.2484
297	5	rs4976217	68437697	SLC30A5	INTRONIC	A	0.000521	1.38	0.3614
298	17	rs2279966	69232956	C17orf54	INTERGENIC	A	0.0005224	0.47	0.2279
299	15	rs2879971	82918472	AC048382.7-2	UPSTREAM	T	0.0005269	1.35	1.011
300	3	rs11707887	140862075	NMNAT3	INTRONIC	A	0.0005301	1.27	0.06165
301	12	rs10842549	25442292	AC092451.12-2	INTERGENIC	C	0.0005325	1.25	0.2301
302	6	rs7775523	21063311	CDKAL1	INTRONIC	C	0.000535	1.26	0.04534
303	5	rs11748946	132775021	FSTL4	INTRONIC	C	0.0005416	1.37	0.1085
304	6	rs4077405	20984662	CDKAL1	INTRONIC	G	0.0005424	1.25	0.0851
305	11	rs1054532	94372055	JMJD2D	3PRIME_UTR	A	0.0005451	1.35	0.1376
306	9	rs7043157	18741638	C9orf94;ADAMTSL1	INTRONIC	G	0.0005466	0.80	0.05248
307	3	rs3774700	62291434	C3orf14	INTRONIC	G	0.000547	0.57	0.05512
308	11	rs10792830	85516456	AP003097.2	INTERGENIC	T	0.0005483	1.25	0.02034
309	5	rs7727192	84447374	AC113412.2-2	INTERGENIC	G	0.0005535	1.26	0.3575
310	6	rs17310261	5093162	LYRM4	INTRONIC	C	0.0005537	0.65	0.9818
311	1	rs10925401	235623753	RYR2	INTRONIC	C	0.0005551	0.80	0.0968
312	10	rs3019500	124426520	AL603764.27	INTRONIC	T	0.0005553	1.32	0.001085
313	2	rs3849346	163210967	KCNH7	INTRONIC	G	0.0005557	1.30	0.3476
314	13	rs1891100	94001278	TGDS	INTERGENIC	T	0.0005562	0.78	0.02895
315	12	rs11836523	13749151	GRIN2B	INTRONIC	C	0.0005567	1.43	1.033
316	14	rs7140539	33150659	NPAS3	INTRONIC	G	0.0005632	1.26	0.175
317	13	rs9512257	26012324	WASF3	INTERGENIC	C	0.0005676	0.79	0.03082
318	9	rs960644	105060805	RP11-341A22.1	INTERGENIC	G	0.0005686	0.70	0.9916
319	6	rs1417667	104909883	LSM10	INTRONIC	G	0.0005708	1.45	0.04004
320	1	rs4653186	36635516	RP1-244F1.1	INTERGENIC	T	0.0005708	0.78	0.06372

321	6	rs1012626	20685540	CDKAL1	INTRONIC	A	0.0005732	0.79	0.1644
322	9	rs1123551	133611630	RAPGEF1	INTERGENIC	T	0.0005789	0.57	0.2388
323	3	rs10937074	183120027	AC007547.26-2	INTERGENIC	A	0.0005819	0.79	0.1125
324	5	rs17733586	73295365	AC093283.3-2	INTERGENIC	T	0.0005835	1.59	0.05668
325	16	rs11649643	59206580	AC018554.7	INTERGENIC	T	0.0005881	1.27	1.016
326	5	rs1862310	107153700	AC008586.6	INTERGENIC	T	0.0005909	1.31	0.01531
327	8	rs2117085	75973042	PI15	INTERGENIC	A	0.000591	0.62	0.1869
328	4	rs12642995	156776705	GUCY1A3	INTERGENIC	G	0.0005941	0.73	1.002
329	12	rs7967304	83766755	SLC6A15	INTERGENIC	G	0.0005967	1.26	0.04071
330	8	rs7002825	101740397	SNX31	INTERGENIC	G	0.0005968	1.29	0.0006819
331	21	rs1389994	17954750	BTG3	INTERGENIC	A	0.0005983	0.79	0.01101
332	4	rs7672650	187508041	AC109517.4	INTERGENIC	G	0.0006017	1.26	0.07013
333	6	rs2767577	156938875	AL158033.18	INTERGENIC	G	0.0006031	1.29	0.1033
334	4	rs7670038	187615001	AC110761.3	INTERGENIC	A	0.0006042	1.30	0.01637
335	14	rs12435645	105108671	IGHA2	INTERGENIC	A	0.0006047	1.48	0.286
336	19	rs1865108	49022693	ZNF283	INTRONIC	C	0.0006055	1.40	0.3212
337	3	rs11708596	14664397	C3orf19	UPSTREAM	T	0.0006125	1.33	0.08658
338	1	rs259588	236537568	AL356010.9	INTERGENIC	C	0.0006156	0.80	0.04742
339	19	rs11878692	1136924	SBNO2	INTERGENIC	C	0.0006166	1.32	0.09159
340	12	rs11535963	18245787	AC087240.17	INTERGENIC	T	0.0006167	0.74	0.4675
341	13	rs2208932	25124174	ATP8A2	INTRONIC	C	0.0006181	1.36	1.004
342	1	rs411238	30559814	RP3-357I16.1	INTERGENIC	A	0.0006199	1.27	0.02182
343	19	rs10425074	50331964	LRRC68	INTRONIC	C	0.0006231	1.29	0.0000191
344	15	rs2242065	56626590	LIPC	INTRONIC	T	0.0006263	1.43	0.225
345	15	rs10520664	85927717	TMEM83	DOWNSTREAM	C	0.0006282	1.54	0.05256
346	5	rs607077	29531321	AC010374.6	INTERGENIC	G	0.0006292	0.78	0.1851
347	8	rs4733037	27149412	STMN4	DOWNSTREAM	A	0.0006296	1.25	0.1635

348	6	rs4510656	20874676	CDKAL1	INTRONIC	A	0.0006331	1.25	0.0603
349	7	rs7798774	28714056	CREB5	INTRONIC	G	0.0006369	1.29	0.01249
350	17	rs4789551	73722674	BIRC5	INTRONIC	C	0.0006389	1.43	0.1017
351	22	rs1883987	35383415	CACNG2	INTRONIC	C	0.0006401	1.26	0.3438
352	14	rs6572182	42387419	n/a	INTERGENIC	T	0.0006406	1.34	0.03403
353	10	rs1890951	61724712	ANK3	INTRONIC	A	0.0006415	0.76	0.0213
354	19	rs12977284	6926032	EMR4P	INTRONIC	A	0.0006432	0.60	0.006861
355	14	rs8005907	95857500	ATG2B	INTRONIC	C	0.0006434	0.64	0.3652
356	4	rs6552500	182579063	n/a	INTERGENIC	C	0.0006445	1.30	0.09871
357	16	rs759831	81421161	AC099506.3	INTERGENIC	A	0.0006475	0.78	0.1277
358	16	rs1369790	59149079	AC018554.7	INTERGENIC	G	0.0006486	1.26	0.9931
359	8	rs11785060	145118650	PLEC1	INTRONIC	T	0.0006516	1.25	0.001063
360	10	rs7077490	108145291	AL133395.21	INTERGENIC	G	0.0006533	1.28	0.03651
361	13	rs7320671	19407203	ZMYM2	INTERGENIC	C	0.0006583	1.24	1.005
362	1	rs11806225	11421873	RP11-149P14.1	INTERGENIC	C	0.0006585	0.55	0.985
363	4	rs17004994	81949609	C4orf22	INTRONIC	G	0.0006591	1.94	0.06421
364	5	rs12189436	29518112	AC010374.6	INTERGENIC	C	0.000661	1.41	0.002539
365	17	rs6502823	4638112	GLTPD2	UPSTREAM	T	0.0006644	1.41	0.2252
366	12	rs6488565	12883409	DDX47	INTERGENIC	A	0.0006645	1.29	0.1035
367	2	rs2664226	220581788	AC114765.6	INTERGENIC	T	0.0006697	1.33	0.003577
368	16	rs6497658	10047257	GRIN2A	INTRONIC	C	0.0006702	1.24	0.3558
369	4	rs1803037	100212174	ADH5P4;ADH5	3PRIME_UTR	A	0.0006706	0.64	0.07578
370	20	rs1018443	8574903	PLCB1	INTRONIC	T	0.0006756	1.28	0.1329
371	19	rs8101040	55255711	AC010624.8-1	INTRONIC	A	0.0006799	1.35	0.0109
372	6	rs9470642	37679431	MDGA1	INTERGENIC	G	0.00068	0.77	0.1246
373	3	rs13072512	71496735	FOXP1	INTRONIC	A	0.0006828	0.80	0.01941
374	1	rs12140361	159588622	SDHC	INTRONIC	C	0.0006829	1.95	0.003019

375	6	rs12663853	40585151	LRFN2	INTRONIC	G	0.0006836	2.67	0.00169
376	11	rs649931	78375935	ODZ4	INTRONIC	T	0.0006863	0.50	0.216
377	18	rs8089316	38722881	RIT2	INTRONIC	C	0.0006911	1.35	0.1073
378	6	rs11754558	123292386	RLBP1L2	INTERGENIC	A	0.0006912	1.84	0.02305
379	9	rs2181156	10491953	RP11-87N24.1	INTERGENIC	G	0.0006913	1.25	0.02215
380	6	rs6935954	26363430	HIST1H2BH	UPSTREAM	A	0.0006935	0.80	0.315
381	9	rs3808708	17684823	SH3GL2	INTRONIC	A	0.0006937	0.79	0.002237
382	14	rs2272550	21661828	AE000660.1-11	NON_SYN	A	0.0006938	1.26	0.9932
383	14	rs12100841	34641402	C14orf10;PPP2R3C	INTRONIC	C	0.0006941	1.31	0.0005634
384	12	rs11107270	92834257	CRADD	INTERGENIC	C	0.0006951	1.83	0.09337
385	10	rs11006970	28592174	CDKAL1	INTRONIC	T	0.0006961	1.40	0.02723
386	6	rs7747724	20859294	MPP7	INTRONIC	C	0.0006961	1.25	0.04912
387	13	rs2217902	101089435	ITGBL1	INTRONIC	C	0.0006961	1.45	0.3725
388	1	rs437722	30564829	RP3-357I16.1	INTERGENIC	A	0.0006965	1.27	0.01147
389	1	rs485660	109824955	SYPL2	3PRIME_UTR	A	0.0006972	1.28	0.3963
390	3	rs340146	113267579	TMPRSS7	INTRONIC	T	0.0006981	1.43	0.01303
391	5	rs11953547	92031385	AC026781.5	INTERGENIC	T	0.0007003	1.34	0.434
392	2	rs2655455	8498335	AC011747.5	INTERGENIC	A	0.0007011	1.24	0.2486
393	7	rs1530680	114194632	FOXP2	INTERGENIC	T	0.0007013	1.30	0.002356
394	16	rs2304469	8803595	PMM2	INTRONIC	C	0.0007046	1.25	0.2075
395	15	rs2412621	39621697	RPAP1	INTRONIC	C	0.0007076	0.79	0.004308
396	8	rs7815950	101728344	SNX31	INTRONIC	C	0.000709	0.65	0.1029
397	20	rs2145280	55788630	CMA1	INTERGENIC	A	0.0007094	1.29	0.01981
398	14	rs12589666	24062459	PMEPA1	INTERGENIC	A	0.0007094	1.25	0.03523
399	8	rs4275231	26137422	PPP2R2A	INTERGENIC	T	0.00071	1.32	0.284
400	12	rs6580784	49754578	CSRNP2	INTRONIC	A	0.0007106	0.75	0.1233
401	3	rs6790913	45058525	CLEC3B	INTERGENIC	T	0.0007202	1.41	1.008

402	18	rs169455	4416360	n/a	INTERGENIC	T	0.0007286	0.77	0.01042
403	10	rs988011	117587767	ATRNL1	INTRONIC	A	0.0007308	1.38	0.01353
404	8	rs7815631	101728102	SNX31	INTRONIC	T	0.0007329	0.65	0.02219
405	14	rs204984	76624887	KIAA1737	INTERGENIC	A	0.000733	0.77	0.02934
406	7	rs1557780	27961372	JAZF1	INTRONIC	C	0.0007332	0.80	0.08359
407	8	rs4397386	141294034	TRAPPC9	INTRONIC	C	0.0007339	1.25	0.998
408	5	rs25923	110824997	CAMK4	INTRONIC	C	0.0007341	1.25	0.4336
409	14	rs7142488	55317763	AL355773.4	INTERGENIC	C	0.0007374	1.26	0.001552
410	17	rs16950116	47022532	CA10	INTERGENIC	T	0.0007383	1.44	0.9888
411	3	rs1242061	144859179	SLC9A9	INTRONIC	A	0.0007388	1.31	0.03286
412	8	rs13277804	18848961	PSD3	INTRONIC	A	0.0007399	0.67	0.1476
413	2	rs10187702	58723279	IK	INTERGENIC	G	0.0007408	0.71	0.001433
414	4	rs4485803	148270902	TTC29	INTERGENIC	A	0.0007409	0.47	0.193
415	5	rs1428609	66716372	AC008459.7	INTERGENIC	G	0.0007418	1.24	1.002
416	15	rs3098538	25501132	GABRG3	INTERGENIC	A	0.0007423	1.30	0.1398
417	2	rs1177265	61192336	KIAA1841	INTRONIC	G	0.0007438	0.80	0.218
418	2	rs17750151	171266344	AC007277.2	INTERGENIC	G	0.0007442	0.69	0.05135
419	6	rs381480	105039869	RP11-427E4.1	INTERGENIC	T	0.00075	1.34	0.1703
420	5	rs9918231	29669784	NAGK	INTERGENIC	A	0.0007506	1.27	0.1345
421	2	rs6724815	71138848	AC010374.6	INTERGENIC	A	0.0007506	1.46	1.001
422	5	rs10042848	92035583	AC026781.5	INTERGENIC	G	0.0007512	1.34	0.3285
423	6	rs9371581	152671279	SYNE1	INTRONIC	G	0.0007551	0.80	0.07961
424	6	rs3778279	26433442	H3F3AP1	UPSTREAM	C	0.000756	1.24	0.2104
425	11	rs4406832	130077513	C11orf44	INTRONIC	A	0.0007569	0.73	0.004991
426	17	rs578065	15705232	AC015922.5	NON_CODING	G	0.000757	1.25	0.0733
427	5	rs10491314	141535648	NDFIP1	INTERGENIC	A	0.00076	1.31	0.01036
428	19	rs3764640	1158238	STK11	INTRONIC	A	0.0007603	0.75	0.9791

429	2	rs1035601	220593213	AC114765.6	INTERGENIC	G	0.0007618	1.32	0.004481
430	4	rs717435	108452512	AC104663.3	INTERGENIC	T	0.0007631	0.80	0.01781
431	12	rs11057929	124006284	DHX37	INTRONIC	C	0.0007656	1.30	0.2553
432	6	rs9366354	20653447	CDKAL1	INTRONIC	G	0.0007676	0.79	0.2312
433	18	rs8098687	30773270	DTNA	INTERGENIC	A	0.0007677	0.73	0.003418
434	8	rs2008555	96802870	AC116154.2	INTERGENIC	T	0.000768	0.76	0.3338
435	10	rs11244887	127892517	ADAM12	INTRONIC	T	0.0007682	0.76	1.011
436	17	rs790097	69115102	SDK2	INTERGENIC	A	0.0007683	0.66	0.1797
437	2	rs16856529	217281937	IGFBP5	INTERGENIC	G	0.0007691	1.33	0.1497
438	20	rs6011530	61112293	AL121673.41	INTRONIC	A	0.0007736	1.36	0.385
439	2	rs7597992	70797524	ADD2	INTRONIC	C	0.0007741	1.32	0.09461
440	6	rs12207523	132027750	ENPP3	INTRONIC	T	0.0007759	0.56	0.1299
441	2	rs888182	217280090	IGFBP5	INTERGENIC	C	0.000776	1.32	0.06355
442	15	rs7177318	48159823	ATP8B4	INTRONIC	T	0.0007764	1.24	0.0006585
443	12	rs3217907	4277097	CCND2	INTRONIC	A	0.0007766	1.25	0.00359
444	6	rs16893540	28066090	RP3-408B20.4	INTERGENIC	T	0.0007824	2.08	0.001104
445	10	rs1471247	61744286	ANK3	INTRONIC	A	0.0007824	0.80	0.06508
446	3	rs651165	173895581	AADACL1	INTRONIC	A	0.0007867	1.28	0.1087
447	12	rs4471501	49711619	SLC11A2	UPSTREAM	A	0.0007868	0.77	0.09591
448	6	rs11155004	139160262	C6orf91;ECT2L	INTRONIC	T	0.000787	0.80	0.2207
449	15	rs7176145	66490322	ITGA11	INTRONIC	G	0.0007882	1.33	0.000597
450	6	rs1395633	54665623	RP3-334F4.1	INTERGENIC	T	0.0007902	1.45	0.1954
451	10	rs11015633	19019475	ARL5B	INTERGENIC	G	0.000792	1.24	0.08936
452	6	rs9480754	107656140	PDSS2	INTRONIC	G	0.0007928	1.27	0.07712
453	3	rs4684237	14685592	C3orf19	INTRONIC	T	0.000799	1.29	0.07652
454	1	rs2294642	11644280	FBXO44	3PRIME_UTR	T	0.0008012	0.60	0.2628
455	15	rs16940650	85926915	TMEM83	DOWNSTREAM	T	0.0008016	1.53	0.04957

456	18	rs9304251	37534376	AC011225.1	INTERGENIC	A	0.0008073	1.45	1.007
457	6	rs2753977	66176184	EGFL10;EYS	INTRONIC	C	0.0008138	1.34	0.007076
458	10	rs1475418	6730153	PRKCQ	INTERGENIC	A	0.0008229	1.26	0.003404
459	6	rs9349437	48017897	C6orf138	INTRONIC	T	0.0008239	1.33	0.3785
460	1	rs953517	36682864	C1orf102	INTRONIC	C	0.0008244	0.79	0.1432
461	21	rs2838000	41568388	BACE2	INTRONIC	T	0.0008251	1.29	1.011
462	12	rs4768967	49792595	TFCP2	INTRONIC	A	0.0008266	0.75	0.1117
463	4	rs931602	79704900	ANXA3	INTRONIC	C	0.0008271	1.27	0.1524
464	12	rs10444509	100738161	GNPTAB	INTRONIC	G	0.0008275	0.73	0.04093
465	1	rs4073574	11725021	AGTRAP	INTRONIC	G	0.0008322	1.25	0.9827
466	3	rs968628	156299605	MME	INTRONIC	G	0.0008367	1.24	0.001567
467	3	rs10460808	106986819	CBLB	INTRONIC	C	0.0008437	1.42	0.004538
468	11	rs516431	131301209	HNT;NTM	INTRONIC	T	0.0008462	1.28	0.3687
469	8	rs12679771	140971097	TRAPPC9	INTRONIC	T	0.0008481	0.75	0.132
470	18	rs623966	30715894	DTNA	INTRONIC	C	0.0008515	0.74	0.0008519
471	13	rs7983069	38379318	RP11-7B3.3	DOWNSTREAM	A	0.0008536	0.80	0.06151
472	3	rs1111516	135552894	RPL39P5	DOWNSTREAM	T	0.0008539	0.79	0.1707
473	10	rs10901559	127888676	ADAM12	INTRONIC	T	0.0008637	0.76	1.014
474	1	rs12408243	46762631	DMBX1	INTERGENIC	C	0.0008653	0.81	0.007723
475	2	rs2392820	221661777	AC019208.8	INTERGENIC	T	0.0008678	1.26	1.02
476	10	rs4747272	6753766	PRKCQ	INTERGENIC	T	0.0008683	1.33	0.03274
477	22	rs7292528	25046227	SEZ6L	INTRONIC	T	0.0008688	1.42	0.2794
478	19	rs7259059	55206290	VRK3	INTRONIC	T	0.0008727	1.29	0.1893
479	3	rs2667469	124371327	PDIA5	INTERGENIC	G	0.0008751	0.76	0.9791
480	7	rs3735219	148144400	EZH2	INTRONIC	A	0.000877	1.34	0.02664
481	16	rs1155970	81422728	AC099506.3	INTERGENIC	C	0.0008791	0.79	0.05162
482	3	rs1461762	111465959	AC117430.3-2	INTERGENIC	A	0.0008797	0.72	0.06479

483	4	rs17039589	110105816	COL25A1	INTRONIC	A	0.0008874	1.39	0.05128
484	6	rs995291	105032852	RP11-427E4.1	INTERGENIC	A	0.0008879	1.34	0.216
485	3	rs7623367	193713253	AC026671.18	INTERGENIC	G	0.000888	0.58	0.02673
486	2	rs13032780	191930845	MYO1B	INTRONIC	T	0.0008932	0.77	0.000455
487	8	rs4872211	23983658	AC023202.8	INTERGENIC	A	0.0008945	0.71	0.2349
488	6	rs1885275	136160513	PDE7B	INTERGENIC	T	0.000896	1.27	0.123
489	18	rs11080606	12857969	PTPN2	INTRONIC	G	0.0008982	1.26	0.2259
490	7	rs7783459	148204831	EZH2	INTRONIC	A	0.0008991	1.34	0.01738
491	22	rs11704749	33942297	HMGXB4	INTERGENIC	A	0.0009009	0.64	0.157
492	7	rs38519	28004411	JAZF1	INTRONIC	T	0.0009025	1.24	0.009706
493	11	rs7938094	39904380	AC021749.6-3	INTERGENIC	C	0.0009057	0.75	0.03027
494	9	rs2860101	70293226	PGM5	INTRONIC	T	0.0009092	0.69	0.00955
495	10	rs10751569	128361479	AL512272.15	INTERGENIC	A	0.0009097	0.80	0.02037
496	12	rs10859283	91145168	AC025164.37-2	INTERGENIC	T	0.0009147	1.27	0.08521
497	14	rs12885638	56461434	AL137100.4	INTERGENIC	C	0.0009156	1.25	0.1334
498	5	rs13165786	83892156	EDIL3	INTERGENIC	C	0.0009195	1.24	0.04904
499	11	rs7118622	11355982	GALNTL4	INTRONIC	C	0.0009246	0.77	1.016
500	3	rs9852993	134093286	TMEM108	INTERGENIC	T	0.0009254	0.75	0.9886
501	1	rs12132152	97523004	DPYD	INTERGENIC	A	0.0009284	2.05	0.00914
502	21	rs7280677	20549970	AP000431.2	INTERGENIC	C	0.0009307	0.79	0.3572
503	1	rs17109254	83289801	RP4-601K24.1	INTERGENIC	A	0.0009324	1.96	0.02495
504	8	rs4240188	12685435	LONRF1	INTERGENIC	A	0.0009327	1.24	0.3855
505	11	rs7943388	39966521	AC021749.6	INTERGENIC	T	0.0009331	0.71	0.08051
506	12	rs149411	51380232	SLC11A2	3PRIME_UTR	T	0.000935	0.80	0.05944
507	6	rs195850	99297801	POU3F2	INTERGENIC	T	0.0009362	0.72	0.5548
508	4	rs998091	5687628	EVC2	INTRONIC	T	0.0009367	1.25	0.08107
509	3	rs500193	182846387	LAMP3	INTRONIC	G	0.0009377	1.48	0.05818

510	1	rs6425838	34254416	CSMD2	INTRONIC	T	0.0009379	1.26	0.1339
511	2	rs7577925	134040519	AC010890.1	INTRONIC	T	0.0009384	0.79	0.01804
512	5	rs10462823	6891874	AC122710.3	INTERGENIC	A	0.0009444	0.70	0.1031
513	6	rs9356756	20844151	CDKAL1	INTRONIC	G	0.0009445	1.24	0.02786
514	5	rs11959584	151854302	NMUR2	INTERGENIC	C	0.0009474	1.24	0.2172

n/a = not applicable

Table 5.5. Breakdown of minor allele frequencies for SNPs $<10^{-4}$ in either LOAD+P vs. LOAD-P or LOAD+P vs. control a
irrespective of analysis. Table shows chromosome, SNP ID, gene name, minor allele, and minor allele frequency in LOA

Chr	SNP	Gene	Minor Allele	MAF LOAD+P	MAF LOAD
19	rs2075650	TOMM40	C	0.30	0.29
19	rs157580	TOMM40	G	0.29	0.31
19	rs6859	PVRL2	C	0.48	0.48
19	rs439401	APOE;PKP2	T	0.27	0.30
19	rs8106922	TOMM40	G	0.30	0.31
15	rs8038077	RORA	G	0.11	0.07
19	rs405509	APOE;PKP2	C	0.43	0.42
7	rs868055	MIRN183	T	0.12	0.08
12	rs1906950	AC092451.12-2	A	0.41	0.35
8	rs4527852	TRMT12	T	0.11	0.08
8	rs2034140	MCPH1	G	0.02	0.07
6	rs11756091	KCNK16	G	0.48	0.42
8	rs10956535	ASAP1	C	0.44	0.33
2	rs1430170	AC010974.1	G	0.23	0.29
8	rs10094093	AC019176.4-2	T	0.16	0.20
4	rs753129	AC110611.3-1	G	0.30	0.21
9	rs960644	RP11-341A22.1	G	0.11	0.18
3	rs9289666	SLC9A9	A	0.15	0.10
19	rs10402948	CDC34	T	0.10	0.07
7	rs4726443	AC005692.1	T	0.05	0.03
2	rs4671328	IK	A	0.39	0.43
8	rs16899656	TATDN1	G	0.11	0.08
12	rs2682778	SUDS3	T	0.17	0.25
10	rs1444400	PRKG1	G	0.37	0.28

10	rs1444401	PRKG1	A	0.37	0.28
16	rs11648322	GRIN2A	T	0.42	0.38
10	rs6482252	AL139815.12-3	T	0.45	0.49
10	rs6480276	PRKG1	G	0.37	0.28
10	rs7902903	AL139815.12-3	T	0.44	0.49
12	rs12372711	AC092451.12-2	G	0.41	0.35
2	rs1861410	IK	C	0.39	0.43
2	rs1519473	VSNL1	C	0.10	0.07
2	rs11680774	CCDC148	C	0.21	0.29
4	rs753129	AC110611.3-1	G	0.30	0.21
2	rs2883854	CCDC148	C	0.23	0.32
6	rs3734618	KCNK16	T	0.48	0.43
2	rs4038129	VSNL1	C	0.10	0.06
15	rs8031076	ATP8B4	A	0.43	0.40
1	rs12045777	EPB41	A	0.47	0.44
2	rs1949510	VSNL1	A	0.10	0.06
16	rs11645153	CDH13	C	0.23	0.18
12	rs1520780	SUDS3	A	0.18	0.27
19	rs4919850	GZMM	T	0.17	0.13
5	rs11740920	FSTL4	C	0.26	0.22
2	rs6741951	IK	T	0.24	0.25
17	rs9904097	TNRC6C	G	0.39	0.34
4	rs2695234	SOD3	T	0.05	0.10
12	rs4759721	RIMBP2	C	0.40	0.46
4	rs12648438	AC115540.3	A	0.23	0.16
7	rs1558542	EGFR	T	0.24	0.19
16	rs922302	AC099506.3	G	0.24	0.21
8	rs12678035	AC091559.7	C	0.01	0.03

2	rs10176091	IK	T	0.28	0.29
6	rs9404549	RP11-427E4.1	T	0.17	0.10
5	rs6870951	FSTL4	A	0.15	0.11
2	rs1978346	IGFBP5	G	0.41	0.35
11	rs2512152	AP002962.2	A	0.23	0.27
19	rs379758	LYPD5	G	0.15	0.12
6	rs13201744	F13A1	T	0.08	0.15
4	rs2100889	IBSP	A	0.27	0.35
3	rs578641	AC055758.23	C	0.17	0.22
15	rs10519257	ATP8B4	G	0.30	0.27
10	rs7079742	ARL5B	T	0.36	0.44
10	rs941856	AFAP1L2	G	0.09	0.06
5	rs10474519	AC108173.1	T	0.13	0.10
17	rs4789523	TNRC6C	A	0.33	0.27
2	rs3771599	PK4P;PKP4	G	0.34	0.43
2	rs11674284	PK4P;PKP4	C	0.25	0.34
16	rs7189966	AC099506.3	C	0.24	0.21
12	rs12366756	SLC11A2	C	0.16	0.18
6	rs7756959	RP11-427E4.1	C	0.17	0.10
2	rs6711582	PK4P;PKP4	T	0.33	0.42
3	rs6800179	CLSTN2	C	0.17	0.11
11	rs1400756	C11orf53	C	0.16	0.11
8	rs12543670	AC019176.4-2	G	0.18	0.20
20	rs747680	DIDO1	G	0.45	0.50
8	rs10956535	ASAP1	C	0.44	0.33
9	rs9314866	RP11-336N8.1	A	0.31	0.35
10	rs6585251	NHLRC2	T	0.23	0.27
2	rs2356186	PK4P;PKP4	T	0.34	0.43

1	rs2640467	MECR	C	0.39	0.31
6	rs9404549	RP11-427E4.1	T	0.17	0.10
18	rs17240415	CCDC102B	A	0.25	0.30
7	rs10235799	AC006008.2	C	0.37	0.45
18	rs280989	n/a	G	0.36	0.40
10	rs2670106	KCNMA1	C	0.08	0.11
12	rs11169654	SLC11A2	A	0.16	0.18
6	rs156235	RP11-427E4.1	C	0.16	0.10
12	rs11535963	AC087240.17	T	0.24	0.32
3	rs7612414	AC026305.20-3	G	0.09	0.06
2	rs848607	AC007401.2	A	0.25	0.31
6	rs7740010	RP11-427E4.1	A	0.16	0.10
12	rs11044223	PIK3C2G	C	0.07	0.13
1	rs3929673	C1orf196	T	0.32	0.41
6	rs7756959	RP11-427E4.1	C	0.17	0.10
4	rs6828769	AC115540.3	G	0.24	0.16
12	rs831429	STAB2	C	0.32	0.28
2	rs3764835	AC005042.2	T	0.13	0.20
16	rs17720179	JMJD5	T	0.11	0.17
13	rs9512519	USP12	C	0.03	0.07
18	rs206749	TMX3	G	0.44	0.37
19	rs745952	VRK3	T	0.16	0.14
2	rs3771635	PK4P;PKP4	G	0.25	0.34
4	rs1989924	ALPK1	A	0.46	0.37
7	rs2693737	MIRN183	C	0.12	0.07
14	rs10143543	AL160237.4-1	A	0.39	0.30
6	rs7740010	RP11-427E4.1	A	0.16	0.10
6	rs156235	RP11-427E4.1	C	0.16	0.10

16	rs8061432	HS3ST4	T	0.09	0.05
2	rs3755365	ADD2	C	0.36	0.27
16	rs17674628	PMM2	A	0.23	0.16
18	rs4799872	FHOD3	A	0.40	0.35
10	rs11252926	DIP2C	A	0.31	0.40
7	rs17156246	CACNA2D1	T	0.16	0.10
1	rs7520521	LRRC7	G	0.34	0.39
15	rs8040030	ATP8B4	A	0.49	0.46
3	rs9867560	AC104471.6	T	0.11	0.18
3	rs17443484	AC104471.6	A	0.11	0.18
16	rs9927466	AC018554.7	T	0.41	0.33
10	rs11252923	DIP2C	T	0.31	0.40
6	rs7759504	ENPP3	T	0.02	0.04
10	rs1576479	NHLRC2	A	0.43	0.47
7	rs219824	SMURF1	A	0.38	0.34
10	rs7913176	NHLRC2	T	0.23	0.27
1	rs1146413	AL158218.11	C	0.38	0.46
9	rs1328533	GNAQ	C	0.32	0.36
10	rs10870210	C10orf93	A	0.11	0.07
3	rs2171513	BTLA	T	0.02	0.03
9	rs11145589	GNAQ	T	0.20	0.25
4	rs10519397	AC006572.2	T	0.06	0.10
3	rs9865618	AC104471.6	G	0.11	0.17
2	rs2080394	FIGLA	C	0.44	0.47
7	rs17155687	NRCAM	T	0.09	0.09
16	rs1436046	AC018554.7	C	0.48	0.40
15	rs17237486	RORA	G	0.21	0.14
6	rs267339	RP11-427E4.1	A	0.19	0.13

10	rs7089944	CCDC3	C	0.22	0.30
2	rs3771408	ADD2	A	0.42	0.49
3	rs2139266	TRIM42	T	0.46	0.45
19	rs17312204	AC010624.8-1	G	0.10	0.09
13	rs9512519	USP12	C	0.03	0.07
6	rs9354570	AL591004.3	T	0.15	0.11
1	rs1768404	AL158218.11	A	0.38	0.46
15	rs8041340	ATP8B4	C	0.35	0.37
8	rs7015252	ASAP1	A	0.39	0.48
1	rs6661589	LRRC7	A	0.34	0.39
2	rs2681031	AC104807.5-2	G	0.48	0.39
5	rs683004	AC010374.6	A	0.27	0.35
5	rs620508	AC010374.6	G	0.27	0.35
3	rs925665	AC090955.3	A	0.31	0.39
4	rs6826655	AC097521.2-2	A	0.41	0.50
14	rs6573225	AL049873.3-2	C	0.15	0.09
14	rs442555	AL049873.3-2	C	0.15	0.09
14	rs399223	AL049873.3-2	C	0.17	0.11
14	rs177726	AL049873.3-2	T	0.17	0.11
2	rs7597989	CALCRL	A	0.42	0.48
2	rs17808559	CCDC148	T	0.25	0.34
10	rs4881399	DIP2C	T	0.31	0.40
6	rs775258	GRIK2	G	0.10	0.16
3	rs4613440	GRIP2	T	0.50	0.41
11	rs598829	HNT;NTM	A	0.34	0.26
13	rs12560710	n/a	A	0.11	0.06
13	rs9284246	MYO16	G	0.44	0.35
2	rs4853462	MYO1B	C	0.36	0.28

10	rs3903435	NRG3	G	0.42	0.49
10	rs9664256	NRG3	G	0.43	0.49
11	rs750270	OPCML	A	0.10	0.05
3	rs13070407	RARB	G	0.25	0.33
6	rs267339	RP11-427E4.1	A	0.19	0.13
6	rs9377643	RP11-427E4.1	C	0.16	0.10
4	rs2303513	SEC24D	A	0.26	0.34
11	rs17794760	TPH1	A	0.15	0.23
8	rs7842680	XKR4	C	0.17	0.11
12	rs10745978	STAB2	C	0.48	0.44
10	rs1107442	RP11-73H14.1	G	0.25	0.20
10	rs1986577	PLXDC2	T	0.40	0.34
12	rs1031477	KIAA1602	C	0.42	0.47
18	rs4798993	AC139070.3	T	0.18	0.19
12	rs7296829	STAB2	C	0.48	0.44
2	rs6716814	AC064872.3	A	0.28	0.24
6	rs6939897	AL591004.3	C	0.15	0.11
19	rs285684	CHST8	T	0.25	0.29
15	rs12909131	ATP8B4	T	0.29	0.25
10	rs35084455	GRID1	T	0.10	0.07
3	rs6806737	CLSTN2	A	0.17	0.12
14	rs17558231	SMOC1	C	0.10	0.09
2	rs6727330	IGFBP5	C	0.41	0.35
2	rs1991108	AC114765.6	A	0.20	0.17
1	rs11122300	GALNT2	T	0.23	0.21
6	rs7756211	CDKAL1	T	0.37	0.30
12	rs17027488	RNU6-2	G	0.29	0.24
2	rs12991296	AC064872.3	C	0.15	0.12

12	rs755598	STAB2	G	0.48	0.44
2	rs885640	TANC1	G	0.39	0.34
12	rs2398518	RIMBP2	A	0.46	0.39
6	rs7773295	PDSS2	A	0.24	0.22
3	rs6808569	SEC22A	T	0.14	0.17
5	rs620508	AC010374.6	G	0.27	0.35
2	rs2861264	AC064872.3	A	0.14	0.12
10	rs2926872	FRMD4A	G	0.13	0.11
3	rs1915728	AC026305.20-3	G	0.07	0.05
3	rs3804592	CASR	T	0.10	0.14
6	rs7758851	CDKAL1	T	0.36	0.30
13	rs1951898	RP11-421P11.8	A	0.16	0.12
16	rs2549159	AC099506.3	C	0.49	0.46
8	rs777801	ADD2	T	0.04	0.07
2	rs3755365	TRPS1	C	0.36	0.27
21	rs2096471	CLIC6	C	0.25	0.20
10	rs7908946	ADAM12	A	0.18	0.25
9	rs1926381	RP11-87M1.1	T	0.16	0.20
18	rs12454977	SALL3	A	0.13	0.18
22	rs760656	BPIL2	C	0.18	0.24
17	rs9909561	GARNL4	A	0.44	0.48
8	rs6578080	TRAPPC9	T	0.50	0.46
5	rs1298248	AC091894.2	T	0.39	0.34
12	rs7979925	AC084398.25-2	C	0.35	0.42
17	rs9889827	TNRC6C	C	0.41	0.35
9	rs3808720	SH3GL2	T	0.33	0.32
4	rs2309472	n/a	A	0.34	0.37
8	rs2034140	AC026305.20-3	G	0.02	0.07

3	rs6788646	MCPH1	A	0.07	0.05
4	rs13108814	C4orf22	A	0.25	0.19
11	rs7936316	FAM181B	A	0.03	0.03
6	rs2745933	CDKAL1	T	0.37	0.43
5	rs905858	AC010374.6	G	0.37	0.44
5	rs607077	AC010374.6	G	0.28	0.35
2	rs12470671	AC011747.5	A	0.36	0.44
5	rs1550825	AC016575.7	A	0.35	0.43
16	rs9927466	AC018554.7	T	0.41	0.33
3	rs7614031	AC069504.7	A	0.45	0.36
2	rs1918901	AC074020.4	C	0.40	0.50
5	rs4527553	AC092373.2-2	T	0.15	0.22
4	rs7687115	AC097521.2-2	G	0.41	0.49
2	rs3755361	ADD2	T	0.42	0.49
8	rs11781115	ADRA1A	G	0.29	0.37
14	rs983449	AL049873.3-2	T	0.11	0.06
9	rs902143	AL512649.6	A	0.42	0.50
1	rs12038526	AL691459.25	G	0.13	0.20
2	rs7563401	ANXA4	G	0.47	0.44
2	rs7567400	ANXA4	G	0.47	0.44
10	rs7079742	ARL5B	T	0.36	0.44
1	rs1894654	CAMTA1	G	0.29	0.37
3	rs9823958	CLSTN2	A	0.14	0.08
7	rs12669501	COL1A2	A	0.08	0.14
7	rs4729127	COL1A2	C	0.08	0.14
5	rs286969	FAM114A2	T	0.43	0.35
13	rs9513354	FARP1	A	0.24	0.17
16	rs4548875	HS3ST4	A	0.18	0.12

16	rs4362392	HS3ST4	C	0.18	0.12
7	rs11767477	MAGI2	C	0.27	0.19
8	rs6470563	MYC	C	0.07	0.12
2	rs7574570	MYO1B	C	0.36	0.28
2	rs10445783	MYO1B	G	0.36	0.28
18	rs8099057	n/a	A	0.15	0.22
3	rs188423	NMNAT3	T	0.47	0.39
3	rs295464	NMNAT3	G	0.47	0.39
2	rs3771644	PK4P;PKP4	A	0.25	0.33
16	rs2304469	PMM2	T	0.48	0.43
19	rs4805441	POP4	T	0.47	0.44
14	rs11851391	PRIMA1	A	0.13	0.08
10	rs10823083	PRKG1	A	0.32	0.40
12	rs2285831	RAPGEF3	A	0.12	0.07
19	rs3810342	RASGRP4	G	0.20	0.13
9	rs16922740	RP11-341A22.1	C	0.07	0.12
13	rs4343137	RP11-65D24.2	T	0.29	0.21
11	rs2468782	SAA3P	G	0.16	0.23
19	rs3764640	STK11	A	0.17	0.23
9	rs7852394	TLR4	C	0.48	0.40
9	rs7854254	TLR4	G	0.48	0.40
9	rs10739508	TLR4	T	0.48	0.44
9	rs1329771	TRPM3	G	0.41	0.49
18	rs7231736	YES1	G	0.46	0.38
19	rs6509701	ZNF320	C	0.33	0.25
19	rs6509698	ZNF468	T	0.17	0.11
2	rs2593708	MGAT5	T	0.43	0.38
3	rs1533393	SEC22A	C	0.14	0.17

6	rs1338715	AL391417.7-2	A	0.33	0.28
4	rs12648767	LAP3	A	0.37	0.33
8	rs2702910	DEFA10P	T	0.24	0.28
11	rs7929650	RASSF10	G	0.11	0.09
6	rs2674394	AL589947.3-2	A	0.18	0.15
5	rs683004	AC010374.6	A	0.27	0.35
16	rs11150535	CDH13	T	0.49	0.41
3	rs1520593	AC026305.20-3	C	0.07	0.05
18	rs7229111	FHOD3	A	0.40	0.36
6	rs2674371	AL589947.3-2	C	0.18	0.15
9	rs3780232	SH3GL2	C	0.33	0.32
11	rs576825	HNT;NTM	C	0.39	0.34
10	rs4339947	PRKCQ	C	0.34	0.32
3	rs7653338	AC095349.10	A	0.07	0.05
17	rs1122634	EPN3	C	0.50	0.44
3	rs7623722	AC097361.2	A	0.45	0.48
3	rs4580491	n/a	C	0.14	0.17
6	rs9342616	AL590874.6	T	0.42	0.47
19	rs3764650	ABCA7	C	0.12	0.11
9	rs10981195	AL138756.23-1	A	0.28	0.24
12	rs11047988	AC092451.12-2	G	0.40	0.35
4	rs6834555	WDR1	C	0.17	0.20
11	rs2085334	CCDC67	G	0.32	0.25
4	rs35495915	PDGFRA	T	0.23	0.28
2	rs12986742	IK	C	0.43	0.45
19	rs10402271	BCAM	G	0.38	0.39
2	rs13425498	AC064872.3	T	0.27	0.22
5	rs3909548	ADAMTS19	A	0.15	0.12

7	rs2693727	MIRN183	G	0.11	0.07
16	rs844395	GRIN2A	A	0.35	0.37
3	rs6770430	AC095349.10	A	0.07	0.05
19	rs377702	PVRL2	T	0.43	0.43
12	rs1871898	TMEM132D	T	0.48	0.46
18	rs11876226	ZNF521	G	0.21	0.17
3	rs9876068	RNF7	C	0.02	0.04
22	rs5753436	AC005005.1-1	G	0.11	0.14
11	rs3096716	PRSS23	T	0.29	0.28
3	rs7648855	AC095349.10	G	0.07	0.05
16	rs10492859	AC099506.3	G	0.18	0.20
10	rs7089454	ADAM12	G	0.21	0.27
16	rs718510	AC018554.7	A	0.33	0.40
10	rs821705	AL133395.21	T	0.36	0.32
3	rs6766386	AC055758.23	T	0.20	0.26
2	rs1517443	AC019051.8	G	0.36	0.45
3	rs7637623	AC069067.17	G	0.42	0.50
4	rs16890321	AC093777.4	T	0.19	0.25
3	rs6441421	AC104471.6	T	0.34	0.42
3	rs1376967	AC129415.1-2	G	0.20	0.14
14	rs6573334	AL160237.4-1	T	0.13	0.08
9	rs10810562	BNC2	A	0.15	0.10
15	rs954432	C15orf53	C	0.37	0.29
1	rs7515917	C1orf196	A	0.47	0.45
7	rs10954664	CACNA2D1	G	0.46	0.38
3	rs6800179	CLSTN2	C	0.17	0.11
8	rs1441249	CNGB3	T	0.43	0.49
12	rs1663588	CPM	A	0.10	0.15

13	rs11617079	DNAJC15	G	0.20	0.27
13	rs2281782	DNAJC15	C	0.20	0.27
11	rs10431058	ELMOD1	T	0.37	0.45
6	rs6931820	EPHA7	T	0.29	0.37
8	rs6559175	MCPH1	C	0.22	0.29
11	rs4758480	MRGPRG	T	0.36	0.45
6	rs855250	OFCC1	C	0.11	0.17
4	rs4699587	PDLIM5	C	0.04	0.08
8	rs10099199	PSD3	G	0.12	0.18
3	rs11706561	RARB	T	0.15	0.21
12	rs678941	RBM19	T	0.10	0.16
13	rs4271475	RP11-217F17.1	T	0.45	0.37
1	rs1041705	RP11-339A11.1	C	0.40	0.32
13	rs1927554	RP11-56M2.1	C	0.38	0.46
13	rs1517897	RP11-56M2.1	T	0.37	0.45
1	rs655146	RP11-575B7.2	G	0.12	0.18
10	rs10827186	RPL7AP53	G	0.18	0.24
1	rs7556195	SIPA1L2	G	0.16	0.23
16	rs12049	TMEM186;C16orf51	C	0.49	0.43
11	rs6592039	FAM181B	A	0.02	0.04
19	rs17801681	SIGLECP3	A	0.08	0.05
1	rs1258033	KNCN	C	0.22	0.27
11	rs2433438	PRSS23	G	0.29	0.28
6	rs9473511	RP1-142O9.2	T	0.09	0.07
6	rs9358383	CDKAL1	G	0.31	0.25
12	rs2301529	SLC11A2	T	0.16	0.18
6	rs2767576	AL158033.18	C	0.20	0.18
8	rs3857946	MTSS1	A	0.11	0.08

16	rs837688	GRIN2A	G	0.45	0.41
10	rs10904321	AKR1CL2	C	0.49	0.43
9	rs12343558	C9orf94;ADAMTSL1	C	0.30	0.27
3	rs9869506	AC117430.3-2	G	0.09	0.13
6	rs9377643	RP11-427E4.1	C	0.16	0.10
18	rs8091319	AP000902.5	T	0.25	0.21
10	rs528320	C10orf49	C	0.16	0.12
5	rs7702830	EPB41L4A	C	0.20	0.15
3	rs966866	CLSTN2	C	0.33	0.28
11	rs1949707	FAM181B	C	0.02	0.04
10	rs7097356	ADAM12	C	0.21	0.27
14	rs2369018	GLRX5	T	0.19	0.24
14	rs6575028	TTC8	T	0.22	0.20
11	rs10831608	GALNTL4	T	0.43	0.39
8	rs3824110	ZNF16	A	0.21	0.25
20	rs6097096	AL049736.10	A	0.15	0.11
10	rs3936497	MPP7	C	0.09	0.08
12	rs10860757	MYBPC1	C	0.09	0.12
19	rs8103315	BCL3	T	0.17	0.16
10	rs10827932	PLXDC2	T	0.45	0.40
18	rs1893311	ZNF521	T	0.20	0.16
16	rs12922279	AC018554.7	C	0.34	0.41
8	rs10104503	XKR5	T	0.35	0.30
6	rs9294750	AC021749.6-3	C	0.43	0.47
11	rs10837311	AL591004.3	T	0.15	0.18
15	rs12903325	ATP8B4	C	0.28	0.25
12	rs12824958	AC008121.43-2	C	0.16	0.18
18	rs4799869	FHOD3	A	0.38	0.34

12	rs10745935	CCDC53	G	0.05	0.09
15	rs6603018	ADAMTSL3	T	0.17	0.12
9	rs504896	NDUFB6	A	0.09	0.07
9	rs10971038	NDUFB6	A	0.10	0.07
12	rs11044223	PIK3C2G	C	0.07	0.13
2	rs1615070	C2orf74	T	0.50	0.45
15	rs906194	AC103965.5	A	0.17	0.12
14	rs17103033	C14orf10;PPP2R3C	G	0.18	0.14
10	rs17684713	ADAM12	A	0.15	0.21
5	rs1345686	EPB41L4A	T	0.20	0.15
1	rs34411680	PARS2	C	0.17	0.14
2	rs10514647	PARD3B	G	0.11	0.07
2	rs6436883	DNER	T	0.44	0.40
2	rs17338519	MYT1L	T	0.13	0.10
22	rs80533	MCHR1	A	0.28	0.25
9	rs7871498	ZCCHC7	A	0.25	0.19
10	rs9888067	RP11-400G3.3	A	0.16	0.21
8	rs1893884	STK3	A	0.49	0.45
4	rs6848311	F11	T	0.16	0.20
2	rs744373	BIN1	G	0.33	0.31
7	rs700316	AC006992.2	A	0.14	0.21
15	rs2711644	AC066611.6	T	0.41	0.33
2	rs7593799	AC074020.4	T	0.41	0.50
2	rs1569135	AC074020.4	G	0.41	0.49
12	rs2130635	AC090680.11	C	0.11	0.16
4	rs4861491	AC108142.1	T	0.12	0.18
14	rs17121169	AL049775.2	G	0.12	0.07
14	rs7160035	AL160237.4-1	T	0.13	0.08

2	rs2312548	ANXA4	A	0.42	0.50
11	rs604967	AP003100.3	C	0.50	0.42
11	rs643122	AP003100.3	T	0.50	0.42
8	rs10088108	ATP6V1B2	G	0.17	0.23
1	rs2365716	BCAN	C	0.32	0.24
2	rs3771084	CALCRL	A	0.38	0.46
2	rs7564839	CCDC148	G	0.29	0.37
16	rs11150535	CDH13	T	0.49	0.41
4	rs9996873	CLCN3	C	0.18	0.24
3	rs1447721	CLSTN2	T	0.13	0.08
10	rs7094118	CUGBP2	C	0.30	0.23
2	rs1583631	ERBB4	T	0.28	0.35
9	rs10963919	FAM154A	G	0.14	0.09
14	rs10484053	GLRX5	T	0.16	0.11
1	rs10493201	GOT2L1	C	0.15	0.10
6	rs1544050	KCNK17	C	0.39	0.47
5	rs2074344	KLHL3	G	0.11	0.06
4	rs4082297	LNX1	C	0.18	0.12
1	rs2990678	LRRC38	A	0.31	0.39
7	rs16885938	MAGI2	T	0.23	0.16
9	rs4877108	NXNL2	C	0.33	0.26
5	rs329320	PHF15	G	0.22	0.16
12	rs621042	PIK3C2G	A	0.43	0.49
10	rs1125478	RP11-448K10.1	T	0.23	0.30
10	rs1930457	RP11-448K10.1	A	0.06	0.11
13	rs2897319	RP11-94N9.1	C	0.21	0.15
6	rs10945617	RP3-393E18.1	A	0.24	0.17
11	rs868344	SCN4B	C	0.39	0.31

5	rs12189367	SEMA5A	A	0.07	0.13
20	rs2427460	SLC17A9	C	0.45	0.47
5	rs2042243	TRPC7	A	0.13	0.08
20	rs1016404	ZFP64	A	0.44	0.48
14	rs11628156	AL355773.4	G	0.39	0.35
14	rs1885186	TTC8	T	0.22	0.20
18	rs12454185	SALL3	G	0.14	0.18
3	rs6790000	PDIA5	C	0.17	0.22
6	rs17445948	RP3-334F4.1	A	0.10	0.08
2	rs17338512	MYT1L	A	0.13	0.10
3	rs6788684	OXSM	A	0.18	0.16
3	rs1705589	PRKCI	C	0.17	0.22
11	rs598829	HNT;NTM	A	0.34	0.26
1	rs11579916	AL359915.14	T	0.14	0.16
8	rs13248917	AC019129.1	T	0.45	0.49
2	rs7607908	IDO2	A	0.17	0.14
9	rs10738482	SH3GL2	A	0.34	0.33
2	rs6753886	SLC5A7	T	0.32	0.37
14	rs2401785	TTC8	A	0.22	0.20
8	rs12541503	XKR5	T	0.28	0.24
10	rs9663603	AL133395.21	C	0.28	0.26
10	rs11006995	AC022021.10-3	A	0.08	0.06
11	rs10899236	SHANK2	G	0.42	0.44
3	rs7616301	AC095349.10	C	0.07	0.05
3	rs7651217	AC095349.10	C	0.07	0.05
7	rs854568	PON1	C	0.26	0.24
13	rs4942106	AKAP11	A	0.27	0.22
11	rs7106873	SHANK2	T	0.48	0.46

11	rs10894589	OPCML	T	0.04	0.07
3	rs9863121	ECT2	T	0.32	0.27
11	rs1545527	AP003778.3-4	G	0.34	0.33
14	rs1958018	NPAS3	G	0.27	0.20
16	rs4541063	FAM92B	C	0.28	0.34
10	rs4748275	PRKCQ	G	0.31	0.29
15	rs12442068	ARNT2	G	0.23	0.20
18	rs12954185	AC139070.3	C	0.15	0.17
18	rs4595890	AC008245.6	A	0.40	0.45
2	rs12467137	EZH2	T	0.13	0.10
7	rs17171118	MYT1L	T	0.17	0.14
11	rs12800502	METT5D1	G	0.13	0.15
10	rs1576480	NHLRC2	T	0.45	0.49
6	rs2206579	CDKAL1	C	0.38	0.31
7	rs2520570	AC005192.1	G	0.14	0.19
3	rs10937074	AC007547.26-2	A	0.30	0.38
5	rs255240	AC008580.7	C	0.42	0.49
5	rs6875311	AC008580.7	C	0.34	0.42
2	rs11690896	AC009303.3-1	C	0.09	0.05
2	rs6705773	AC019051.8	G	0.31	0.39
15	rs779885	AC032011.15	G	0.37	0.30
16	rs1532472	AC040170.9	T	0.29	0.36
16	rs246174	AC040173.5	A	0.35	0.42
4	rs10516985	AC093828.3	C	0.09	0.14
11	rs7939810	AC104563.14	G	0.38	0.30
3	rs12638515	AC104637.5	G	0.02	0.05
4	rs7438821	AC115540.3	A	0.19	0.13
10	rs17684713	ADAM12	A	0.15	0.21

8	rs10097956	ADAM28	G	0.23	0.31
2	rs3771449	ADD2	A	0.49	0.42
14	rs7155550	AL121819.6	C	0.21	0.15
14	rs10149208	AL121819.6	A	0.43	0.35
14	rs12889310	AL163171.4	C	0.47	0.39
1	rs7511712	AL390877.1	G	0.31	0.38
18	rs7236716	ALPK2	T	0.10	0.06
2	rs12622388	ANXA4	C	0.34	0.27
2	rs7597155	ANXA4	C	0.47	0.45
8	rs11774901	ASAP1	T	0.04	0.08
14	rs8005907	ATG2B	C	0.06	0.11
3	rs9868790	BZW1L1	C	0.34	0.27
2	rs4851161	C2orf55	C	0.06	0.03
5	rs6555968	C5orf50	A	0.44	0.48
2	rs13407117	CCDC148	A	0.29	0.37
18	rs12232768	CHST9	C	0.03	0.06
10	rs7913161	CPXM2	G	0.09	0.14
4	rs1872270	DMP1	T	0.28	0.35
1	rs126013	EPB41	A	0.41	0.33
9	rs7036118	FAM154A	T	0.44	0.36
2	rs7596564	FIGLA	A	0.45	0.37
11	rs687572	FLI1	C	0.29	0.37
1	rs13353041	GOT2L1	A	0.15	0.10
3	rs4234525	GRIP2	T	0.48	0.40
1	rs2481665	INADL	C	0.38	0.46
21	rs12482075	KCNJ6	T	0.28	0.35
1	rs12035356	KCNN3	C	0.01	0.04
11	rs11030026	LIN7C	A	0.19	0.13

1	rs12569332	LPGAT1	C	0.14	0.09
6	rs911182	MAP3K5	G	0.50	0.42
8	rs2912004	MCPH1	A	0.26	0.33
14	rs1263805	METTL3	C	0.22	0.29
7	rs2693727	MIRN183	G	0.11	0.07
6	rs12207681	OFCC1	C	0.11	0.16
11	rs4565912	OR10G8	C	0.35	0.27
11	rs2445290	OR51L1	A	0.13	0.19
10	rs1414395	PHYH	T	0.36	0.44
3	rs9831813	RAB7A	A	0.08	0.13
19	rs8102902	RAX2	G	0.16	0.23
13	rs9519340	RP11-217F17.1	A	0.46	0.39
1	rs1426786	RP11-2B19.1	A	0.31	0.38
6	rs267333	RP11-427E4.1	A	0.42	0.34
10	rs16911207	RP11-448K10.1	C	0.07	0.12
10	rs1999505	RP11-448K10.1	C	0.06	0.11
9	rs7047010	RP11-54D18.4	C	0.24	0.32
9	rs10125646	RRAGA	G	0.45	0.37
16	rs1864	TMEM186;C16orf51	G	0.39	0.31
3	rs11712892	TRIM42	A	0.34	0.42
8	rs7835207	TSNARE1	G	0.14	0.20
16	rs11863067	XYLT1	T	0.05	0.09
18	rs1544241	YES1	T	0.49	0.42
16	rs4889197	C16orf47	G	0.48	0.48
12	rs1190655	AC025164.37-2	C	0.37	0.34
8	rs17282526	ASAP1	C	0.49	0.46
1	rs12043001	SEC16B	G	0.11	0.13
3	rs9289466	RPL39P5	C	0.32	0.38

11	rs1048099	ABCC8	T	0.46	0.49
4	rs17001970	SHROOM3	T	0.08	0.06
4	rs1374621	CDKAL1	T	0.06	0.05
6	rs4712540	STK32B	T	0.44	0.50
14	rs2363506	AC007686.5	A	0.42	0.48
17	rs1052507	HELZ	G	0.12	0.09
12	rs1861786	GRIN2B	T	0.33	0.38
4	rs6850106	AC096756.3-2	C	0.05	0.04
12	rs1356083	RIMBP2	T	0.48	0.47
5	rs4976217	SLC30A5	A	0.23	0.19
17	rs2279966	C17orf54	A	0.03	0.05
15	rs2879971	AC048382.7-2	T	0.17	0.12
3	rs11707887	NMNAT3	A	0.32	0.26
12	rs10842549	AC092451.12-2	C	0.46	0.41
6	rs7775523	CDKAL1	C	0.39	0.33
5	rs11748946	FSTL4	C	0.26	0.22
6	rs4077405	CDKAL1	A	0.47	0.46
11	rs1054532	JMJD2D	A	0.18	0.14
9	rs7043157	C9orf94;ADAMTSL1	G	0.44	0.46
3	rs3774700	C3orf14	G	0.04	0.06
11	rs10792830	AP003097.2	T	0.50	0.47
5	rs7727192	AC113412.2-2	G	0.39	0.33
6	rs17310261	LYRM4	C	0.07	0.11
1	rs10925401	RYR2	C	0.39	0.44
10	rs3019500	AL603764.27	T	0.39	0.37
2	rs3849346	KCNH7	G	0.25	0.22
13	rs1891100	TGDS	T	0.28	0.32
12	rs11836523	GRIN2B	C	0.12	0.09

14	rs7140539	NPAS3	G	0.37	0.33
13	rs9512257	WASF3	C	0.37	0.43
9	rs960644	RP11-341A22.1	G	0.11	0.18
6	rs1417667	LSM10	G	0.11	0.07
1	rs4653186	RP1-244F1.1	T	0.28	0.35
6	rs1012626	CDKAL1	A	0.32	0.38
9	rs1123551	RAPGEF1	T	0.04	0.06
3	rs10937074	AC007547.26-2	A	0.30	0.38
5	rs17733586	AC093283.3-2	T	0.06	0.05
16	rs11649643	AC018554.7	T	0.31	0.25
5	rs1862310	AC008586.6	T	0.23	0.20
8	rs2117085	PI15	A	0.05	0.07
4	rs12642995	GUCY1A3	G	0.14	0.19
12	rs7967304	SLC6A15	G	0.36	0.31
8	rs7002825	SNX31	G	0.26	0.21
21	rs1389994	BTG3	A	0.36	0.40
16	rs1079348	ABAT	C	0.32	0.25
19	rs718133	AC002511.1-2	T	0.21	0.28
17	rs481648	AC015922.5	C	0.33	0.26
15	rs8039372	AC032011.15	C	0.29	0.36
4	rs9307041	AC093895.3	A	0.28	0.35
4	rs17615522	AC096739.3	C	0.04	0.08
1	rs7556538	AC098656.2	C	0.08	0.12
3	rs1499508	AC121493.1	A	0.25	0.18
14	rs754131	AL121819.6	G	0.20	0.14
14	rs7149631	AL121819.6	T	0.21	0.15
14	rs1112627	AL163171.4	T	0.40	0.32
10	rs7067598	ARMC3	G	0.28	0.21

16	rs896401	C16orf68	G	0.28	0.21
6	rs4707016	C6orf59;RIPPLY2	A	0.37	0.45
11	rs11234095	DLG2	T	0.07	0.12
11	rs10898274	DLG2	G	0.07	0.12
11	rs532859	ELMOD1	T	0.48	0.44
9	rs10811094	FAM154A	A	0.43	0.49
13	rs1360974	FGF14	T	0.35	0.27
2	rs6707940	FIGLA	G	0.44	0.36
5	rs6873055	GRIA1	A	0.19	0.13
20	rs11574739	HNF4A	C	0.23	0.16
11	rs4579932	HNT;NTM	C	0.42	0.50
2	rs934287	ICA1L	T	0.14	0.20
14	rs1547350	KIF26A	G	0.48	0.44
1	rs2999865	LRRC38	A	0.22	0.30
18	rs9807134	n/a	A	0.18	0.25
10	rs4748737	NEBL	G	0.36	0.29
6	rs12213004	OFCC1	T	0.11	0.16
2	rs3771604	PK4P;PKP4	T	0.37	0.45
2	rs3771669	PK4P;PKP4	G	0.26	0.33
10	rs7897633	PRKG1	G	0.44	0.48
1	rs2152391	RP11-339A11.1	A	0.40	0.32
1	rs10493658	RP11-339A11.1	T	0.40	0.32
9	rs1124647	RP11-341A22.1	G	0.08	0.12
13	rs912521	RP11-57H24.1	A	0.29	0.22
6	rs10806716	RP3-393E18.1	A	0.24	0.17
3	rs6764249	SCN10A	G	0.18	0.12
6	rs7748110	SOBP	A	0.06	0.10
18	rs17805412	TXNDC2	A	0.12	0.18

4	rs7672650	AC109517.4	G	0.36	0.31
6	rs2767577	AL158033.18	G	0.26	0.24
4	rs7670038	AC110761.3	G	0.46	0.50
14	rs12435645	IGHA2	A	0.14	0.12
19	rs1865108	ZNF283	C	0.13	0.10
3	rs11708596	C3orf19	T	0.20	0.16
1	rs259588	AL356010.9	C	0.40	0.43
19	rs11878692	SBNO2	C	0.20	0.18
12	rs11535963	AC087240.17	T	0.24	0.32
13	rs2208932	ATP8A2	C	0.17	0.13
1	rs411238	RP3-357I16.1	A	0.30	0.28
19	rs10425074	LRRC68	C	0.26	0.23
15	rs2242065	LIPC	T	0.11	0.09
15	rs10520664	TMEM83	C	0.07	0.05
5	rs607077	AC010374.6	G	0.28	0.35
8	rs4733037	STMN4	A	0.38	0.34
6	rs4510656	CDKAL1	A	0.50	0.46
7	rs7798774	CREB5	G	0.25	0.23
17	rs4789551	BIRC5	C	0.11	0.08
22	rs1883987	CACNG2	C	0.39	0.36
14	rs6572182	n/a	T	0.17	0.15
10	rs1890951	ANK3	A	0.34	0.41
19	rs12977284	EMR4P	A	0.05	0.05
14	rs8005907	ATG2B	C	0.06	0.11
4	rs6552500	n/a	C	0.24	0.20
16	rs759831	AC099506.3	A	0.25	0.26
16	rs1369790	AC018554.7	G	0.37	0.30
8	rs11785060	PLEC1	T	0.41	0.37

10	rs7077490	AL133395.21	G	0.29	0.26
13	rs7320671	ZMYM2	C	0.46	0.38
1	rs11806225	RP11-149P14.1	C	0.03	0.06
4	rs17004994	C4orf22	G	0.06	0.04
5	rs12189436	AC010374.6	C	0.12	0.08
17	rs6502823	GLTPD2	T	0.12	0.08
12	rs6488565	DDX47	A	0.25	0.22
2	rs2664226	AC114765.6	T	0.19	0.17
16	rs6497658	GRIN2A	C	0.47	0.43
4	rs1803037	ADH5P4;ADH5	A	0.08	0.10
20	rs1018443	PLCB1	T	0.28	0.25
19	rs8101040	AC010624.8-1	A	0.17	0.15
6	rs9470642	MDGA1	G	0.22	0.27
3	rs13072512	FOXP1	A	0.44	0.49
1	rs12140361	SDHC	C	0.05	0.05
6	rs12663853	LRFN2	G	0.03	0.03
11	rs649931	ODZ4	T	0.03	0.04
18	rs8089316	RIT2	C	0.16	0.13
6	rs11754558	RLBP1L2	A	0.06	0.05
9	rs2181156	RP11-87N24.1	G	0.44	0.43
6	rs6935954	HIST1H2BH	A	0.38	0.40
9	rs3808708	SH3GL2	A	0.32	0.31
14	rs2272550	AE000660.1-11	A	0.35	0.28
14	rs12100841	C14orf10;PPP2R3C	C	0.22	0.17
12	rs11107270	CRADD	C	0.06	0.04
10	rs11006970	CDKAL1	T	0.12	0.10
13	rs2217902	ITGBL1	C	0.10	0.08
6	rs7747724	MPP7	C	0.50	0.46

1	rs437722	RP3-357I16.1	A	0.30	0.28
1	rs485660	SYPL2	A	0.28	0.24
3	rs340146	TMPRSS7	T	0.11	0.08
2	rs7577964	AC009228.4-2	A	0.33	0.41
18	rs1789597	AC009271.7	G	0.25	0.19
12	rs1351681	AC024153.22	T	0.39	0.31
15	rs12443269	AC066611.6	C	0.40	0.33
4	rs7688470	AC093735.2	T	0.18	0.24
4	rs2869703	AC093895.3	C	0.28	0.35
5	rs10055973	AC113427.2	C	0.29	0.23
5	rs11738161	AC113427.2	T	0.29	0.23
5	rs13188129	AC113427.2	A	0.29	0.23
5	rs11738216	AC113427.2	A	0.29	0.23
10	rs7908946	ADAM12	A	0.18	0.25
10	rs7079003	ADARB2	T	0.37	0.30
2	rs3771450	ADD2	A	0.49	0.43
14	rs9972193	AL049873.3-2	C	0.25	0.19
2	rs35907069	ANXA4	A	0.06	0.11
2	rs12612409	ANXA4	T	0.06	0.11
18	rs11081236	AP005202.3	T	0.41	0.48
20	rs6067566	BCAS4	T	0.08	0.12
2	rs1157699	CALCRL	T	0.38	0.31
15	rs11635381	CHSY1	A	0.48	0.41
9	rs3780256	CNTLN	C	0.49	0.43
17	rs16968987	COIL	T	0.06	0.09
10	rs7083429	CTNNA3	C	0.35	0.28
21	rs4818184	DSCAM	A	0.11	0.16
10	rs4880336	JAKMIP3	T	0.20	0.27

4	rs7655220	KCNIP4	G	0.26	0.32
1	rs11240240	LRRN2	C	0.05	0.02
1	rs4653186	LSM10	T	0.28	0.35
3	rs6783373	MCF2L2	A	0.31	0.24
7	rs2693737	MIRN183	C	0.12	0.07
13	rs9300636	NALCN;VGCNL1	C	0.34	0.42
10	rs7094054	NRG3	T	0.42	0.50
6	rs16888746	PARK2	T	0.16	0.10
3	rs9853945	RAB7A	G	0.08	0.13
9	rs10867685	RP11-553J6.1	A	0.49	0.43
17	rs6503608	SOCS7	T	0.39	0.47
9	rs11142705	TRPM3	A	0.38	0.46
4	rs12509170	TSPAN5	G	0.48	0.40
13	rs7327207	ZMYM2	C	0.38	0.46
5	rs11953547	AC026781.5	T	0.17	0.14
2	rs2655455	AC011747.5	G	0.50	0.43
7	rs1530680	FOXP2	T	0.23	0.19
16	rs2304469	PMM2	T	0.48	0.43
15	rs2412621	RPAP1	C	0.31	0.34
8	rs7815950	SNX31	C	0.07	0.09
20	rs2145280	CMA1	A	0.24	0.21
14	rs12589666	PMEPA1	A	0.42	0.35
8	rs4275231	PPP2R2A	T	0.20	0.16
12	rs6580784	CSRNP2	A	0.16	0.18
3	rs6790913	CLEC3B	T	0.12	0.09
18	rs169455	n/a	T	0.40	0.42
10	rs988011	ATRNL1	A	0.13	0.09
8	rs7815631	SNX31	T	0.07	0.09

14	rs204984	KIAA1737	A	0.22	0.26
7	rs1557780	JAZF1	C	0.34	0.40
8	rs4397386	TRAPPC9	C	0.50	0.45
5	rs25923	CAMK4	C	0.40	0.34
14	rs7142488	AL355773.4	C	0.36	0.32
17	rs16950116	CA10	T	0.10	0.07
3	rs1242061	SLC9A9	A	0.45	0.42
8	rs13277804	PSD3	A	0.08	0.12
2	rs10187702	IK	G	0.11	0.12
4	rs4485803	TTC29	A	0.02	0.03
5	rs1428609	AC008459.7	G	0.49	0.43
15	rs3098538	GABRG3	A	0.21	0.18
2	rs1177265	KIAA1841	G	0.41	0.45
2	rs17750151	AC007277.2	G	0.09	0.13
6	rs381480	RP11-427E4.1	T	0.17	0.12
2	rs6724815	AC010374.6	A	0.09	0.07
5	rs9918231	NAGK	A	0.29	0.24
5	rs10042848	AC026781.5	G	0.17	0.14
6	rs9371581	SYNE1	G	0.42	0.46
6	rs3778279	H3F3AP1	T	0.49	0.47
11	rs4406832	C11orf44	A	0.14	0.16
17	rs578065	AC015922.5	G	0.41	0.35
5	rs10491314	NDFIP1	A	0.21	0.18
19	rs3764640	STK11	A	0.17	0.23
2	rs1035601	AC114765.6	G	0.19	0.17
4	rs717435	AC104663.3	T	0.40	0.42
12	rs11057929	DHX37	C	0.22	0.19
6	rs9366354	CDKAL1	G	0.32	0.38

18	rs8098687	DTNA	A	0.13	0.16
8	rs2008555	AC116154.2	T	0.19	0.25
10	rs11244887	ADAM12	T	0.19	0.24
17	rs790097	SDK2	A	0.07	0.10
2	rs16856529	IGFBP5	G	0.19	0.15
20	rs6011530	AL121673.41	A	0.15	0.13
2	rs7597992	ADD2	C	0.18	0.14
6	rs12207523	ENPP3	T	0.03	0.06
2	rs888182	IGFBP5	C	0.20	0.16
15	rs7177318	ATP8B4	T	0.46	0.43
12	rs3217907	CCND2	A	0.40	0.34
10	rs1471247	ANK3	A	0.35	0.42
6	rs16893540	RP3-408B20.4	T	0.04	0.04
3	rs651165	AADACL1	A	0.27	0.23
12	rs4471501	SLC11A2	A	0.22	0.24
6	rs11155004	C6orf91;ECT2L	T	0.45	0.49
15	rs7176145	ITGA11	G	0.30	0.25
6	rs1395633	RP3-334F4.1	T	0.10	0.08
10	rs11015633	ARL5B	G	0.47	0.40
6	rs9480754	PDSS2	G	0.31	0.30
3	rs4684237	C3orf19	T	0.25	0.21
3	rs13096015	AC007547.26-2	G	0.30	0.38
2	rs2702089	AC009228.4-2	A	0.36	0.44
2	rs7599725	AC019051.8	T	0.26	0.34
4	rs7697101	AC093735.2	C	0.44	0.48
4	rs6829972	AC093777.4	G	0.12	0.17
3	rs6794105	AC099544.2	A	0.35	0.43
3	rs1580800	AC104441.2	A	0.10	0.15

5	rs7707671	AC116337.2	C	0.26	0.33
16	rs17671037	AC125796.2	C	0.32	0.25
16	rs7188565	AC125796.2	A	0.41	0.34
10	rs7912903	ADARB2	T	0.03	0.06
9	rs10964182	AL158206.8-2	T	0.27	0.20
1	rs1752221	AL158218.11	T	0.42	0.48
13	rs9575351	AL512782.6-2	C	0.06	0.10
11	rs12224791	AP001884.4	G	0.35	0.28
16	rs860380	C16orf47	C	0.40	0.47
1	rs2003046	C1orf127	A	0.26	0.19
8	rs2249963	C8orf14	C	0.42	0.50
15	rs723988	CHSY1	T	0.47	0.39
7	rs42523	COL1A2	C	0.21	0.27
1	rs7513428	CREG1	A	0.18	0.13
5	rs7718958	DDX46	C	0.22	0.16
1	rs12039988	EPB41	A	0.39	0.47
6	rs1324103	EPHA7	C	0.43	0.50
14	rs4569181	ESRRB	G	0.50	0.43
5	rs1461243	FAM114A2	C	0.19	0.13
9	rs16937267	FAM154A	C	0.15	0.09
15	rs13380359	GABRG3	G	0.06	0.03
15	rs4778147	GABRG3	T	0.50	0.43
4	rs17539365	GBA3	A	0.42	0.35
13	rs1333464	GPC6	T	0.16	0.11
13	rs1927522	GPR12	T	0.17	0.12
5	rs17113869	GRIA1	C	0.22	0.29
5	rs13189947	GRIA1	A	0.15	0.10
3	rs2090702	GRIP2	T	0.47	0.39

2	rs7603997	ITSN2	T	0.31	0.39
10	rs6482189	MLLT10	A	0.35	0.28
10	rs1243194	MLLT10	T	0.33	0.26
18	rs9956147	n/a	C	0.18	0.24
6	rs9502396	NRN1	G	0.15	0.21
11	rs871437	OPCML	C	0.13	0.08
13	rs1517881	RP11-56M2.1	T	0.36	0.44
1	rs12741645	RP5-1198O20.5	T	0.12	0.17
22	rs2235171	SLC5A4	T	0.05	0.02
4	rs17211183	TMSL3	A	0.03	0.06
3	rs1353021	TNIK	A	0.43	0.48
17	rs759974	VPS53	C	0.47	0.45
4	rs9999510	ZNF827	C	0.42	0.34
1	rs2294642	FBXO44	T	0.05	0.06
15	rs16940650	TMEM83	T	0.07	0.05
18	rs9304251	AC011225.1	A	0.10	0.06
6	rs2753977	EGFL10;EYS	C	0.17	0.12
10	rs1475418	PRKCQ	A	0.31	0.29
6	rs9349437	C6orf138	T	0.19	0.14
1	rs953517	C1orf102	C	0.31	0.38
21	rs2838000	BACE2	T	0.25	0.20
12	rs4768967	TFCP2	A	0.16	0.18
4	rs931602	ANXA3	C	0.30	0.24
12	rs10444509	GNPTAB	G	0.13	0.14
1	rs4073574	AGTRAP	G	0.39	0.33
3	rs968628	MME	G	0.47	0.46
3	rs10460808	CBLB	C	0.11	0.08
11	rs516431	HNT;NTM	T	0.26	0.22

8	rs12679771	TRAPPC9	T	0.17	0.22
18	rs623966	DTNA	C	0.15	0.18
13	rs7983069	RP11-7B3.3	A	0.34	0.38
3	rs1111516	RPL39P5	T	0.31	0.37
10	rs10901559	ADAM12	T	0.19	0.24
1	rs12408243	DMBX1	C	0.45	0.50
2	rs2392820	AC019208.8	T	0.32	0.25
10	rs4747272	PRKCQ	T	0.30	0.28
22	rs7292528	SEZ6L	T	0.11	0.10
19	rs7259059	VRK3	T	0.22	0.20
3	rs2667469	PDIA5	G	0.19	0.25
7	rs3735219	EZH2	A	0.17	0.14
16	rs1155970	AC099506.3	C	0.28	0.29
3	rs1461762	AC117430.3-2	A	0.11	0.16
4	rs17039589	COL25A1	A	0.13	0.12
6	rs995291	RP11-427E4.1	A	0.17	0.12
3	rs7623367	AC026671.18	G	0.05	0.06
2	rs13032780	MYO1B	T	0.21	0.25
8	rs4872211	AC023202.8	A	0.11	0.15
6	rs1885275	PDE7B	T	0.29	0.26
18	rs11080606	PTPN2	G	0.33	0.29
7	rs7783459	EZH2	A	0.17	0.14
7	rs17169762	AC005582.1	C	0.08	0.13
5	rs29645	AC008580.7	T	0.35	0.43
18	rs1944351	AC009271.7	A	0.45	0.47
16	rs1369790	AC018554.7	G	0.37	0.30
12	rs10844642	AC024153.22	C	0.41	0.33
3	rs9843001	AC092981.3-1	T	0.20	0.14

4	rs13152723	AC093735.2	G	0.21	0.15
4	rs11725282	AC104070.3	C	0.15	0.10
2	rs6718240	AC104807.5-2	G	0.30	0.38
8	rs2013265	ADAM28	A	0.22	0.29
14	rs1955429	AL049775.2	T	0.19	0.14
9	rs1411355	AL389915.19	A	0.24	0.18
7	rs2392581	AMPH	G	0.45	0.38
9	rs7041706	BNC2	C	0.27	0.20
6	rs421424	BX255934.7	A	0.25	0.32
11	rs1400756	C11orf53	C	0.16	0.11
6	rs7766161	C6orf70	G	0.15	0.21
10	rs11014358	CACNB2	G	0.17	0.11
5	rs12651858	CCDC125	C	0.10	0.06
12	rs2555319	CCDC60	C	0.37	0.30
1	rs7520966	CYB5RL	T	0.23	0.30
5	rs17704210	EBF1	G	0.09	0.14
1	rs1591785	ETV3	T	0.38	0.46
15	rs11634818	FAM148A	G	0.09	0.05
13	rs9319313	GPR12	G	0.17	0.12
11	rs4930103	H19	G	0.43	0.50
16	rs11644878	HS3ST4	A	0.43	0.49
13	rs4771929	HS6ST3	T	0.03	0.06
11	rs1124847	LRRC55	G	0.46	0.38
11	rs287756	n/a	T	0.39	0.46
13	rs1517896	RP11-56M2.1	A	0.37	0.45
6	rs434310	RP3-399J4.2	A	0.24	0.31
11	rs4756930	SAAL1	T	0.41	0.48
6	rs718868	SAMD5	T	0.36	0.29

2	rs6730730	SLC19A3	G	0.09	0.15
9	rs3739482	SLC24A2	A	0.36	0.43
3	rs182568	SUMF1	T	0.11	0.06
7	rs4256491	TMEM195	A	0.11	0.16
7	rs4615456	TMEM195	G	0.45	0.37
3	rs9861350	TRIM42	A	0.38	0.46
8	rs777801	TRPS1	T	0.04	0.07
9	rs880242	USP20	G	0.44	0.37
6	rs9688888	XXyac-YX65C7_A.4	G	0.10	0.16
8	rs9642799	ZFPM2	A	0.32	0.26
3	rs6551273	ZNF654	G	0.17	0.11
22	rs11704749	HMGXB4	A	0.06	0.09
7	rs38519	JAZF1	T	0.46	0.40
11	rs7938094	AC021749.6-3	C	0.15	0.18
9	rs2860101	PGM5	T	0.09	0.10
10	rs10751569	AL512272.15	A	0.42	0.45
12	rs10859283	AC025164.37-2	T	0.28	0.27
14	rs12885638	AL137100.4	C	0.34	0.29
5	rs13165786	EDIL3	C	0.46	0.41
11	rs7118622	GALNTL4	C	0.22	0.27
3	rs9852993	TMEM108	T	0.16	0.20
1	rs12132152	DPYD	A	0.04	0.03
21	rs7280677	AP000431.2	C	0.29	0.36
1	rs17109254	RP4-601K24.1	A	0.05	0.03
8	rs4240188	LONRF1	A	0.44	0.37
11	rs7943388	AC021749.6	T	0.11	0.13
12	rs149411	SLC11A2	T	0.38	0.40
6	rs195850	POU3F2	C	0.13	0.14

4	rs998091	EVC2	T	0.36	0.32
3	rs500193	LAMP3	G	0.08	0.06
1	rs6425838	CSMD2	T	0.33	0.29
2	rs7577925	AC010890.1	T	0.31	0.35
5	rs10462823	AC122710.3	A	0.10	0.13
6	rs9356756	CDKAL1	G	0.46	0.40
5	rs11959584	NMUR2	T	0.46	0.50

n/a = not applicable.

Table 5.6. Full results for the SNP based analysis of the szgene markers. Table shows gene name, gene position in 'top
r^2 values, p-value and OR in within case analysis and case-control analysis, and szgene OR and 95% confidence interva

						LOAD+P vs. LOAD-P		LOAI Cc
Gene	'top result' position	SNP	Proxy	D'	r^2	*P*	OR	*P*
DISC1	1	rs3737597	rs16856351	1	1	0.76	1.09	0.29
DISC1	1	rs821597	rs864752	1	0.97	0.95	0.99	0.32
DISC1	1	rs821616	rs821596	1	1	0.76	1.03	0.36
DISC1	1	rs6675281	n/a	n/a	n/a	0.53	1.09	0.44
DISC1	1	rs3738398	rs1417585	0.93	0.78	0.19	1.14	0.55
DISC1	1	rs2812393	rs2812389	1	0.84	0.17	1.14	0.58
DISC1	1	rs1322784	rs2255340	1	0.95	0.33	1.11	0.62
DISC1	1	rs2255340	n/a	n/a	n/a	0.33	1.11	0.62
DISC1	1	rs2738864	rs2255340	1	1	0.33	1.11	0.62
DISC1	1	rs3738401	n/a	n/a	n/a	0.70	1.04	0.66
DISC1	1	rs1000731	n/a	n/a	n/a	0.81	0.97	0.78
DISC1	1	rs2492367	rs17766087	0.74	0.31	0.69	0.95	0.84
DISC1	1	rs1322783	n/a	n/a	n/a	0.35	1.05	0.86
DISC1	1	rs9432024	rs9431714	1	0.89	0.81	0.98	0.89
DISC1	1	rs1934909	n/a	n/a	n/a	0.85	1.03	0.95
DISC1	1	rs999710	rs1073179	0.96	0.69	0.95	1.01	0.96
DISC1	1	rs1984895	n/a	n/a	n/a	n/a	n/a	n/a
DISC1	1	rs751229	n/a	n/a	n/a	n/a	n/a	n/a
SLC18A1	2	rs2270637	n/a	n/a	n/a	0.95	1.01	0.47
SLC18A1	2	rs2270641	rs1497022	1	1	0.25	0.89	0.93
GABRB2	3	rs6556547	rs7702598	0.73	0.26	0.46	1.14	0.40
GABRB2	3	rs187269	rs173766	1	1	0.87	0.98	0.83
GABRB2	3	rs1816072	rs252957	0.93	0.83	0.67	1.04	0.84
GABRB2	3	rs1816071	rs252957	0.93	0.83	0.67	1.04	0.84

GABRB2	3	rs194072	n/a	n/a	n/a	0.72	0.95	0.89
GABRB2	3	rs252944	n/a	n/a	n/a	n/a	n/a	n/a
DRD2	4	rs1079597	rs2471857	1	1	0.42	0.90	0.07
DRD2	4	rs1800497	n/a	n/a	n/a	0.50	0.92	0.29
DRD2	4	rs6275	rs2242592	1	1	0.35	1.10	0.37
DRD2	4	rs6277	rs754672	0.96	0.81	0.75	1.03	0.83
DRD2	4	rs1801028	n/a	n/a	n/a	n/a	n/a	n/a
DRD2	4	rs1799732	n/a	n/a	n/a	n/a	n/a	n/a
10q26.13	5	rs11248526	n/a	n/a	n/a	0.86	1.03	0.85
10q26.13	5	rs17101921	n/a	n/a	n/a	n/a	n/a	n/a
AKT1	6	rs1130214	rs4983559	1	0.66	0.26	1.11	0.24
AKT1	6	rs2498799	n/a	n/a	n/a	n/a	n/a	n/a
AKT1	6	rs3803300	n/a	n/a	n/a	n/a	n/a	n/a
AKT1	6	rs3730358	n/a	n/a	n/a	n/a	n/a	n/a
AKT1	6	rs2494732	n/a	n/a	n/a	n/a	n/a	n/a
GRIN2B	7	rs1806201	n/a	n/a	n/a	0.16	1.16	0.046
GRIN2B	7	rs7301328	rs2216344	0.96	0.69	0.54	1.06	0.11
GRIN2B	7	rs1805502	n/a	n/a	n/a	0.57	0.93	0.25
GRIN2B	7	rs1019385	n/a	n/a	n/a	n/a	n/a	n/a
DGCR2	8	rs2073776	rs2238743	0.95	0.54	0.86	0.98	0.15
DGCR2	8	rs807759	n/a	n/a	n/a	n/a	n/a	n/a
PLXNA2	9	rs841865	n/a	n/a	n/a	0.87	0.98	0.12
PLXNA2	9	rs752016	n/a	n/a	n/a	0.59	0.94	0.45
PLXNA2	9	rs1327175	n/a	n/a	n/a	n/a	n/a	n/a
PLXNA2	9	rs2498028	n/a	n/a	n/a	n/a	n/a	n/a
RPGRIP1L	10	rs9922369	rs2111119	1	0.25	0.78	0.96	0.85
TPH1	11	rs1800532	rs2670765	1	1	0.07	1.19	0.31
TPH1	11	rs1799913	n/a	n/a	n/a	n/a	n/a	n/a
DRD4	12	rs4646983	n/a	n/a	n/a	n/a	n/a	n/a
DRD4	12	rs1800955	n/a	n/a	n/a	n/a	n/a	n/a
DRD4	12	120-bp TR	n/a	n/a	n/a	n/a	n/a	n/a
DRD4	12	48-bp VNTR	n/a	n/a	n/a	n/a	n/a	n/a

DAOA	13	rs778293	n/a	n/a	n/a	0.42	0.80	0.24
DAOA	13	rs3916966	n/a	n/a	n/a	0.76	1.03	0.36
DAOA	13	rs3916967	rs3916966	1	1	0.76	1.03	0.36
DAOA	13	rs2391191	n/a	n/a	n/a	0.81	1.02	0.40
DAOA	13	rs1421292	rs1362886	1	0.88	0.58	1.05	0.65
DAOA	13	rs947267	rs701567	1	1	0.64	1.04	0.73
DAOA	13	rs3916970	rs2893229	0.96	0.77	0.81	0.98	0.78
DAOA	13	rs778294	n/a	n/a	n/a	0.17	1.03	0.87
DAOA	13	rs3916971	n/a	n/a	n/a	0.47	0.93	0.90
DAOA	13	rs3918342	rs9519707	1	1	0.58	1.05	0.98
DAOA	13	rs3916965	n/a	n/a	n/a	n/a	n/a	n/a
11p14.1	14	rs1602565	rs7938219	1	1	0.99	1.00	0.45
DRD1	15	rs4532	n/a	n/a	n/a	0.24	1.08	0.55
HTR2A	16	rs6314	n/a	n/a	n/a	0.55	0.91	0.46
HTR2A	16	rs6311	n/a	n/a	n/a	0.72	1.04	0.74
HTR2A	16	rs6313	n/a	n/a	n/a	0.77	1.03	0.75
RELN	17	rs7341475	n/a	n/a	n/a	0.58	0.93	0.73
APOE	18	rs7412	n/a	n/a	n/a	n/a	n/a	n/a
APOE	18	rs429358	n/a	n/a	n/a	n/a	n/a	n/a
NRG1	19	rs4733376	rs3757934	1	1	0.15	1.25	0.038
NRG1	19	rs6988339	n/a	n/a	n/a	0.47	0.93	0.66
NRG1	19	rs10503929	n/a	n/a	n/a	0.71	1.05	0.80
NRG1	19	rs2466058	rs4602844	1	0.67	0.99	1.00	0.88
NRG1	19	rs3924999	n/a	n/a	n/a	0.33	0.91	0.96
NRG1	19	rs2439272	n/a	n/a	n/a	n/a	n/a	n/a
NRG1	19	rs35753505	n/a	n/a	n/a	n/a	n/a	n/a
NRG1	19	rs6994992	n/a	n/a	n/a	n/a	n/a	n/a
NRG1	19	420M9-1395	n/a	n/a	n/a	n/a	n/a	n/a
NRG1	19	478B14-642	n/a	n/a	n/a	n/a	n/a	n/a
NRG1	19	478B14-848	n/a	n/a	n/a	n/a	n/a	n/a
NRG1	19	SNP8NRG22132	n/a	n/a	n/a	n/a	n/a	n/a
NRG1	19	SNP8NRG241930	n/a	n/a	n/a	n/a	n/a	n/a

NRG1	19	SNP8NRG433E1006	n/a	n/a	n/a	n/a	n/a	n/a
1L1B	20	rs1143634	n/a	n/a	n/a	0.09	1.20	0.2
1L1B	20	rs16944	rs10169916	1	1	0.36	1.10	0.4
MTHFR	21	rs1801131	n/a	n/a	n/a	0.21	0.88	0.0
MTHFR	21	rs1801133	n/a	n/a	n/a	0.98	0.99	0.6
COMT	22	rs4633	rs4680	1	0.97	0.93	0.99	0.3
COMT	22	rs4680	n/a	n/a	n/a	0.93	0.99	0.3
COMT	22	rs737865	rs2020917	1	1	0.40	0.92	0.5
COMT	22	rs165599	n/a	n/a	n/a	0.67	1.06	0.8
COMT	22	rs4818	n/a	n/a	n/a	n/a	n/a	n/a
COMT	22	rs769224	n/a	n/a	n/a	n/a	n/a	n/a
HP	23	Hp ½	n/a	n/a	n/a	n/a	n/a	n/a
DAO	24	rs3741775	n/a	n/a	n/a	0.98	1.00	0.4
DAO	24	rs4623951	n/a	n/a	n/a	0.87	1.02	0.4
DAO	24	rs3825251	n/a	n/a	n/a	0.67	0.95	0.6
DAO	24	rs2111902	n/a	n/a	n/a	0.60	0.95	0.9
DAO	24	rs3918346	n/a	n/a	n/a	n/a	n/a	n/a
TP53	25	rs1042522	rs7141	0.81	0.31	0.59	0.95	0.4
ZNF804A	26	rs1344706	rs1366840	1	0.69	0.23	1.12	0.9
16p13.12	27	rs7192086	rs7189560	1	0.96	0.16	0.87	0.9
DTNBP1	28	rs1018381	rs9296985	1	1	0.25	1.20	0.0
DTNBP1	28	rs2619538	rs9296985	1	0.92	0.67	1.20	0.0
DTNBP1	28	rs742106	n/a	n/a	n/a	0.97	1.00	0.1
DTNBP1	28	rs2619539	rs4236167	1	0.97	0.51	0.94	0.2
DTNBP1	28	rs1011313	n/a	n/a	n/a	0.32	0.85	0.4
DTNBP1	28	rs2619522	rs1474605	1	0.95	0.82	0.97	0.5
DTNBP1	28	rs2619528	rs1474605	1	0.95	0.82	0.97	0.5
DTNBP1	28	rs3213207	rs1474605	1	0.56	0.82	0.97	0.5
DTNBP1	28	rs760761	rs1474605	1	1	0.82	0.97	0.5
DTNBP1	28	rs1047631	n/a	n/a	n/a	0.14	0.83	0.5
DTNBP1	28	rs760666	rs875462	1	1	0.90	0.99	0.9
DTNBP1	28	rs2005976	n/a	n/a	n/a	n/a	n/a	n/a

DTNBP1	28	rs909706	n/a	n/a	n/a	n/a	n/a	n/
OPCML	29	rs3016384	n/a	n/a	n/a	0.76	1.03	0.3
RGS4	30	rs951436	rs951437	1	1	0.93	0.99	0.5
RGS4	30	rs951439	rs6678136	1	1	0.93	1.01	0.7
RGS4	30	rs2661319	n/a	n/a	n/a	n/a	n/a	n/
RGS4	30	rs10917670	n/a	n/a	n/a	n/a	n/a	n/

n/a = not applicable.

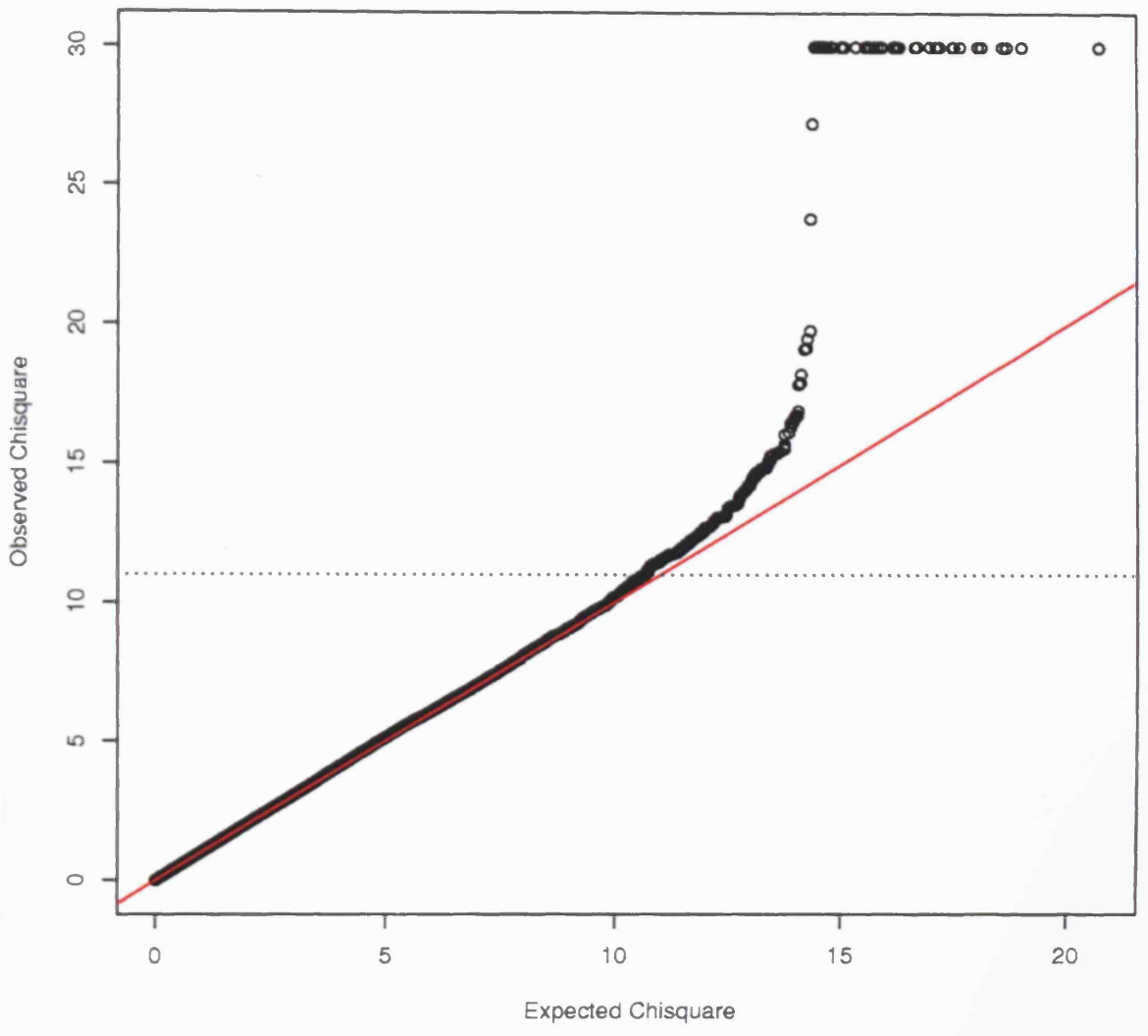


Figure 5.1. *Example of quantile-quantile plot when comparing different sets of controls.* In this example, screened controls from the UK and Ireland, genotyped on the Illumina 610-quadchip are compared with controls from the 1958 British birth cohort, genotyped on the Illumina HumanHap550. The observed association χ^2 test statistics (y-axis) have been plotted against those expected under the null expectation (x-axis). The y-axis was limited at 30 although higher values were observed. The line of equality is colored red. An exclusion χ^2 threshold of 11 was employed (indicated by the dotted horizontal line). [Figure taken from Harold et al. 2009}.

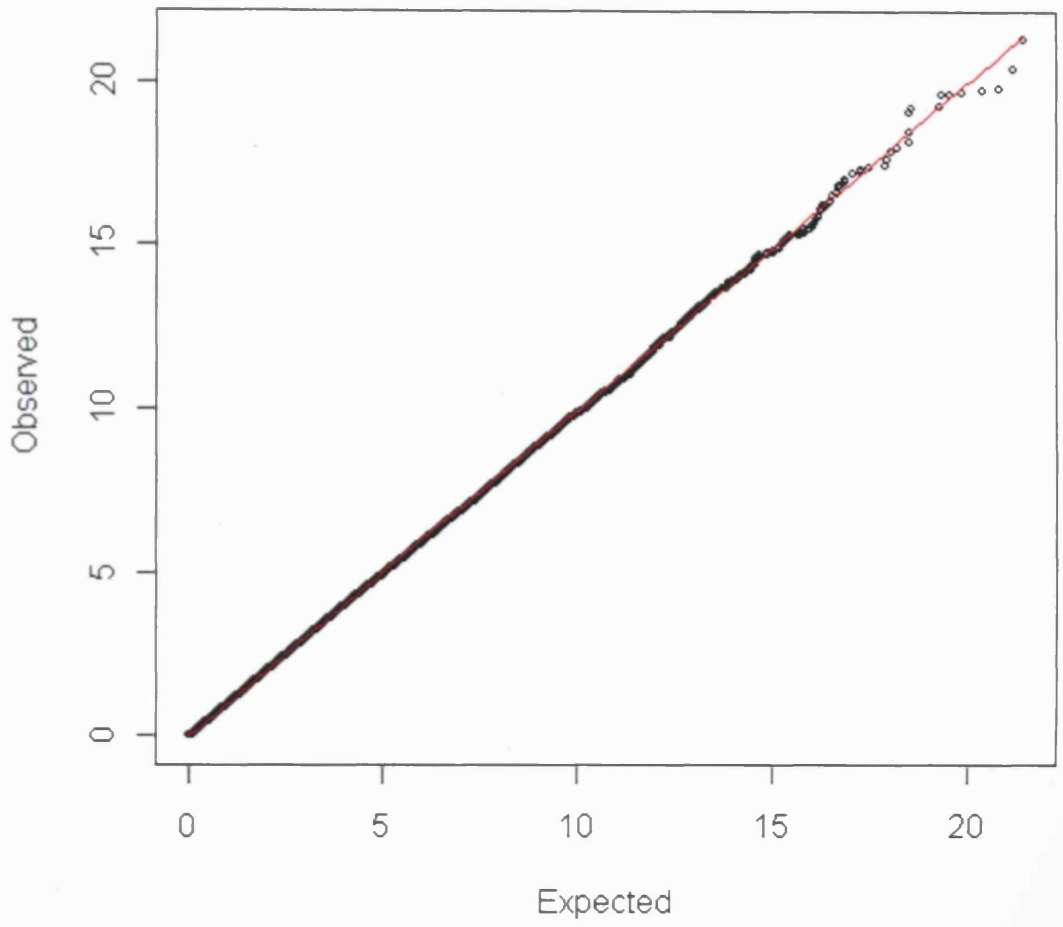


Figure 5.2. *Example of quantile-quantile plot when comparing different sets of controls.* In this example, 1958 British birth cohort population controls, genotyped as part of the WTCCC study are compared with 1958 British birth cohort population controls, genotyped as part of the T1DGC study. The observed association χ^2 test statistics (y-axis) have been plotted against those expected under the null expectation (x-axis). The line of equality is colored red. No exclusion χ^2 threshold was warranted.

Abbreviations

AAO	age at onset
Aβ42	amyloid-βeta 42
AD	Alzheimer's disease
AD+P	Alzheimer's disease with psychosis
AD-P	Alzheimer's disease without psychosis
ALIGATOR	Association LIst Go AnnoTatOR
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
APOE	apolipoprotein E
APP	β-amyloid precursor protein
Arg	arginine
ARID4A	AT rich interactive domain 4A
ARP	affected relative pair
ASAP1	development and differentiation enhancing factor
ASP	Affected Sibling Pair
BBS	Bardet–Biedl syndrome
BDNF	brain-derived neurotrophic factor
bHLH	basic helix-loop-helix
Blat	basic local alignment tool
BLOC-1	biogenesis of lysosome-related organelles complex 1
bp	base pairs/base position
CAMDEX	Cambridge mental disorders of the elderly examination
CCDC60	coiled-coil domain containing 60 gene
CDS	protein-coding regions
CEPH	centre d'etude du polymorphisme humain
CEU	centre d'etude du polymorphisme humain from Utah
CHRNA7	nicotinic acetylcholine receptor gene
CI	confidence interval
CLSTN2	calsyntenin 2
CLU	clusterin
CNP	2', 3'-cyclic nucleotide 3' phosphodiesterase
CNTLN	centlein, centrosomal protein

CNV	copy number variation
COMT	catechol-O-methyltransferase
cM	centimorgans
CNS	central nervous system
CSF2RA	colony stimulating factor, receptor 2 alpha gene
CT	computerised tomography
Cx31	connexin 31
Cys	cysteine
c16orf5	chromosome 16 open reading frame 5
DAOA	D-amino acid oxidase activator
ddH_2O	double distilled water
DISC1	disrupted in schizophrenia 1
ddNTPs	dideoxy-nucleotide triphosphates
DLB	dementia with lewy bodies
DLPFC	dorsolateral prefrontal cortex
dNTPs	deoxy-nucleotide triphosphates
DPC	dystrophin-associated protein complex
DRD1	dopamine receptor D1
DRD2	dopamine receptor D2
DRD3	dopamine receptor D3
DRD4	dopamine receptor D4
DTNBP1	dystrobrevin binding protein 1/dysbindin 1
DZ	dizygotic
DSM-IV	4^{th} edition of diagnostic and statistical manual of mental disorders
DNA	deoxyribonucleic acid
ECR	evolutionary conserved region
EDMD-AD	autosomal dominant Emery–Dreifuss muscular dystrophy
EDTA	ethylenediaminetetraacetic acid
EM	expectation-maximization
ENCODE	ENCyclopedia Of DNA Elements
EOAD	early onset Alzheimer's disease
ERE6	oestrogen response element 6
Exo I	exonuclease I

FDR	false discovery rate
GABA	gamma-aminobutyric acid
GALNT2	polypeptide N-acetylgalactosaminyltransferase 2
GDS	global deterioration scale
GJB3	connexin 31
Gln	glutamine
GO	gene ontology
GRIK2	glutamate receptor, ionotropic, kainate 2
GRIN2B	N-methyl-D-aspartate receptor subunit 2B
GRM2	glutamate receptor metabotropic 2
GRM3	glutamate receptor metabotropic 3
GWA	genome-wide association
GWAS	genome-wide association study
GWS	genome-wide significance
HD	Huntington's disease
HIST1H2BJ	histone cluster 1, H2BJ
HIST1H2BL	histone cluster 1, H2BL
hr	hour
HWE	Hardy-Weinberg equilibrium
IBD	identity by descent
IBS	identity by state
IL-1β	interleukin-1β
IL3RA	interleukin 3 receptor alpha
Kb	kilobase
KCNK16	potassium channel, subfamily k, member 16
kDa	kiloDalton
LASER-AD	London and the south east region AD
Leu	leucine
LGMD	Limb-Girdle muscular dystrophy
LMNA	lamin A/C
LD	linkage disequilibrium
LOAD	late onset Alzheimer's disease
LOAD+P	late onset Alzheimer's disease with psychosis
LOAD-P	late onset Alzheimer's disease without psychosis

LOD	logarithm of the odds
MAF	minor allele frequency
MALDI-ToF	matrix assisted laser desorption ionisation – time of flight
Max	maximum
Mb	megabase
MCPH1	microcephalin
MDS	multi-dimensional scaling
Met	methionine
$MgCl_2$	magnesium chloride
Min	minute/minimum
MLS	maximum LOD score
mM	micromole
MMSE	mini mental state examination
MRC	medical research council
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MS	mass spectometry
MZ	monozygotic
NAP5	nck associated protein 5
NCBI	national center for biotechnology information
NHS	national health service
NIMH	national institute for mental health
NINCDS-ADRDA	national institute of neurological and communication disorders and stroke and the Alzheimer's disease and related disorders associations
ng	nanogram
NKX2-2	nk2 homeobox 2
nm	nanometre
NMDA	N-methyl-D-aspartic acid
NPI	neuropsychiatric inventory
NRG1	neuregulin-1
NRGN	neurogranin
OCD	obsessive compulsive disorder
OLIG1	oligodendrocyte lineage transcription factor 1

OLIG2	oligodendrocyte lineage transcription factor 2
OMIM	online mendelian inheritance in man
OMR	oligodendrocyte/myelin related
OPCML	opioid binding protein/cell adhesion
OR	odds ratio
PC	principal component
PCA	principal components analysis
PCLO	piccolo
PCR	polymerase chain reaction
PD	Parkinson's disease
PDE4B	phosphodiesterase 4B
Phe	phenylalanine
PICALM	phosphatidylinositol binding clathrin assembly protein
PLXNA2	plexin A2
pmol	picomole
PS	power and sample size calculation
PSEN1	presenilin 1
PSEN2	presenilin 2
QC	quality control
QQ	quantile-quantile
RELN	reelin
RORA	RAR-related orphan receptor A
RPGRIP1L	retinitis pigmentosa GTPase regulator interacting protein 1 – like
rpm	revolutions per minute
SAP	shrimp alkaline phosphatase
Ser	serine
SLC9A9	solute carrier family 9
SNAP	SNP annotation and proxy search
SNP	single nucleotide polymorphism
STRING	known and predicted protein-protein interactions database
Szgene	schizophrenia gene
Taq	*Thermus Aquatics*
TBE	tris borate ethylenediaminetetraacetic acid

TCF4	transcription factor 4
TCF7L2	transcription factor 7-like 2
TE	tris ethylenediaminetetraacetic acid
Tm	melting temperature
TPH1	tryptophan hydroxylase1
TRIM32	tripartite motif-containing 32
T1DGC	type 1 diabetes genetics consortium
T2D	type 2 diabetes
UCSC	University California, Santa Cruz
UK	United Kingdom
USA	United States of America
US	United States
UTR	untranslated region
UV	ultraviolet
V	version
Val	valine
VCFS	Velocardiofacial syndrome
WGA	whole genome association
WTCCC	Wellcome Trust case control consortium
ZNF804A	zinc finger protein 804A
°C	degrees centigrade
µg	microgram
µl	microlitre
µM	micromole
1958BBC	1958 British birth cohort
5HT-2A	serotonin receptor
5HTTLPR	serotonin gene-linked promoter region

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