Family factors and child and adolescent depression symptoms: testing environmental and genetic risk effects using longitudinal and genetically sensitive research designs

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Summary

Child and adolescent depression is a complex and multifactorial disorder that is likely due to the co-action and interaction of multiple psychosocial and genetic risk factors. Identifying the specific psychosocial factors which confer risk is a paramount aim of depression research since this is essential to informing the design and implementation of preventive interventions. Prior research has suggested that a range of family factors are associated with the development of child and adolescent depression. However, the extent to which these associations are likely to represent causal influences is unclear due to a range of issues such as establishing the direction of effects and estimating the role of potential confounding factors which include genetic influences and alternative psychosocial risks. In this thesis I use different research designs to examine the "environmental" effects of two specific family factors which have established associations with child and adolescent depression symptoms: parent-child hostility (study 1) and parent depression (study 2). I also explore whether there is moderation of the putative environmental effects of these family factors by specific gene variants (gene-environment interaction; study 3). The role of both parent and child gender was examined across studies. Using genetically sensitive and longitudinal designs, environmental effects on child and adolescent depression symptoms were detected for mother-child hostility and parent depression. For both family factors, effects appeared to be stronger for girls suggesting their increased susceptibility to these stressors. No evidence of gene-environment interaction was detected.

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Abstract

Background: The aetiology of child and adolescent depression is attributed to both psychosocial and genetic risk factors and to gene-environment interplay. Identifying specific psychosocial risk factors which contribute to aetiological pathways is essential for elucidating malleable intervention targets and is therefore important to scientific research and clinical practice. Prior research has suggested that a range of family factors are associated with child and adolescent depression. However, the extent to which associations are explained by environmental risk processes is unclear due to uncertainty regarding the direction of effects (child and adolescent depression may result in family adversity as part of a reverse causation or reciprocal model of effects) and the likely presence of both genetic and environmental confounds. Due to the limited availability of experimental designs, different research methods with complementary strengths are needed to test putative environmental risk effects. The role of parent and child gender has not been extensively studied and may be an important source of variation in response to family environmental risk effects.

Aims: The aim of this thesis was to use different research designs and statistical methods to critically test environmental effects of family risk factors on child and adolescent depression symptoms. I examine two specific family factors which have established associations with child and adolescent depression symptoms: parent-child hostility (study 1) and parent depression (study 2). I also explore whether there is moderation of the putative environmental effects of these family factors by specific gene variants (gene-environment interaction; study 3). The role of both parent and child gender was examined across studies.

Methods: Questionnaire data derived from four, independent, community based samples were used. Study 1 used two different samples and designs 1) a longitudinal sample of 316 families with children and adolescents aged 11-13 years was used to test the direction of effects and 2) a cross-sectional genetically informative sample of 1075 twin pairs aged 12-20 years was used to test environmental effects. Study 2 used a different genetically informative sample of 852 families each with one participating child conceived using assisted reproductive technology (aged 4-10 years) to test environmental links in the intergenerational transmission of depression symptoms. Study 3 used two samples to test for gene-environment interaction 1) a high-risk sample of 271 mothers with recurrent major depression each with one participating child or adolescent aged 9-17 years and 2) a control sample drawn from a population based twin study (one twin randomly selected from each pair) which consisted of 165 children and adolescents aged 12-16 years who were unexposed to maternal depression.

Results: In study 1, a bidirectional longitudinal association was found between mother-daughter hostility and child and adolescent depression symptoms. This association was accounted for by environmental and genetic factors. Father hostility had no predictive link with daughter depression symptoms. Rather, daughter depression symptoms appeared to have risk effects on father hostility. The twin analyses revealed that this association was due to genetic effects alone. For sons, longitudinal associations were not significant and cross-sectional associations were due to genetic effects with no evidence of environmental processes. In study 2, environmental links were found between parent depression and child and adolescent depression symptoms. Little evidence of inherited effects was detected and environmental effects were found to be stronger for girls. In study 3, a significant association was found between a) mother-child hostility and child and adolescent

depression symptoms and b) maternal depression and child and adolescent depression symptoms. However, there was no association of child and adolescent depression symptoms with specific genetic factors and no evidence of gene-environment interaction for either family factor.

Conclusion: Using different research designs, environmental effects on child and adolescent depression symptoms were detected for two family risk factors - mother-child hostility and parent depression. For both family factors, effects appeared to be stronger for girls suggesting their increased susceptibility to these stressors. No evidence of gene-environment interaction was detected for the selected gene variants and psychosocial factors. However, power in the current sample was limited.

Chapter 1

Depression and depression symptoms in children and adolescents

This introductory chapter will describe the importance of child and adolescent depression, referring firstly to its clinical presentation and the wide range of negative outcomes associated with both symptoms and disorder. The definition and assessment of depression is discussed next before considering epidemiology and the issues associated with establishing exact prevalence rates. Finally, gender differences in the prevalence of depression are discussed.

Clinical presentation and negative outcomes

Depression refers to a continuous and pervasive state of low mood that is often accompanied by a range of other symptoms (e.g. loss of interest and pleasure in activities that would usually be enjoyed, disrupted sleep patterns and appetite disturbance) which interfere with a person's ability to function across multiple domains and contexts. The manifestation of depression and depression symptoms is highly heterogeneous resulting in a complex and multifaceted psychopathology which can often be difficult to detect and diagnose (Hankin, 2006). In defining the clinical disorder of depression in children and adolescents, traditional diagnostic criteria (DSM-IV and ICD-10) require the presence of a range of symptoms in addition to low mood. These include insomnia or hypersomnia, weight loss or weight gain, fatigue, feelings of worthlessness, reduced self-esteem, bleak and pessimistic views of the future and ideas or acts of self-harm and suicide. Both clinical depression and subthreshold depression symptoms in childhood and adolescence are associated with significant impairment to interpersonal relationships, social competence and academic performance (Birmaher, Ryan, Williamson, Brent, & Kaufman, 1996; Hankin, 2006). Increased levels of substance use and comorbid anxiety and behavioural problems are also reported (Hankin, 2006). A range of prospective

longitudinal studies have also found that depression in adolescence predicts increased risk of suicide which is the third leading cause of adolescent fatality (Gould, Greenberg, Velting, & Shaffer, 2003). Depressive episodes occurring in childhood and adolescence have been found to last for an average of 3-6 months in community samples and 5-8 months in clinic referred samples with an earlier study finding the average episode duration to be as long as 7-9 months (Birmaher, Ryan, Williamson, Brent, & Kaufman, 1996).

Adding to the impact of depression, a range of studies have found major depression and depression symptoms in childhood and adolescence to persist across the lifecourse and predict the onset of adult depressive disorder (Copeland, Shanahan, Costello, & Angold, 2009; Fergusson, Horwood, Ridder, & Beautrais, 2005; Harrington, Fudge, Rutter, Pickles, & Hill, 1990; Maughan & Kim-Cohen, 2005; Pine, Cohen, Gurley, Brook, & Ma, 1998; Rao & Chen, 2009). It is important to note that predictive links vary according to whether prospective versus retrospective designs are used, whether comorbid diagnoses are considered and whether depression pre- versus post-puberty is examined. For example, both childhood and adolescent depression have been found to predict future depression episodes in adulthood (Birmaher et al., 2004; Harrington et al., 1997) however, some studies evidence stronger predictive links between adolescent and adult depression than child and adult depression (Rutter, Kim-Cohen, & Maughan, 2006). This is likely due to the significantly lower prevalence rates of depression in childhood versus adolescence and also to the difficulties associated with assessing the presence of emotional disorders in cognitively less mature age groups since reliance on self-reports is the most accurate method of assessing internal emotional states (Luby et al., 2003) and this is often not practical with young children.

Another important issue in assessing the recurrence of depression is whether comorbid disorders are considered. Lewinsohn et al., (2000) found that multiple depressive episodes in adolescence predicted depression in early adulthood after controlling for comorbid anxiety disorder, substance use, conduct disorder and oppositional defiant disorder. Pine et al., (1998) report an initial predictive association, however, in multivariate analyses when comorbid conduct disorder was included, adolescent depression was no longer predictive of adult depressive disorder. Similarly, in the Great Smoky Mountains study, Copeland et al., (2009) report a significant link between adolescent and young adult depression, however, this was accounted for by comorbidity with oppositional defiant disorder, anxiety and substance use. Despite several negative findings, depression in adolescence remains an important predictor of depression in adulthood and rather than explaining away findings, comorbidity may be more usefully considered as an important aspect of the depression 'syndrome' which increases the likelihood of recurrence.

Importantly, predictive links between adolescent and adult depression have also been found for subthreshold adolescent depression symptoms. Pine et al., (1999) found that subclinical depressive disorder in adolescents predicted adult major depression. In a longitudinal study of adolescents, Fergusson et al., (2005) assessed depression symptoms in addition to diagnosis level disorder at 17-18 years and major depressive disorder at 25 years. Both symptoms and diagnoses in adolescence were predictive of major depressive disorder in young adulthood after controlling for comorbidity. In conjunction, these findings demonstrate that depression in childhood and adolescence shows continuity over time which suggests a stable and chronic lifetime course that has its aetiological roots in early development (Lau & Eley, 2010). This highlights the importance of predicting and preventing depression symptoms when they emerge in childhood and adolescence.

Defining and assessing depression

Depression can be defined and assessed according to clinical interviews based on DSM-IV/ICD-10 diagnostic criteria or according to questionnaire based methods which assess depressive symptomatology. It has become increasingly acknowledged that depression can be meaningfully defined along a continuum of increasing symptom severity (Gotlib, Lewinsohn, & Seeley, 1995; Hankin, Fraley, Lahey, & Waldman, 2005) as well as being classified as a clinical disorder that is either absent or present. Whether depression is best classified according to a categorical or a dimensional approach has been an issue of debate for some time. Recent research has suggested that statistically, in terms of underlying latent structure, a dimensional definition best applies (Rusico & Rusico, 2000). Using taxometric analyses which employ statistical procedures to determine whether the underlying structure of observed variables represents a categorical or a continuous construct, Hankin et al., (2005) found that depression symptoms in a population based sample of children and adolescents were best described according to a continuum of symptom severity. The dimensional structure was obtained for DSM-IV major depression symptoms, for various domains of depression including emotional distress, vegetative symptoms and involuntary defeat and was present for both youth and parent reports, for boys and girls and for younger and older children and adolescents. In the population based Great Smoky Mountains study, Angold et al., (1999) found that a diagnosis of depression was not necessary for significant impairment to children's functioning; children and adolescents who did not meet full DSM-III-R criteria for disorder still reported symptoms of significant psychosocial impairment. Similarly, using the Virginia Twin Study of Adolescent Behavioural Development, Pickles et al., (2001) found that many children and adolescents who fell below the DSM-III-R diagnostic threshold for depression had symptom-related impairment. This study reported no evidence of any additional impairment at the diagnostic threshold suggesting that

diagnostic boundaries, although undoubtedly clinically useful, do not necessarily represent theoretically meaningful categories which aid an understanding of the aetiology of depression. In practical terms it is generally acknowledged that both approaches have utility and are relevant to the classification, diagnosis and treatment of depression. However, current findings suggest that continuous approaches to defining depression may more accurately reflect the underlying construct and present a useful approach for research that remains clinically relevant.

Depression epidemiology

Major depressive disorder is projected to become the second leading cause of disability adjusted life years by the year 2020 (Murray & Lopez, 1997) and the leading cause of disability worldwide in high-income countries such as the United Kingdom and the United States (Mathers & Loncar, 2006; McKenna, Michaud, Murray, & Marks, 2005). The exact prevalence of clinical depression and depressive symptomatology is difficult to ascertain for a variety of reasons; wide heterogeneity in the manifestation of symptoms, differences in the methods used to calculate prevalence estimates, variation in the measurement instruments used and in the interpretation of symptoms and cross-cultural differences (Kessler et al., 2001). In identifying precise prevalence rates, it is important to consider factors which affect epidemiological estimates. Rates of major depression vary according to a number of factors which include age (children versus adolescents and adults), ethnic group (Gonzalez, Tarraf, Whitfield, & Vega, 2010), whether prospective longitudinal versus retrospective survey data are utilised (Moffitt et al., 2010), whether symptoms versus diagnoses are considered, the method used to calculate prevalence (lifetime versus 12-month), and importantly by gender with twice as many women affected as men.

An important epidemiological finding is that depression in childhood is consistently less common than depression in adolescent and adult populations. For example in a longitudinal community study, Costello et al., (2003) found that 3-month prevalence rates of major depression increased from between 0.5% - 1.9% for 9-12 years olds to 2.7% - 3.7% for 14-16 year olds. A range of studies report that the 12-month prevalence of childhood depression ranges from 0.4% - 2.5% (Birmaher et al., 1996) whilst for adolescent depression it ranges from 0.4% - 8.3% (Birmaher et al., 1996). A range of prospective longitudinal studies have been conducted and report lifetime prevalence rates of 1-3% in children rising to 17% in adolescence (Costello, et al., 2003; Reinherz, Giaconia, Lefkowitz, Pakiz, & Frost, 1993; Reinherz et al., 1993). Using a longitudinal sample, Lewinsohn et al., (1999) found point prevalence estimates for adolescent depression of 2.9% at the first time point and 3.1% at the second time point. Lifetime prevalence rates were 20.4% and 24.0% at the respective time points. Lifetime estimates based on a different study, the National Comorbidity Survey (Kessler, McGonage, Zhao et al., 1994), have been reported as slightly lower - 14% in 15-18 year olds.

Prevalence rates also vary according to whether symptoms versus diagnoses are assessed with significantly higher rates reported for subthreshold symptoms. For example, community epidemiologic surveys of self-reported depression symptoms have found that between 20% and 50% of children and adolescents exceed recommended clinical cut-off scores over symptom recall periods of 1-6 months (Hankin, 2006; Kessler, Avenevoli, & Merikangas, 2001). However, despite consistent findings that the prevalence of depression increases with age, it is important to note that pubertal status and developmental stage may be more accurate predictors of depression onset than age per se (Angold & Costello, 2001; Silberg et al., 1999). Despite variation in prevalence rates according to numerous factors, the consistent finding is that depression is one of the most common forms of

psychopathology in children and adolescents and represents a significant burden to society as well as to the individuals experiencing the disorder.

Gender differences

Relevant to an understanding of the prevalence and aetiology of depression and depression symptoms, a highly consistent female preponderance in prevalence rates emerges from early adolescence (most studies report age 12-13 years) and persists into adulthood (Nolen-Hoeksema, 2001; Piccinelli & Wilkinson, 2000) with approximately twice as many women affected than men. The 2:1 female to male ratio is based on two early large-scale epidemiological survey studies which produced estimates of 2.4:1 (Weissman, Bruce, Leaf, Florio, & Holzer, 1991) and 1.7:1 Kessler et al., (1994). The gender difference is observed for both subclinical depression symptomatology and for diagnosed disorder (Nolen-Hoeksema & Girgus, 1994) and is consistent across cultural and ethnic groups (Cyranowski, Frank, Young, & Shear, 2000; Wolk & Weissman, 1995). In pre-adolescent children, depression symptoms and disorder are found to occur at the same rate in boys and girls (Brooks-Gunn & Petersen, 1991; Nolen-Hoeksema, 1987) with some early studies suggesting that boys may be more likely to exhibit depression symptoms than girls before the age of 12 years (Anderson, Williams, McGee, & Silva, 1987). Following the emergence of the gender difference in early adolescence, the predominance of the disorder in females has been found to persist until old age (Cyranowski et al., 2000).

The reasons underlying the gender difference in depression are unclear but may cast light on the aetiology of depression in general. Various theoretical models have been advanced to account for the female preponderance and refer to social, cultural, interpersonal, cognitive, biological and hormonal factors. Earlier models referred predominantly to social and interpersonal gender differences in personality and

behavioural style although references to biological changes in the endocrine system in females were proposed as early as 1989 (Brooks-Gunn & Warren, 1989). Such gender differences in personality and behavioural style refer to findings that girls are more socially oriented and communal in their interpersonal relationships than boys. For example, girls' style of interaction in groups is focused on cooperation and the maintenance of social and interpersonal relationships whereas boys' interaction style has been found to be more focused on competition and dominance (Nolen-Hoeksema & Girgus, 1994). In line with 'diathesis-stress' theories, such pre-existing gender differences have been proposed to act as diatheses which interact with the increased challenges of adolescence and result in greater depression levels in females.

More recent theoretical models accounting for the gender difference in depression have been advanced and adopt an integrative approach which takes into account social, biological, hormonal and genetic factors (Cyranowski, et al., 2000; Hankin & Abramson, 2001; Hyde, Mezulis, & Abramson, 2008; Nolen-Hoeksema, 2001; Piccinelli & Wilkinson, 2000). Cyranowski et al., (2000) propose that heightened affiliative needs such as a preference for close emotional relationships, intimacy and responsiveness along with hormonal mechanisms such as increased oxytocin levels interact to result in the gender difference in depression and depression symptoms. Cyranowski et al., (2000) also emphasise the role of negative life events. In support of this, there is evidence to suggest that adolescent females report more exposure to negative life events than adolescent males and also display greater levels of stress and heightened responsivity to stress (Ge, Lorenz, & Conger, 1994). For example, Ge et al., (1994) found that whereas pre-adolescent boys reported more negative life events and depression symptoms than girls, after age 13 years, girls reported significantly more negative life events and changes in depressed mood were associated with changes in the number of life stressors girls experienced. In contrast

to the interpersonal and social emphasis of the model proposed by Cyranowski et al., (2000), Hankin & Abramson, (2001) propose a cognitive vulnerability-stress theory in which girls are more cognitively vulnerable to negative events in terms of dysfunctional attitudes, negative inferential styles and ruminative response tendencies which contribute to elevations of negative affect. Girls are hypothesized to ruminate more than boys, make more negative inferences about life events and exhibit more depressogenic cognitive inferential styles. This has been supported in subsequent research. Stone et al., (2011) for example found that gender differences in co-rumination – the tendency to frequently discuss and rehash problems with peers, mediated the gender difference in the onset of clinically significant depression episodes. The models proposed by Cyranowski et al., (2000) and Hankin & Abramson (2001) are informative in that they provide detailed accounts and specific hypotheses which have been supported in subsequent research (Hyde, et al., 2008). However, although each model considers multiple factors, they focus predominanlty on either social/interpersonal (Cyranowski et al., 2000) or cognitive factors (Hankin & Abramson, 2001). The most recent account of the emergence of the gender difference in depression has been proposed by Hyde et al., (2008) and advances an integrative model incorporating affective factors such as emotional reactivity, biological factors such as genetic vulnerability, pubertal hormones, pubertal timing/development and cognitive factors including cognitive style and rumination. Given the well established gender difference, a crucial question addressed in this thesis is whether and how putative environmental risk factors for child and adolescent depression differ in their effects between boys and girls.

Chapter summary

The topics covered in this chapter have demonstrated the importance of child and adolescent depression and depression symptoms and highlighted the need for early prevention and treatment of symptoms. Predicting and preventing child and adolescent depression is contingent upon the identification of likely causal environmental risk factors which precede the onset of symptoms and can therefore be targeted in preventive intervention strategies since environmental influences are potentially malleable (Rutter, 2007b). A greater understanding of specific psychosocial risk factors which confer their effects via environmental processes is therefore a paramount goal of research in this area.

Depression and depression symptoms have consistently been found to run in families (Weissman, Wickramaratne, et al., 2006), have numerous biological correlates (Gillespie, Phifer, Bradley, & Ressler, 2009) and evidence strong associations with a wide range of putative environmental risk factors such as exposure to psychosocial stress (Hammen, 2005). The finding that depression runs in families provides important clues that aetiology is likely due to the action and interaction of both genetic and environmental risk factors. However, disentangling environmental from genetic effects on intergenerational transmission requires a range of methodological strategies and statistical techniques.

In addition to separating the independent effects of genes and environments to identify direct environmental effects, an important area of research is the role of gene-environment interplay. The identification of environmental effects that are distinct from genetic influences along with tests of gene-environment interplay requires the use of genetically sensitive research designs that permit a separation of environmental from genetic effects. Genetically sensitive research designs are

therefore necessary for identifying putative environmental risk factors. The next chapter will introduce the various types of genetic design and describe how they have been used to investigate the heritable basis of depression aetiology and the role of gene-environment interplay. An understanding of the nature of genetic influences on depression and gene-environment interplay is necessary before proceeding to a description of the issues associated with testing whether putative psychosocial influences confer potentially causal risk effects over and above the influence of genetic factors. This is discussed in Chapter 3.

Chapter 2

The importance of genetically sensitive designs

Identifying psychosocial influences which confer risk effects via environmental pathways requires the potential contribution of genetic factors to be taken into consideration. A range of putative "environmental" risk factors for depression have been found to show genetic influence (Kendler & Baker, 2007; McGue, Elkins, Walden, & Iacono, 2005; Neiderhiser et al., 2004; Plomin, Reiss, Hetherington, & Howe, 1994). Since depression is also influenced by genetic factors, observed associations between putative psychosocial risk factors and depression as an outcome may in fact be accounted for by an unmeasured heritable component (Plomin, et al., 1994). Thus, genetically informative studies and other types of quasi-experimental designs have been important for testing whether associated risk factors have environmentally mediated effects (Kendler & Gardner, 2010; van Os, Kenis, & Rutten, 2010). Such designs include quantitative and molecular genetic techniques and have primarily been used to test the magnitude of the heritable component to depression and to attempt to identify the specific gene variants which may account for this influence. This chapter will describe key genetically sensitive designs and summarize the evidence that heritable influences contribute to the aetiology of child and adolescent depression.

Genetics of child and adolescent depression

Genetic research on child and adolescent depression has moved beyond testing the presence of genetic influences and quantifying the magnitude of heritability estimates to elucidating the relative extent of genetic versus environmental effects, the specific genetic and environmental factors which confer risk and the mechanisms which underlie their interplay (Lau & Eley, 2010). Evidence has been gathered using a range of designs including family, twin, adoption and molecular genetic studies.

These designs have demonstrated that child and adolescent depression and depression symptoms show moderate but significant genetic influences and have also identified an important contribution of environmental factors and gene-environment interplay.

Family studies were the first design to suggest a possible genetic contribution to the aetiology of child and adolescent depression. Such studies examine the odds of developing a disorder in individuals with a family history of the illness relative to a control group of individuals without a family history (Thapar & Stergiakouli, 2008). Family studies have suggested that depression is on average twice as likely to occur in the relatives of depressed versus non-depressed children (Goodyer, Cooper, Vize, & Ashby, 1993; Harrington, et al., 1997; Klein, Lewisohn, Seeley, & Rohde, 2001; Kovacs, Devlin, Pollock, Richards, & Mukerj, 1997; Kutcher & Marton, 1991; Weissman, Wolk, Wickramaratne, et al., 1999; Wickramaratne, Greenwald, & Weissman, 2000a; Williamson et al., 1995). In addition, family studies of the children of depressed parents have found an approximate 2-3 fold increase in risk relative to the children of non-depressed controls (Beardslee, Versage, & Gladstone, 1998; Weissman et al., 1987; Weissman, Leckman, Merikangas, Gammon, & Prusoff, 1984; Wickramaratne, et al., 2000a; Wickramaratne & Weissman, 1998). The family design permits a test of whether a disorder 'runs' in families. However, since parents and children are genetically related, environmental versus genetic modes of intergenerational transmission cannot be distinguished and consequently this design cannot be described as truly genetically sensitive. Alternative genetic designs including twin and adoption studies along with a range of extensions of these methods such as the Children of Twins (COT) and Extended Twin designs permit a separation of environmental and genetic influences and therefore constitute a more useful tool for investigating aetiological pathways.

The classic twin design is based on the fact that monozygotic (MZ) and dizygotic (DZ) twins differ in their degree of genetic relatedness; whereas MZ twins are genetically identical, DZ twins are no more genetically alike than non-twin siblings and share on average half their genes. If a trait is genetically influenced, MZ twins will therefore be expected to show greater similarity on measures of the trait relative to DZ twins. A range of twin studies on child and adolescent depression have been conducted (Eaves et al., 1997; Gjone & Stevenson, 1997; Hewitt, Silberg, Neale, Eaves, & Erickson, 1992; Rende, Plomin, Reiss, & Hetherington, 1993; Schmitz, Fulker, & Mrazek, 1995; Thapar & McGuffin, 1994; Wierzbicki, 1987) and report moderate but significant heritability estimates. In childhood, heritability estimates have been found to vary widely from 15% (Rice, Harold & Thapar, 2002) to 43% (Happonen et al., 2002). In adolescence they are more consistently estimated at approximately 42% (Glowinski, Madden, Bucholz, Lynskey, & Heath, 2003) to 55% (Rice et al., 2002) which is closer to the average heritability estimate of 37% for adults (Sullivan, Neale, & Kendler, 2000). In addition, the influence of the shared environment is consistently found to be greater in children than in adolescents (Rice et al., 2002). The finding that the magnitude of heritability estimates is lower in children and the influence of the shared environment greater than in adolescents has been replicated across twin studies (Scourfield et al., 2003), although some do not report such an effect (Gjone & Stevenson, 1997). In accounting for this difference in heritability, Lau & Eley (2006) found that new genetic risk factors related to depression emerged in adolescence which, together with increased gene-environment correlation (Rice, Harold, & Thapar, 2003) may explain age-related increases in heritability estimates.

Child and adolescent gender has also been found to affect twin study heritability estimates but findings are inconsistent. Some studies report greater genetic influences for girls (Silberg, et al., 1999) while others report higher heritability for

boys (Eley & Stevenson, 1999). Rice et al., (2002) report a significantly larger heritability estimate for adolescent boys (43% for boys versus 31% for girls) and a significantly greater shared environmental effect for girls (30% for girls versus 10% for boys).

Adoption studies provide a different, cross-generation genetically sensitive design which examines trait similarity between relatives by comparing genetically related individuals in biologically intact families with genetically unrelated individuals in adoptive families. Genetic influences are inferred when individuals are more similar to biological relatives with whom they have a genetic but not a social relationship than adoptive relatives with whom they have a social but not a genetic relationship (Thapar & Stergiakouli, 2008). In addition to the adoption and twin method, other quantitative genetic designs based on differences in genetic relatedness have been used to test the presence of environmental risk effects. These include cross-generational designs such as the COT method and a more novel strategy which has been recently developed and utilises the differing degrees of genetic relatedness among children born via assisted reproductive technology such as In Vitro Fertilization (IVF). This technique is based on the same principle as the adoption design; if an association between a putative environmental risk variable and an outcome is found in genetically unrelated parent-child pairs then this provides evidence for the presence of environmental effects whereas, if the association is present in related but not unrelated families, genetic effects only are implicated.

Three adoption studies of child and adolescent depression have been conducted and all suggest an important contribution of environmental risk effects (Eley, Deater-Deckard, Fombonne, Fulker, & Plomin, 1998; Tully, Iacono, & McGue, 2008; van den Oord, Pickles, & Waldman, 2003). Eley et al., (1998) found significant environmental links between maternal neuroticism and child depression symptoms but no evidence

for genetic contribution which concurs with a study that examined internalising symptoms in a sample of adoptees using a sibling based design (van den Oord et al., 2003). In accordance with these studies, Tully et al., (2008) found a significant environmental contribution to the intergenerational transmission of maternal depression symptoms with some evidence also for genetic links since associations were observably larger in genetically related than unrelated parent-child pairs. However, the difference between the related and unrelated groups was not statistically significant. Using the COT design, Silberg et al., (2010) found that the intergenerational transmission of depression symptoms was due to the influence of shared family environmental factors with no evidence for the role of genetic contribution. Using a sample of children conceived via IVF, Harold et al., (2010) found that the association between parent and child depression was present in both genetically related and unrelated mother-child pairs again supporting the role of a significant environmental contribution to intergenerational links.

The lack of consistent evidence for genetic contribution in adoption studies contrasts with the results of twin research which suggests moderate genetic effects. The presence of passive gene-environment correlation has been referred to as a potential explanation for the discrepant findings across twin and adoption studies (Rice & Thapar, 2008). Passive gene-environment correlation refers to the fact that parents provide their children with a family environment that is correlated with their own genetically influenced characteristics (Price & Jaffee, 2008). Passive gene-environment correlation is present in the twin design because parents and children are genetically related whereas in adoption designs it is removed by virtue of the adoptive group wherein parents and children are genetically unrelated. This suggests that heritability estimates in twin studies may in part be accounted for by the effects of passive gene-environment correlation. This is plausible because in the classic twin design the effects of passive gene-environment correlation are subsumed within the

heritability estimate so may inflate the role of genetic influences relative to adoption designs.

Following the identification of heritable influences using the twin design, molecular genetic studies have been used to attempt to identify the specific gene variants which account for this effect. These have mainly focused on adult depression. To date, molecular genetic studies have provided little consistent evidence that specific genes confer direct risk effects for major depression. This includes recent genome-wide association studies (GWAS) (Muglia et al., 2010; Shi et al., 2010; Shyn et al., 2011; Sullivan et al., 2009; Wray et al., 2010). One potential reason for the lack of significant effects across molecular genetic studies may be that genetic susceptibility for depression does not manifest as a direct main effect of a specific gene variant but is contingent upon exposure to conditions of psychosocial stress. Increasing attention has therefore been paid to the role of gene-environment interplay with specific focus on gene-environment interaction. Gene-environment interplay is important to elucidating the ways in which genes and environments work together to influence susceptibility for depression and is therefore relevant to establishing the role of environmental risk effects. Gene-environment interaction in particular has become an important topic in depression research. The role of gene-environment interplay in the aetiology of child and adolescent depression symptoms will be discussed next.

Gene-environment interplay in child and adolescent depression

The concept of gene-environment interplay offers two ways in which genetic susceptibility to depression may be expressed: 1) by resulting in a greater exposure of individuals to environmental stressors associated with risk for depression such as negative life events - gene-environment correlation (rGE) and 2) by resulting in increased susceptibility to the effects of such stressors - gene-environment

interaction (GxE). rGE can be defined as an association between a person's genotype and the environmental influences to which they are exposed or experience (Rutter, 2007) whereas GxE is defined as genetically influenced sensitivity to the environment or genetic moderation of the effects of environmental influences (Rutter, 2007a; Rutter & Silberg, 2002). Both forms of gene-environment interplay have been studied in relation to child and adolescent depression and depression symptoms and have been found to be important to understanding the ways in which environmental risk effects manifest and confer their influence. rGE in relation to child and adolescent depression will be discussed first before a detailed description of GxE which is a main focus of this thesis.

rGE and child and adolescent depression

In addition to being a mechanism through which genetic susceptibility may confer its effects, rGE is relevant to an understanding of exposure to family risk factors and how such exposure is associated with the development of depression and depression symptoms (McGue, et al., 2005; Neiderhiser et al., 1994; Plomin, et al., 1994). There are three identified subtypes of rGE (Price & Jaffee, 2008): active, evocative and passive. Passive rGE is relevant to an understanding of how the family environment impacts upon risk for child and adolescent depression and occurs when certain aspects of the family environment are associated with heritable parental characteristics (Jaffee & Price, 2007).

Consistent with passive rGE, twin and adoption studies have demonstrated genetic influences on numerous measures of the family environment including a range of parenting behaviours such as; positivity, negativity, control and monitoring (Neiderhiser, et al., 2004), conflict, regard, involvement and support (Elkins, McGue, & Iacono, 1997a), warmth (Lichtenstein et al., 2003; McGue, et al., 2005), global

family conflict (Horwitz et al., 2010) and marital satisfaction (Spotts et al., 2004). In a meta-analysis of 55 studies assessing the heritability of environmental influences related to depression, Kendler & Baker (2007) found significant genetic estimates in the range of 7%-39% with the majority falling between 15% and 35% and an aggregate estimate of 27% across measures for both parent and child reports. Genetic influences on measures of the family environment arise because these measures assess, in part, genetically influenced characteristics of the respondents and are not therefore 'pure' measures of environmental influence (Plomin, et al., 1994).

Genetic influences on measures of the family environment have implications for studies of the association between family factors and child and adolescent depression symptoms. Since depression symptoms also show genetic influence, it is possible that the same set of genes may influence the origin of both risk factor and outcome variables. This raises the possibility that observed associations between the putative environmental risk (family factors) and the outcome (child and adolescent depression) are accounted for through a genetic pathway common to both variables. This is referred to as genetic correlation or genetic mediation (Rutter et al., 2001). rGE therefore has important implications for elucidating environmental effects. However, it is important to note that the origins of a risk factor do not necessarily determine its mechanism of action (Rutter, 2007). For example, family risk factors may arise in part through genetic factors (rGE) but still have environmental effects in the same way that smoking confers an environmentally mediated risk effect on lung cancer despite the propensity to smoke being partly under genetic influence (Rutter, 2007). The existence of rGE points to the fact that genes do not directly affect environments. Rather, genetic vulnerability for depression is expressed through the creation of high- risk environmental exposure (Lau & Eley, 2010). rGE is therefore assumed to play an important role in the aetiology of depression and is important to

consider when attempting to establish the processes underlying putative environmental effects on risk for depression.

GxE and child and adolescent depression

GxE has become an important topic in research on the aetiology of depression and offers an interesting framework for potentially understanding the links between psychosocial and biological risk factors. GxE in relation to child and adolescent depression has been investigated using both twin and molecular genetic studies. A range of twin studies have demonstrated that GxE is relevant to the aetiology of child and adolescent depression using a variety of different methods. Silberg et al., (2001) found that maternal ratings of independent negative events predicted depression and anxiety only among adolescents whose parents had an emotional disorder. This demonstrated significant GxE between family liability which was used to index genetic susceptibility and exposure to negative life events. However, the presence of parent psychopathology could represent either shared genetic or shared environmental liability which could not be distinguished using this particular method of indexing genetic susceptibility. An alternative method of testing GxE using the twin design utilises statistical twin modelling techniques (Neale, Boker, Xie, & Maes, 1999) which enable a mathematical estimation of the significance of a specific GxE parameter. Using this method, Rice et al., (2006) found that the influence of family conflict in predicting depression symptoms was greater in individuals at higher genetic risk of depression. Rice et al., (2006) also found that the genetic variance of depression increased according to increasing levels of family conflict which provided an additional indication of the presence of GxE using a different method. The genetic estimate is presumed to increase in the presence of psychosocial stressors because exposure to environmental risk triggers latent genetic vulnerabilities (Lau & Eley, 2010).

An important issue in assessing quantitative GxE is to control for the simultaneous presence of rGE. The presence of rGE may lead to false conclusions of GxE - observable interactions may in fact be due to an interaction between the genetic factors influencing each variable and not to interaction between genetic and environmental influences. Lau & Eley (2008b) investigated dependent negative life events and maternal punitive discipline in an adolescent twin and sibling sample. After controlling for the presence of rGE, genetic variance for adolescent depression symptoms was found to increase according to increasing levels of exposure to negative life events and maternal punitive discipline. Quantitative genetic methods that assess overall genetic liability therefore suggest an important role for GxE in relation to the aetiology of child and adolescent depression. However, since they index overall genetic risk, quantitative genetic studies do not point to the specific genes and therefore the biological systems responsible.

Molecular genetic GxE studies

Molecular genetic GxE studies test for interaction between a specific gene variant and exposure to environmental risk. The first molecular genetic study to support a role for GxE in the aetiology of depression was conducted by Caspi et al., (2003). This study investigated the role of a functional polymorphism (5-HTTLPR) in the serotonin transporter gene (*SLC6A4*) in moderating the association between stressful life events and depression in an adult sample. Although this study focused on adult depression, it is relevant to an understanding of GxE since it triggered the corpus of GxE research currently available including that on child and adolescent depression. Caspi et al., (2003) found that individuals possessing one or two copies of the short allele of 5HTTLPR displayed significantly more depression symptoms, DSM-IV diagnoses of depression and suicidal ideation or suicide attempts than individuals with the long allele version of 5-HTTLPR. Importantly, this finding was made

biologically plausible by the fact that the short allele of 5-HTTLPR is known to be associated with lower transcriptional efficiency of the serotonin promoter and reduced uptake and reuptake at the synapse compared with the long allele and could therefore confer biological risk for depression. The moderating role of 5-HTTLPR has been replicated in both adult (Aguilera et al., 2009; Bukh et al., 2009; Cervilla et al., 2007; Dick et al., 2007; Goldman, Glei, Lin, & Weinstein, 2010; Grabe et al., 2005; Jacobs et al., 2006; Kendler, Kuhn, Vittum, Prescott, & Riley, 2005; Mandelli et al., 2007; Scheid et al., 2007; Surtees et al., 2006; Taylor et al., 2006; Zalsman et al., 2009) and child and adolescent samples (Aslund et al., 2009; Benjet, Thompson, & Gotlib, 2010; Cicchetti, Rogosch, & Sturge-Apple, 2007; Eley et al., 2004; Kaufman et al., 2004; Kim et al., 2007; Kumsta et al., 2010; Sjoberg et al., 2006; Sugden et al., 2010). There have also been a series of non-replications in children and adolescents and a certain amount of variation found according to gender with some studies reporting stronger effects for females (Eley, et al., 2004; Hammen, Brennan, Keenan-Miller, Hazel, & Najman, 2010; Sjoberg, et al., 2006).

In addition to replications of the original GxE finding, studies have demonstrated GxE between the short allele of the serotonin transporter gene variant and maltreatment (Caspi, Hariri, Holmes, Uher, & Moffitt, 2010), parental nurturing behaviour such as unresponsiveness, organization of attachment style and a range of other outcomes relevant to the development of depression; behavioural inhibition (Fox, Henderson, Marshall, Nichols, & Ghera, 2005), self-regulation (Kochanska, Philibert, & Barry, 2009), attachment security (Barry, Kochanska, & Philibert, 2008), negative emotionality, and fear (Pauli-Pott, Friedel, Hinney, & Hebebrand, 2009). These studies suggest that gene-environment interactions related to 5-HTTLPR and stressors in early childhood are related to later risk for depression and associated phenotypes.

Despite the fact that positive replications outweigh negative ones, there has been controversy regarding the reliability of GxE findings on 5-HTTLPR and the broader issue of whether GxE exists on a biological versus a statistical level (Risch et al., 2009; Rutter, Thapar, & Pickles, 2009). Risch et al., (2009) conducted a meta-analysis of studies on 5-HTTLPR and stressful life event exposure and concluded that there was little support for the existence of an interaction effect. A series of issues have been raised regarding the methodology used by Risch and colleagues and the approach taken by the authors such as the unnecessary exclusion of studies based on stringent statistical criteria (only 14 of 34 relevant studies were included), the recoding of original data, and reliance on a purely statistical approach to GxE. However, this meta-analysis drew attention to several important methodological issues in the study of GxE such as whether a main genetic effect is required, which statistical interaction model is preferable (additive versus multiplicative) and how to conceptualize and asses environmental risk exposure.

Uher & McGuffin (2010) have recently conducted a meta-analysis of all 34 studies on 5HTTLPR, environmental adversity and depression and found evidence for significant interaction. This study draws attention to the importance of the methods used to assess environmental adversity such that 5HTTLPR appears to be more strongly related to objectively occurring adversity rather than assessments based on retrospective self-report. Variation according to gender has also been reported with inconsistent findings for adolescent males (Uher & McGuffin, 2010). A recently conducted meta-analysis by Karg et al., (2011) used a different analytic method and included a broader range of studies than Risch and colleagues. Karg et al., (2011) found significant evidence for the presence of interaction between 5-HTTLPR, stress exposure and depression. Karg et al., (2011) also re-ran analyses using the sub-group of studies examined by Risch and colleagues and did not find support for an

interaction effect suggesting that differences between meta-analyses were not due to the methodology used but to the distinct set of studies included in each.

Another gene which has been found to interact with environmental influences to predict depression in children and adolescents is the corticotrophin-releasing hormone receptor 1 gene (*CRHR1*). Corticotrophin-releasing hormone plays a predominant role in the regulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis. The HPA axis is a collection of neural and endocrine structures that regulate the response to stress (Gillespie et al., 2009). Stressors are perceived by the cortex of the brain and stimulate a hormonal response which prepares the organism for 'fight or flight.' In response to stress, the hypothalamus secretes corticotrophin releasing hormone (CRH). CRH is transported to the anterior pituitary gland where it activates *CRHR1* receptors which results in the release of adrenocorticotrophic hormone (ACTH). ACTH stimulates the production and release of the adrenocorticosteroid hormone cortisol. The main role of cortisol is to restore homeostasis through 'negative feedback' or 'feedback inhibition.' Feedback inhibition by cortisol is mediated by its action on glucocorticoid and mineralocorticoid receptors which are widely spread across the brain in the hippocampus, parvaventricular nucleus and the pituitary gland. Feedback inhibition serves to reduce stress-induced activation of the HPA axis by limiting excess secretion of the stress hormones. Dysregulation of the HPA axis is thought to be a key component in the biological underpinnings of depression (Nemeroff & Vale, 2005).

CRHR1 has been investigated in several GxE studies assessing the effects of child abuse and maltreatment on child and adolescent depression. Bradley et al., (2008) examined whether the effects of child abuse were moderated by a selection of 10 polymorphisms in the *CRHR1* gene. Depression was assessed dimensionally using the Beck Depression Inventory (BDI) and past history was also considered using the

Structured Clinical Interview (SCID) for DSM-IV. After corrections for multiple testing, 2 SNPs in the *CRHR1* gene remained significant moderators of the association: no significant association between the SNPs and child abuse was observed in the 'none to mild' child abuse group but the association was significant in the 'moderate to severe' child abuse group. Significant GxE was also observed with a haplotype formed by the three most significant SNPs. Both SNPs and the haplotype were associated with a protective effect in that individuals possessing the protective allele were less likely to develop depression following exposure to child abuse and trauma. Significant GxE for the haplotype has been replicated in several subsequent studies in relation to childhood maltreatment and diagnoses of past-year and recurrent major depressive disorder (Polanczyk et al., 2009; Ressler et al., 2009). However, in the study by Polanczyk et al., (2009) which utilised two samples, one using the same measure of child abuse as the study by Bradley and colleagues and another using a different measure, the GxE was only replicated using the same measure. This was likely due to the emotive nature of the measure used by Bradley and colleagues - the Child Trauma Questionnaire (CTQ) which assessed perceptions and feelings regarding the experience of childhood maltreatment as opposed to more objective measures of child abuse which assess frequency of occurrence. This suggests that the protective effect of *CRHR1* may stem from its role in the consolidation of emotion laden memories. In addition, this points to the importance of the nature of the environmental variable used in GxE studies.

The 5-HTTLPR and *CRHR1* variants are currently the only gene variants which have demonstrated replicated interactions with environmental stressors that have been implicated in the aetiology of depression; stressful life events and child abuse and trauma.

Chapter Summary

The support for a heritable influence on child and adolescent depression is drawn predominantly from quantitative genetic twin studies which suggest a moderate but significant genetic component to aetiology (Rice et al., 2002). However, specific candidate genes which account for this effect have not been successfully identified in molecular genetic studies. This has suggested that genetic risk effects are likely to span the whole genome, involve multiple genes each of small effect and possibly be contingent upon exposure to environmental stressors. In addition to supporting genetic influences, twin research concurs with the results of cross-generational designs such as the adoption method, the COT design and a novel strategy based on children conceived via IVF in suggesting a significant role for non-inherited risk factors. This is supported by the fact that genetic influences account for less than half the variance in adolescent and adult depression and even less for child depression (Rice et al., 2002; Scourfield et al., 2002).

In addition to implicating a role for environmental as well as genetic factors, genetically sensitive research has been essential in highlighting the fact that genes and environments do not work in isolation and confer joint effects in increasing risk for depression via processes of gene-environment interplay. Gene-environment interplay holds important promise for elucidating the processes by which environmental and genetic effects operate with regard to increasing susceptibility and explaining the origin of individual differences in stress responsivity. However, specific attention needs to be paid to the selection of candidate environmental risk factors (Moffitt et al., 2006; Rutter, 2010). Although a wide range of psychosocial factors have been linked with the development of depression, it is necessary that statistical associations represent likely causal relationships. This is a necessary prerequisite for GxE studies and is essential to understanding the nature of environmental risk

effects for child and adolescent depression symptoms. The next chapter will describe psychosocial risk factors for child and adolescent depression along with the challenges associated with establishing whether such risk factors confer potentially causal environmental risk effects.

Chapter 3

Psychosocial risk factors and testing environmental effects

The aetiological pathways to depression are due to the co-active and interactive effects of both genetic and psychosocial risk factors. Although significant links with depression have been demonstrated for a range of psychosocial stressors, the extent to which such associations represent predictive environmental effects is unclear and cannot be discerned on the basis of observational or cross-sectional data (Rutter et al., 2007). In differentiating between correlation and a potential cause and effect relationship, an understanding of the nature of statistical association is required. An association between variables may arise through one of several pathways: 1) the hypothesized effect of the proposed risk factor on the outcome variable 2) the reverse effect of the outcome variable on the risk factor 3) via the influence of a third unmeasured variable which accounts for the occurrence of both risk factor and outcome rendering any observed association spurious and 4) an association may be due to the reciprocal effects of both the risk factor influencing the outcome and vice versa. In ascertaining whether associations between psychosocial risk factors and depression are likely to represent a cause and effect relationship, two major conceptual and methodological issues are therefore pertinent: the possibility of reverse causation or bidirectional effects wherein the assumed outcome variable (e.g. depression symptoms) results in the occurrence of negative psychosocial experiences (e.g. hostility or rejection in the parent-child relationship) and the potential role of unmeasured confounds which may refer to either genetic and/or alternative environmental factors (Rutter et al., 2001; Rutter, 2006). The current chapter focuses on putative psychosocial risk factors for depression and the methodological issues inherent in establishing whether observed associations represent predictive environmental effects.

Psychosocial risk factors

The main focus of research into the psychosocial antecedents of child and adolescent depression has centred on negative life events such as bereavement, parental divorce, physical/sexual abuse, trauma, maltreatment, negative parenting, the parent-child relationship and parent depression (Hammen, 2005). Interpersonal stressors involving disruptions to close relationships, especially those stemming from the family environment, have emerged as highly important (Hammen, et al., 2010; Hammen, Brennan, & Shih, 2004). Due to the importance of exposure to stress in the aetiology of depression, the present chapter begins with a brief discussion of the role of stressful life events before proceeding to the putative psychosocial risk factors which comprise the main focus of this thesis: the parent-child relationship and parent depression. The methodological issues associated with establishing the presence of environmental risk effects and the evidence that these risk factors have a potentially causal influence on child and adolescent depression are then described.

Stressful life events

The term stressful life events has been used to describe a wide range of acute and chronic risk exposures such as bullying, bereavement, illness, injury, and moving schools or neighbourhoods (Hammen, 2005, 2009). In addition, stressful life events include those which stem from the family environment such as childhood abuse and maltreatment (Bifulco, Brown, & Harris, 1994; Duggal et al., 2000; Goodyer & Altham, 1991; Goodyer, Kolvin, & Gatzanis, 1985; Hops, Lewinsohn, Andrews, & Roberts, 1990; Rudolph et al., 2000), negative parent-child relationships (Branje, Hale, Frijins, & Meeus, 2010; Hipwell et al., 2008; Weich, Patterson, Shaw, & Stewart-Brown, 2009), parenting (McLeod, Weisz, & Wood, 2007), inter-parental conflict (Nomura, Warner, & Wickramaratne, 2001), poor quality attachment relationships (Abela et al., 2005) and parent depression (Silberg, Maes, & Eaves, 2010; Tully, et al., 2008).

Exposure to stressful life events, especially when multiple events are experienced, has long been recognised as a robust and well established risk factor for child and adolescent depression (Hammen, 2005). Stress exposure in childhood has also been found to predict depression in adulthood (Bifulco, Bernazzani, Moran, & Ball, 2000; Hammen, 2005, 2009; Tennant, 2002). It is necessary to consider the heterogeneity inherent within the construct of stressful life events which involves many facets that have been differentially related to depression (Hammen, 2005). The issues associated with conceptualizing stressful life events will first be outlined before a discussion of research pertaining to their association with child and adolescent depression symptoms.

Conceptualizing stressful life events

Stressful life events can be conceptualized either as 'acute' - with a distinct beginning and end point or 'chronic' - lasting for 12 months or more (McGonagle & Kessler, 1990). In addition, life events can be described either as 'independent' - outside of the individual's influence or 'dependent' - arising in part due to characteristics of the individual (Brown & Harris, 1978). Acute, chronic, independent and dependent life events have all been associated with depression and depression symptoms (Hammen 2005). However, there is considerable variation according to the type of stress exposure examined. For example whereas the majority of early work on stress and depression focused on acute stressors, it has now been shown that exposure to ongoing chronic stressors is more strongly predictive (Hammen, et al., 2010). However it is often difficult to distinguish acute from chronic stress. For example stressors involving a discrete event such as parental divorce may be conceptualized as acute, however, if the effects of such stressors persist over time, they may be chronic in impact. Brown & Harris (2008) therefore suggest that the effects of multiple acute stressful life events may be better conceptualized as chronic stress exposure.

There is also evidence that chronic stressors experienced in childhood such as maltreatment are predictive of future acute events (Hazel, Hammen, Brennan, & Najman, 2008). The observed effects of acute stressors may therefore mask underlying chronic stress exposure (Hammen et al., 2010).

Dependent life events have been found to be more strongly associated with depression than independent life events (Kendler et al., 1999; Silberg et al., 2001). For example, Kendler et al., (1999) found an association between both independent and dependent life events and major depression in a sample of adult female twins. However, when the severity of the event was controlled, dependent events were more strongly associated with onset than independent events. This is related to the issue of rGE; individuals do not experience stressful life events at random but vary in their tendency to select themselves into high-risk environments and such a tendency may be under genetic influence. In support of the contribution of rGE, Kendler, Karkowski, & Prescott (1999) found that part of the association between stressful life events and major depression was mediated by genetic factors but also involved a significant environmental component. Finally, it is important to consider that predictors of first and later episodes of major depression are likely to be different (Daley, Hammen, & Rao, 2000). Most studies of clinical depression find support for higher rates of stressful life events before first onset compared to recurrence and that the association between stressful life events and major depression declines in magnitude as the number of previous depression episodes increases (Kendler, Thornton, & Gardner, 2000). This may be due to a kindling effect wherein following first onset, subsequent depression episodes occur with less provocation possibly due to neurobiological mechanisms (Kendler, Thornton, & Gardner, 2001). Kendler, Thornton, & Gardner, (2001) have tested the kindling hypothesis in a sample of adult female twins and found that it applies more to those at low genetic risk who evidenced a stronger association between stressful life events and major depression

than those at high genetic risk who were likely to be already predisposed to depression regardless of stress exposure.

Stressful life events and child and adolescent depression

One of the earliest studies in this area (Goodyer et al., 1985) found that adolescents with depression were 3-6 times more likely than non-depressed controls to have experienced a stressful life event during the 3 months preceding onset of the depression episode (life events included those related to family and marriage, accident, illness, school and separations or additions to children's social network) and this finding has been replicated in subsequent studies. Mayer et al., (2009) found that a clinic-based sample of children and adolescents with major depression had experienced twice the number of stressful life events (4 event clusters; parental health, bereavement, sociodemographic factors and intrafamilial factors such as family arguments and parental separation) than a non-depressed control group with a large number occurring before age 7-9 years. Stressful life events have also been shown to predict depression in a range of community (Lewinsohn, Rohde, & Gau, 2003; Monroe, Rohde, Seeley, & Lewinsohn, 1999) and genetically sensitive (Kendler, Karkowski, & Prescott, 1999; Kendler, Thornton, & Gardner, 2001; Silberg, et al., 1999) studies.

Genetically sensitive studies have found that genetic liability to depression overlaps with genetic liability to experiencing stressful life events (Kendler & Karkowski-Shuman, 1997; Silberg et al, 1999) but have also demonstrated a direct environmental component to the association, suggesting that stress exposure is a likely causal risk factor for the development of depression (Kendler & Gardner, 2010; Kendler, Gardner, Neale, & Prescott, 2001; Kendler, Karkowski, Corey, Prescott, & Neale, 1999; Thapar, Harold, & McGuffin, 1998). Parental loss in childhood and

adolescence has also been found to predict depression (Cerel, Fristad, Verducci, Weller, & Weller, 2006; Dowdney, 2000) although many studies in this area have used relatively small sample sizes and the evidence that this risk factor is causal is weak (Gersten, Beals, & Kallgren, 1991; Weller, Weller, Fristad, & Bowes, 1991). Whilst studies have found that the rate of depression within a year following the death of a parent is approximately 30% (Brent & Weersing, 2007), there is much greater heterogeneity in response than generally found for exposure to physical and sexual abuse and parental loss has also been found to be a less consistent and specific risk factor for depression. For example, diagnosable depression in bereaved children has been found to occur mostly in those already at increased risk due to alternative factors such as personal or family history of depression (Cerel, et al., 2006). In addition, it may be the case that the effects of exposure to chronic stressful life events are mediated through more proximal processes such as the parent-child relationship (Hammen, 2004).

In examining the association between stress exposure and depression, it is important to consider the potentiality of a reverse causal relationship. Depressed individuals or individuals with depression symptoms may experience increased negative life events due to the nature of their symptoms (Hankin & Abramson, 2001, 2002; Harkness, Lumley, & Truss, 2008; Kercher & Rapee, 2009; Liu & Alloy, 2010). The experience of such stressors then predicts depression onset or recurrence forming a maladaptive cycle. This is referred to as the 'stress generation hypothesis' (Hammen, 1991) and postulates that previous depression episodes result in the generation of interpersonal, dependent, stressful life events via maladaptive personality characteristics, behaviour and psychosocial impairment. There is some evidence that stress generation in response to depression is most pronounced for first onsets of clinical depression as opposed to recurrent episodes and is exacerbated by prior history of chronic stress exposure. For example, Harkness et al., (2008) found

significantly higher rates of interpersonal events in the 3-month period immediately following depression onset compared with the 3-month period preceding depression onset in adolescents with a history of childhood maltreatment. In a sample of depressed adolescents who had not experienced childhood maltreatment, the rate of life events remained constant suggesting that stress generation is potentiated by early developmental risk factors (Harkness et al., 2008). This may because individuals who have experienced childhood abuse may already be compromised in terms of their interpersonal functioning and may also lack certain coping skills necessary to deal effectively with the challenges associated with depression (Harkness et al., 2008). In the study by Harkness et al., (2008) the generation of interpersonal life events only occurred in adolescents experiencing the first onset of depression.

The stress generation hypothesis is also linked to rGE which suggests that certain individuals are likely to select themselves into stress inducing environments due in part, to the influence of genetic factors (Kendler et al., 2001; Silberg et al., 1999). A recent study found mixed support for the stress generation hypothesis. Pettit et al., (2010) investigated 1-year reciprocal paths between depression symptoms and two stress constructs: minor hassles and major life events. Significant cross-lagged paths were found from minor hassles at Time 1 to depression symptoms 1-year later but the opposite pathway wherein depression symptoms predicted minor hassles was not significant. This provided support for stress exposure but not stress generation and is consistent with a study by Carter et al., (2006) which found that the association between depression symptoms and school based hassles was best conceptualized according to a stress exposure model. In contrast, major life events were not found to predict depression symptoms a year later, however, there was modest support for depression symptoms predicting major life events. The difference in cross-lagged effects according to the stress construct examined may be attributed

to the persistent and ongoing nature of minor hassles which are likely to be more proximally related to depression symptoms over a 1-year period than major life events which occur less frequently (Pettit et al., 2010). In addition, it is possible that depression symptoms led to an increase in risk taking behaviour which resulted in the subsequent occurrence of life events but not daily hassles (Pettit et al., 2010).

Research therefore suggests that stressful life events have a robust and consistent association with the development of child and adolescent depression but heterogeneity in the definition, experience and response to such events are important factors to consider when assessing the precise nature of risk effects. Research has suggested that the type of stressor experienced is important in triggering a depression episode (Hammen, 2009) with stronger associations observed for interpersonal stress involving disruptions to close relationships and social networks, especially for females (Hammen, 2004). This implicates the family environment as particularly important for the development of child and adolescent depression and much research has been conducted in this area which will now be described.

The importance of the family environment

The family environment has been extensively studied in relation to child and adolescent psychopathology and is recognised as an important context for child development (Cummings & Davies, 1996; Downey & Coyne, 1990). In addition to child maltreatment and abuse, a range of family factors which involve more normative variations in functioning such as inter-parental conflict, parent-child conflict/negativity and parent depression symptoms have been implicated in the aetiology of child and adolescent depression symptoms (Kaslow, Deering, & Racusin, 1994; Sheeber, Hops, & Davis, 2001; Weich, et al., 2009). The parent-child relationship has been identified as particularly salient for child development

(Sheeber, et al., 2001) and has consistently been associated with child and adolescent depression. A recent meta-analysis of 45 studies identified differential associations for various parent-child dimensions with parent-child hostility - defined as high levels of aversiveness, negativity and conflict, emerging as most strongly associated with child and adolescent depression symptoms (McLeod et al., 2007). However measures of parent-child hostility have not been extensively researched in longitudinal studies and whether this represents a predictive environmental risk factor for child and adolescent depression symptoms has not been tested within a genetically sensitive framework.

A related but distinct aspect of the family environment which has emerged as a highly consistent risk factor for child and adolescent depression is the presence of parent depression (Weissman, Wickramaratne, et al., 2006). Links with offspring depression have been established for both maternal (Bureau, Easterbrooks, & Lyons-Ruth, 2009; Weissman, Pilowsky, et al., 2006) and paternal depression (Klein, Lewinsohn, Rohde, Seeley, & Olino, 2005; Lieb, Isensee, Hofler, Pfister, & Wittchen, 2002; Ramchandani, Stein, Evans, & O'Connor, 2005) but stronger and more consistent evidence has been found in support of the former (Klein, et al., 2005; Tully, et al., 2008). The role of paternal depression is however becoming more widely considered (Brennan, Hammen, Katz, & Brocque, 2002). As is the case for parent-child relationship factors, whether parent depression confers effects via a predictive environmental risk process remains unclear due to the potential presence of reverse causation and the possible role of unmeasured genetic and environmental confounds. I will now describe evidence pertaining to the direction of effects and the role of genetic factors in the association between 1) the parent-child relationship and child and adolescent depression and 2) parent depression and child and adolescent depression symptoms.

The parent-child relationship and depression: what is the direction of effects?

The potential presence of reverse causation and bidirectional effects in the association between family factors and child development is an important conceptual and methodological issue (Pardini, 2008; Pettit & Arsiwalla, 2008) and has been recognised for some time (Bell, 1968; Scarr & McCartney, 1983). Prior to a now widely cited re-appraisal of existing research in this area by Bell (1968), cross-sectional associations between family factors and child behaviour were largely assumed to represent the effects of parental behaviour on children (Pardini, 2008). However this is now understood to be an oversimplification and an inaccurate representation of the dynamic nature of family interactions. According to bidirectional models of effect, children are not passive recipients of parental behaviour, they actively influence the family environment and shape the behaviour of individuals around them (Pettit, & Arsiwalla, 2008). For example, antisocial and aggressive behaviour exhibited by children may elicit hostility and rejection from parents which in turn reinforces the negative behaviour in the child, resulting in a continuous and reciprocal pattern of effects (Patterson et al., 1989). Testing the direction of associations is therefore important to establishing environmental effects. In line with a reverse causation model, observed associations may in fact be driven solely by child effects in which case the parenting behaviour is not a true precipitating risk factor.

The direction of effects cannot be ascertained through the use of cross-sectional data which represents a single snap-shot of associations as they exist at an individual time point (Pardini, 2008). In order to establish the direction of effects between variables, longitudinal data comprising measurements collected over multiple time points used in conjunction with a range of appropriate statistical techniques are

required. The presence of a bidirectional or reciprocal model of effects in the association between family factors and child development is accepted as the likelihood due to the interactive and dynamic nature of family relationships (Pardini, 2008). However, little is understood about the conditions under which bidirectional versus unidirectional effects are more or less likely to be detected and the underlying processes which may account for effects. For example, little is known about whether bidirectional models are more relevant to certain dimensions of the parent-child relationship or certain child outcomes and also, whether the likelihood of child versus parent effects is influenced by potential moderating factors such as child and parent gender and child age and/or developmental stage. A corpus of research has examined associations between the parent-child relationship and child and adolescent depression using a wide range of research designs and statistical methods. However, a large amount of research has utilised cross-sectional data (Restifo & Bogels, 2009; Sheeber, et al., 2001) so the extent to which observed associations can be said to represent predictive environmental risk processes is unclear.

Several prospective longitudinal studies have been conducted to examine the direction of the association between parent-child relationship factors and child and adolescent depression and depression symptoms and report a mix of findings which suggest the presence of both bidirectional and unidirectional effects. Several studies report evidence for reciprocal models of influence. In a 6-year longitudinal study of 7-12 year old girls, Hipwell et al., (2008) tested reciprocal effects between parent ratings of parent-child warmth and harsh punishment and child depression symptoms assessed using children's self-reports. Both parental warmth and harsh punishment predicted girls' depressed mood after controlling for age and single parent status. In addition, both reverse pathways representing the effects of child depression symptoms on parent-child warmth and harsh punishment were significant. However

when the effects of race and poverty were taken into account, parent-child harsh punishment no longer predicted child depression symptoms so reciprocal effects were only reported for parent-child warmth. This study therefore suggests that parent-child warmth is a more robust and proximal predictor of child depression symptoms than parent-child harsh punishment. The effects of child depression symptoms on harsh punishment remained significant suggesting that the parenting effect was weaker than the child effect. Since effects varied according to whether parent-child warmth versus harsh punishment was examined, this study demonstrates heterogeneity according to the particular aspect of the parent-child relationship examined. As the authors note, effect sizes in this study were small and models testing the effects of parenting variables accounted for relatively little variance in child depression symptoms. In addition, the sample consisted only of girls. However the use of cross-rater reports and robust statistical modelling techniques constitute significant advantages and add to the reliability of findings.

In contrast to the study by Hipwell et al., (2008) which focused on specific aspects of the parent-child relationship, Branje et al., (2010) examined overall level of perceived parent-child relationship quality - a composite measure based on assessments of communication, trust and alienation. This was a 4-wave study of adolescents aged 10-14 years at wave one and 13-17 years at wave 4. The study utilised adolescent reports of depression symptoms and adolescent perceptions of the parent-child relationship so the potentiality for shared method variance was a limitation. However Branje et al., (2010) tested the roles of both parent and child gender which was a significant strength as few studies have tested associations separately for mothers and fathers and sons and daughters. Similar to the findings by Hipwell and colleagues, reciprocal models were reported for both mother and father parent-child relationship quality. For mothers, reciprocal paths were found consistently across child gender. However for fathers, perceived parent-child relationship quality was

related to depression symptoms only for boys. The non-significant finding for girls contrasts with prior research suggesting that girls are more sensitive to the depressogenic effects of exposure to family based interpersonal stressors (Hammen, 2004). The significant finding for boys may be due to greater identification and affiliation between fathers and sons. The authors also speculate that girls may have closer relationships with their mothers and therefore be less amenable to paternal influences than boys (Branje et al., 2010).

Hale et al., (2008) examined perceived parental rejection and child and adolescent depression symptoms in a 4-wave longitudinal study (children and adolescents were aged 10-14 years at Time 1) and also tested the effects of child and adolescent gender. At the first two waves, perceived parental rejection predicted child and adolescent depression symptoms and vice versa supporting a bidirectional model. However at waves 3 and 4, child and adolescent depression predicted parental rejection but not vice versa suggesting that as adolescence progresses, parental rejection becomes less important to the aetiology of depression symptoms. However this study simultaneously examined adolescent depression symptoms and aggressive behaviour. Parental rejection predicted aggressive behaviour at all four waves so it may be that later in adolescence, adolescents are more likely to respond to parental rejection with aggressive behaviour than depression symptoms. Results were similar across all waves for boys and girls, however between waves 3 and 4, depression symptoms predicted perceived parental rejection only for girls. This may be to do with the fact that girls scored significantly higher on depression symptoms than boys at waves 3 and 4. Although this study tested the role of child gender, they did not explore the potential influence of parent gender. This would have provided an interesting comparison to the results of Branje and colleagues who used a similar parent-child construct but examined both parent and child gender.

Unidirectional parent-effects models have also been reported in relation to the parent-child relationship and child and adolescent depression symptoms. In a 2-year longitudinal study of 12-year old children, Chen et al., (2004) found that perceptions of parental warmth predicted emotional problems but not vice versa. This is in contrast to the study by Hipwell and colleagues which reports a bidirectional model for the association between parent-child warmth and child depression symptoms. The discrepancy in these results could be due to the fact that Chen et al., (2004) used child reports of parent-child warmth whereas Hipwell et al., (2008) used parent reports. It could also be accounted for by population and cultural differences since Chen et al., (2004) used a wholly Chinese sample. In a sample of 11-15 year old girls, Stice et al., (2004) found that deficits in perceived parental support were related to increases in depression symptoms and also, first onset major depression 2 years later but not vice versa. Stice and colleagues examined diagnoses as well as symptoms which was an advantage since the majority of studies have focused on symptoms only. Focusing on more general measures of the overall family climate rather than the parent-child relationship specifically, Sheeber et al., (1997) found that family conflict and support predicted adolescent depression symptoms at 1-year follow-up but the reverse pathway was not significant. The same pattern of results was found for boys and girls. Rueter, Scaramella, Wallace & Conger, (1999) also report a unidirectional parent effects model and like Stice et al., (2004), examined depression diagnoses in addition to symptoms: increases in parent-reported parent-adolescent disagreements from age 12-13 years to age 14-15 years predicted adolescent reported internalising symptoms which in turn predicted first onset depressive disorder at age 19 years. The reverse pathway from internalising symptoms to increased parent-adolescent disagreements was not significant.

There have also been studies which find support for unidirectional child effects models: At the last two waves of the 4 wave study by Hale et al., (2008), adolescent

effects only were found for parental rejection and depression symptoms (for girls not boys). In a 2-year longitudinal study of adolescents aged 12-19 years, Albrecht et al., (2004) found that adolescent perceptions of parental psychological control at the first time point did not predict adolescent internalising symptoms at the second time point. However, adolescent internalising symptoms predicted their perceptions of parental psychological control 2 years later. A main limitation of this study was reliance on adolescent reports of both internalising behaviour and parental control. It is also necessary to note that internalising rather than depression symptoms were considered. However there is considerable overlap between these constructs and internalising symptoms often precede depression (Zahn-Waxler, Klimes-Dougan, & Slattery, 2000).

Variation in findings regarding the presence of bidirectional versus unidirectional effects may be due to a variety of factors including the specific aspect of the parent-child relationship assessed, the definition of outcome measures, child and adolescent age, parent and child gender and the reporters used. The majority of studies assessing the direction of the association between parent-child relationship factors and child and adolescent depression symptoms appear to support a bidirectional model of effects. This is important because, although child effects have been found to be significant which is an interesting finding in its own right, parent effects are also consistently reported. This suggests that the parent-child relationship is a potentially predictive risk factor for child and adolescent depression symptoms. Some studies report unidirectional child effects, however, these appear to be less numerous. In addition, results have been found to vary according to both parent and child gender. However, previous concerns that associations between family relationships and child depression symptoms may be driven entirely by child effects have not been empirically substantiated. This provides preliminary evidence for the potential role of

the parent-child relationship as an environmental risk factor that may contribute to the aetiological pathways to child and adolescent depression.

The parent-child relationship and depression: what is the contribution of genetic and environmental factors?

There is a second important issue to consider when examining environmental risk effects and that is the possibility of association arising because of genetic links between risk and outcome variables. To test whether the association between family relations and child depression is accounted for, in part, through a genetically influenced pathway, genetically sensitive designs are required. Such designs allow environmental pathways to be estimated independently of genetic factors. A range of genetically sensitive designs are available and have been described in Chapter 2. Bivariate twin designs have been used to test whether the association between family environmental factors and child depression is accounted for through a genetic and/or an environmental pathway.

Pike, McGuire, Hetherington, Reiss, and Plomin (1996) found that the non-shared environment (environmental factors that contribute to dissimilarity between twins) significantly contributed to the association between composite (parent and child) ratings of maternal but not paternal negativity and depression symptoms in a sample of twin and sibling pairs aged 10-18 years. There was also a substantial genetic contribution to the association for both maternal and paternal negativity with genetic factors accounting for approximately 70% of the association. The cross-sectional nature of this study precluded examination of the direction of effects. Also using a cross-sectional sample and focusing on anxiety rather than depression symptoms, Eley, Napolitano, Lau & Gregory (2010) found that in 8-year old twins, the association between maternal control and self-rated child anxiety problems was

accounted for by genetic and non-shared environmental effects. Although it examined anxiety symptoms, this study focused on a younger sample of children wherein anxiety problems are often found to precede depression symptoms (Eley & Stevenson, 1999). To date there are few studies which have simultaneously tested both the contribution of genetic factors and the direction of effects. Using longitudinal twin data (adolescent twins aged 10-18 years), Neiderhiser, Reiss, Hetherington, and Plomin (1999) also found that genetic factors accounted for a substantial proportion of the association between composite ratings of maternal and paternal conflict-negativity and offspring depression symptoms (the genetic parameter dropped below significance for maternal conflict-negativity and depression but still accounted for 70% of the association which concurred closely with the findings by Pike and colleagues). The non-shared environment contributed approximately 30% to the association for both maternal and paternal conflict-negativity. Whilst a bidirectional model of effects was supported for paternal conflict-negativity and depression symptoms, a unidirectional model in which parental negativity predicted increased child depression symptoms but not vice versa was the most parsimonious for maternal conflict-negativity suggesting that mothers are less likely to respond with increased levels of conflict and negativity in response to child depression symptoms than fathers. Although longitudinal and genetically sensitive, this study did not explore the role of child and adolescent gender which has been found to be important in non-genetic as well as genetically sensitive studies.

Only one study to date has examined the role of genetic and environmental factors in the association between family environment and adolescent (aged 10-20 years) depression symptoms separately for sons and daughters (Jacobson & Rowe, 1999). The best fitting model was one which allowed parameters to vary by adolescent gender: Genetic factors contributed more substantially to the association between family connectedness and depressed mood for females which is concordant with

evidence suggesting that interpersonal stressors are more important for girls whilst the influence of the non-shared environment was greater for males. However this study used a very global measure of general family functioning so did not specifically examine the parent-child relationship and relied on adolescent self-reports of both their depression symptoms and the family environment. In addition, this study did not explore the role of parent gender which the previous studies have highlighted as important. Genetically sensitive studies on the link between family relationships and child and adolescent depression therefore suggest that genetic factors contribute somewhat substantially to the association. This emphasizes the importance of using genetically sensitive designs to test environmental risk effects. However genetically sensitive studies also suggest the presence of significant environmentally mediated links between negative family relationships and child and adolescent depression symptoms.

Parent depression and child and adolescent depression

In addition to a range of parent-child relationship factors, an aspect of the family environment which has been consistently associated with the development of child and adolescent depression symptoms is parent depression. A range of top-down family studies report that children of depressed parents are 2-3 times more likely than offspring of non-depressed controls to exhibit depressive disorder and elevated levels of depression symptoms along with a range of other types of symptomatology including conduct and behavioural problems (Weissman et al., 1997; Weissman et al., 1999; Beardslee et al., 1998). Both maternal and paternal depression have been associated with child depression and depression symptoms, however, the role of maternal depression has been paid most attention due to higher depression prevalence rates among women and the predominant role played by mothers in child rearing (Ramchandani, Stein, Evans & O'Connor, 2005). This does not dismiss the

importance of paternal depression which is being increasingly recognised as a risk factor for child and adolescent depression (Ramchandani et al., 2005; Klein et al., 2005; Lieb et al., 2002).

A large body of literature examining outcomes in children of depressed parents has accumulated. These studies have examined the association between parent depression and child outcomes both cross-sectionally and longitudinally (Weissman, Wolk, Goldstein, et al., 1999), the effects of severity, chronicity and timing (Hammen & Brennan, 2003) and have also tested potential mediating mechanisms which may account for the association between parent and child depression (Foster, Garber, & Durlak, 2008; Pilowsky et al., 2008). It has been found that the severity of maternal depression symptoms confers greater risk to children than either the chronicity of episodes or the timing; children exposed to 1-2 months of severe, diagnosed maternal depression have been found to display increased symptoms of depression whereas a more prolonged period of exposure to milder maternal depression symptoms (greater than 12 months) was required before the onset of child symptoms (Hammen & Brennan, 2003). However the processes through which parental depression confers its risk effect on children remain unclear as does the question of whether it is a truly precipitating risk factor which confers risk via environmental mechanisms.

Parent depression and child and adolescent depression: what is the direction of effects?

In contrast to longitudinal research examining the direction of effects in the association between parent-child relationship factors and child and adolescent depression, comparatively little research has been conducted testing the direction of the association between parent and child depression. However, in addition to

maternal depression conferring risk for children, it is also likely that negative symptoms displayed by children will increase risk for or exacerbate existing symptoms of depression in parents. Using 3-wave longitudinal data, Elgar et al., (2003) examined the direction of effects in the association between maternal depression symptoms and child adjustment problems (aggression, hyperactivity and emotional problems). Results showed that maternal depression symptoms tended to coincide with or precede child emotional problems but changed as a function of exposure to child aggression and hyperactivity. This concurs with a study by Gross et al., (2009) which found that disruptive behaviour by toddlers predicted more chronic and elevated trajectories of maternal depression over the course of 8 years in addition to maternal depression predicting disruptive child behaviour. Consistent evidence exists for bidirectional models of effect between maternal depression and child symptoms which involve disruptive behaviour such as externalising, hyperactivity and aggression (Gross, et al., 2009; Gross, Shaw, & Moilanen, 2008), however, the evidence for bidirectional effects involving child emotional problems such as depression is less convincing. Ge et al., (1995) found that symptoms of parent and adolescent distress were reciprocally related over time whilst in a longitudinal treatment study, Pilowsky et al., (2009) found that decreases in the severity of maternal depression tended to precede symptom and functional improvements in children - decreases in maternal depression scores were not associated with decreases in child symptoms during the previous 3 months making it unlikely that mothers in this study showed signs of remission following improvements in their children's symptoms.

Parent depression and child and adolescent depression: what is the contribution of genetic and environmental factors?

In understanding the intergenerational link between parent and child depression, associations could in part be due to the influence of reverse causation and could also arise through the effects of common genetic factors which result in the development of depression in both generations. Few studies have used genetic designs to test the extent of heritable versus environmental influences on the intergenerational transmission of depression symptoms. A range of non-genetically sensitive treatment studies (Foster et al., 2008; Pilowsky, et al., 2008; Weissman, Pilowsky, et al., 2006) report that improvements in maternal depression symptoms resulted in a subsequent amelioration of child and adolescent (children/adolescents aged 7-17 years) symptoms and diagnoses suggesting the influence of a direct environmental effect: Remission of maternal depression symptoms over 3 months was significantly associated with a reduction in children's current symptoms after controlling for child age, gender, baseline symptoms and maternal depression severity. However since this study design was not genetically sensitive, it does not provide a direct test of environmental mediation in that the possibility that mothers and children who remitted were less genetically vulnerable than mothers who did not could not be ruled out.

Two studies have used genetic designs to directly test the presence of environmental links in the intergenerational transmission of maternal depression (Silberg, et al., 2010; Tully, et al., 2008). Using an adoption design, Tully et al., (2008) report significant associations between maternal and child and adolescent (children/adolescents aged 10 – 14 years) major depression in genetically unrelated and related mother-child pairs suggesting the presence of environmental links. Since associations were observably larger (32% greater) in genetically related than unrelated mother-child pairs, the influence of both genetic and environmental risk

factors was suggested. However, the difference across relatedness groups was not significant so conclusions of a genetic contribution could not be statistically substantiated. Associations were not significant in genetically unrelated father-child pairs suggesting the presence of parent gender differences in environmental effects in that genetic rather than environmental risk factors may be more important for father to child intergenerational links.

Using a COT design (children and adolescents aged 9-17 years), Silberg et al., (2010) found that the intergenerational transmission of parental depression symptoms was due primarily to the influence of shared family environmental factors. This provided support for the study by Tully and colleagues. However, whereas the presence of a genetic contribution was suggested in the adoption study by Tully et al., (2008), Silberg et al., (2010) did not find any evidence for the role of genetic factors. A recent study of our own based on a sample of children aged 4-10 years conceived via assisted reproduction examined depression and conduct symptoms in genetically related and unrelated parent-child dyads (Harold et al., 2010). Environmental links were found between maternal and child depression but not paternal and child depression (child gender differences were not examined). This suggests that the previously reported findings of environmental mediation which used samples wherein the mean age was in adolescence also apply to younger age groups. Across different genetically sensitive designs, a range of findings therefore suggest that the intergenerational transmission of depression symptoms is likely to involve an environmentally mediated component. However, in addition to the presence of genetic confounds, in testing environmental causality it is also necessary to consider the potential role of environmental third variables. For example, exposure to adverse life events which impact upon parents and children such as bereavement, divorce or poverty may increase risk for depression in both parents and children and thereby account for observed "transmission effects." To my knowledge the role of

environmental confounds has not been examined in a genetically sensitive study of the intergenerational transmission of depression symptoms.

The role of parent and child gender

A common theme which has emerged across longitudinal, and genetically sensitive studies on the parent-child relationship and parent depression is the importance of testing associations separately for both parent and child gender. For example, Branje et al., (2010) found that relationship quality with mothers predicted depression symptoms for boys and girls but that relationship quality with fathers only predicted depression symptoms in boys. In contrast, Hale et al., (2008) found that depression symptoms predicted parental rejection only for girls. The genetic study by Pike et al., (1996) also reports differences according to parent gender - environmental effects of parental negativity were found for the mother-adolescent relationship only. With regard to parent depression, two genetically sensitive studies find environmental effects only for maternal, not paternal depression (Harold et al., 2010; Tully et al., 2008) and this coincides with a range of non-genetic studies which also report stronger associations for maternal depression and child depression (Klein et al., 2005; Lieb et al., 2002).

A range of studies have reported that the association between parent depression and child depression is stronger for girls than boys (Cortes, Fleming, Catalano, & Brown, 2006; Davies & Windle, 1997; Fergusson, Horwood, & Lynskey, 1995; Jenkins & Curwen, 2008). Differential effects have therefore been found according to both parent and child gender in response to parent-child relationship factors and parent depression. This highlights the importance of considering mothers and fathers and sons and daughters separately in order to gain a full understanding of precisely how family factors confer environmental risk effects (Raley & Bianchi, 2006; Russell &

Saebel, 1997). Few studies have considered the role of both parent and child and adolescent gender in assessing the effects of family factors on depression symptoms and even fewer have done so whilst simultaneously testing the direction of effects and the role of genetic factors. This is important because gender differences which have emerged across studies are inconsistent and require substantiation but may be important sources of variation in environmental effects. The role of parent and child gender therefore constitutes an important aim of this thesis. Identifying factors which affect environmental risk effects is necessary for establishing what environmental variables have what effects for which individuals and is therefore important to developing interventions which can be targeted at specific subgroups (Rutter, 2009). This is an important extension to studies which just test the presence versus absence of environmental risk effects as the aetiology of depression symptoms is known to be due to both environmental and genetic effects and to GxE. What is less clear is how genetic and environmental risk mechanisms operate in conjunction along with the specific environmental and genetic risk factors which confer risk, why and for whom.

Chapter Summary

Negative, conflictual and hostile parent-child relationships and parent depression are two aspects of the family environment which have evidenced consistent associations with child and adolescent depression symptoms. However, despite well replicated associations, the extent to which risk effects are due to environmental processes remains unclear and is important to advancing existing research on the aetiology of depression and informing the design of preventive interventions. Key issues when testing predictive environmental links are 1) the direction of effects 2) the potential role of genetic factors and 3) parent and child gender. The next chapter outlines in detail the aims of the present thesis with regard to addressing these gaps in the literature and testing whether specific aspects of the family environment – the parent-

child relationship and parent depression, confer predictive environmental risk effects on child and adolescent depression.

Chapter 4

The Present Thesis

The previous chapters have outlined the complex nature of the aetiology of depression and have described existing research on genetic influences, psychosocial risk factors and the role of gene-environment interplay. Although it is established that both environmental and heritable risk factors contribute to depression, little is currently known about specific psychosocial and genetic risk factors and the processes underlying their effects. Prior research has demonstrated associations of the parent-child relationship and parent depression with child and adolescent depression symptoms, however, the extent to which links are accounted for through predictive environmental effects remains unclear as does the extent to which these environmental exposures interact with specific genetic factors (GxE). In this thesis I aim to address these issues by testing whether exposure to two specific family risk factors for child and adolescent depression - parent-child hostility and parental depression, confer predictive environmental risk effects and whether these risk factors interact with specific gene variants involved in the regulation of the stress response. I also explore whether environmental risk effects and GxE varies according to parent and child gender as this is a potentially important moderator of effects that has not been extensively studied within a genetically sensitive framework.

A problem with addressing these issues is the limited availability of experimental methods for testing causal risk effects. An important methodological strategy is therefore to use different research designs with complementary strengths to attempt to approximate cause and effect inferences (van Os et al., 2010; Kendler et al., 2010; Rutter et al., 2001). When findings are replicated across different designs, this increases validity and adds strength to conclusions of potential cause and effect processes (Rutter, 2007b; Rutter, Pickles, Murray, & Eaves, 2001; van Os, et al.,

2010). This thesis will therefore employ a number of different yet complementary research designs to test environmental risk effects and GxE. The present chapter outlines the aims of this thesis and describes the samples, research designs, and statistical techniques used to address the key research questions.

Aims

The first aim of this thesis was to test whether parent-child hostility and parent depression confer risk for child and adolescent depression symptoms through an environmental pathway after testing the direction of effects and the potential role of genetic and environmental confounds. Since depression is due to both environmental and genetic risk factors but genetic main effects have not been demonstrated, the role of GxE has attained importance. The second aim was therefore to test whether, in addition to conferring direct environmental effects, these family risk factors interact with specific gene variants to predict child and adolescent depression symptoms. Finally, establishing environmental and genetic risk effects is an important first step towards addressing these gaps in the literature, however, another key goal is to identify for whom and under what circumstances such effects are most pertinent (Rutter et al., 2001). This is relevant to the development of targeted preventive and treatment strategies. The third aim was therefore to examine the role of both parent and child gender since which may be important moderators of environmental and genetic effects but have not been extensively studied using quasi-experimental designs. The 4 different research designs used to address these aims were a longitudinal community study, a cross-sectional twin study, a novel genetic design based on a sample of children conceived via IVF and a high-risk sample of children exposed to recurrent parent depression.

The thesis is divided into three empirical chapters which each comprise separate studies addressing different questions relating to the role of family environmental risk effects in the aetiological pathways to child and adolescent depression symptoms. The chapters are stand-alone papers that are either currently in press or under review.

The specific aims of each study were as follows:

Study 1: The first aim of study 1 was to use longitudinal data to test the direction of effects in the association between a specific aspect of the parent-child relationship - parent-child hostility and child and adolescent depression symptoms, and genetically sensitive twin data to test whether associations were accounted for by environmental processes after the influence of genetic factors had been estimated. The second aim was to examine the role of both parent and child gender: associations were therefore tested separately for mother and father hostility and for sons and daughters.

Study 2: The aim of study 2 was to use an alternative genetically sensitive design based on a sample of children conceived via IVF to investigate environmental links between a different family risk factor - parent depression symptoms, and child depression symptoms. Environmental links were examined after testing the potential contribution of genetic factors, the role of exposure to shared adverse life experiences and the effects of child age and gender.

Study 3: The aim of study 3 was to investigate whether the association between 1) parent-child hostility and child and adolescent depression symptoms and 2) recurrent parent depression and child and adolescent depression symptoms was moderated by specific gene variants involved in the regulation of the HPA axis. A high-risk sample of children and adolescents exposed to parental depression and a control

sample drawn from the twin study, both with available molecular genetic data, were used to test GxE.

Methods

The details of the samples used in each study will now be described. Details of the measures used, specific sample specifications and analyses are provided in later chapters that are a series of papers which are either in press or under review.

Samples

Sample 1: The South Wales Family Study (SWFS): a longitudinal design

The South Wales Family Study (SWFS) was a three-wave longitudinal community study which recruited families (parents and one participating child) from across South Wales for a study focused primarily on experiences of family life and psychosocial development. Families were recruited through 12 secondary schools in South Wales which were selected on the basis of their economic and social characteristics to obtain a demographically representative range. Following initial contact with the schools, parents were sent a letter inviting them to participate in a research project investigating the link between family life and child development. Parents were then given further information at a scheduled parent-teacher evening and were sent an additional follow-up letter and consent form. The children whose parents had consented to participate completed a questionnaire during the school day under the supervision of a visiting researcher. Parent questionnaire packages which included instructions for completion and a pre-paid envelope were mailed to the family home. Mothers and fathers were sent separate packages and were asked to participate by completing the questionnaires independently. The three waves of data collection were conducted a year apart: Time 1 (1999); Time 2 (2000) and Time 3 (2001). A range of measures assessing the family environment and psychosocial functioning

were administered at each time point and were consistent across waves. Children and adolescents were asked to report on the family environment, the parent-child relationship, family interaction, the school environment, behaviour, mood and substance use. Parents were asked to report on the family environment, the inter-parental relationship, the parent-child relationship, their mood and behaviour, and their child's mood and behaviour. During the three-year period, contact with families was maintained through Christmas cards mailed to family homes with an accompanying letter thanking them for their participation. Contact details for the principal investigator, Professor Gordon Harold, were provided at each point of contact and a summary booklet outlining key research findings was distributed to all families upon completion of the study. 652 children and their families were initially approached and 543 families consented to participate representing an 83% response rate. 543 children aged 11 – 13 years and 387 parents (at least one parent from a family) initially took part. A sub-sample was selected for use in this thesis which is described in Chapter 5 (Paper 1).

Sample 2: Cardiff Study of All Wales and North West of England Twins (CaStANET): a genetically informative design

The CaStANET is based on a general population twin register established in 1991 which included twins born between 1976 and 1991 in the Cardiff and South Wales area. The register was expanded in 1996 to include twins born between 1976 and 1991 across the whole of Wales and in nine health districts in Greater Manchester and Lancashire. Twin births were originally identified from birth register records accessed through the National Health Service (NHS). The addresses of the twin families were obtained through health authorities and general practitioners and the families were contacted by post to ask if they would be willing to participate in a study on the development of twin pairs. The twin register contains names and addresses

for over 6000 twin families. Twins were aged 5-20 years when the register was established. All data has been collected via postal questionnaire mailed to the family homes. There have been five waves of data collection to date. Across waves, data have been collected from parents, twin pairs and teachers. A range of measures have been administered across the different waves of data collection, each of which has focused on a different overall aim (see van den Bree et al., 2007 for a list of all measures administered across waves).

This thesis is based on cross-sectional data from wave 4 when twins were aged 16 to 20 years as this was when data on a range of aspects of the family environment were collected. A range of strategies have been implemented in order to maintain contact with families. Addresses have been traced through the NHS and general practitioners prior to each mail-out and each time they are contacted, families are asked to provide change of address notifications. The zygosity of twin pairs was ascertained using parent responses to a 'twin similarity' questionnaire (Cohen, Dibble, Grawe, & Pollin, 1975; Nichols & Bilbro, 1966) which was sent to parents at waves I, 2 and 3. The twin similarity questionnaire consists of items such as 'do the twins share the same hair/eye colour' and 'are they as alike as peas in a pod?' and has been shown to correctly classify zygosity in over 90% of cases (Scourfield, Martin, Lewis & McGuffin, 1999; Thapar, Harrington, Ross & McGuffin, 2000). In a subsample of twins, zygosity has been validated using genotyped DNA markers (Payton et al., 2001). The twin sample has been found to be representative of the local population in terms of ethnicity and socioeconomic status (Thapar & McGuffin, 1996a; Thapar & McGuffin, 1996c; Scourfield, McGuffin, & Thapar, 1997).

Sample 3: The Cardiff In Vitro Fertilization (IVF) Study: a cross-generational genetically informative design.

The Cardiff IVF sample was recruited through 18 assisted reproduction clinics based in the United Kingdom and one in the United States (Thapar et al., 2007). Inclusion criteria for eligibility in the study stipulated that families had a child born between 1994 and 2002 (aged 5-9 years at the time of the study) following successful IVF treatment. It was required that the donors of gametes and also the surrogates in the case of gestational surrogacy were unrelated to either parent. Families meeting the inclusion criteria for the study were invited to participate via a package mailed from the clinic to the family home on behalf of the researchers. Packages contained a letter which explained that the clinic had been asked to contact families in their care with children aged 5-9 years and briefly outlined the aims of the research. Participants were given thank you payments if they chose to participate. Packages also contained an information letter which outlined full details of the project, information about the researchers, data confidentiality and instructions on how to participate. Questionnaire booklets containing the measures to be completed were also provided in the package. Separate questionnaires were included for mother and fathers. Data were collected from parents only due to the young age of the children at the time of assessment and because of concerns about children being unaware of the method of conception. Clinic staff recruited 888 families who provided questionnaire data; 873 questionnaires were returned from mothers and 605 questionnaires were returned from fathers. Complete family responses from mothers and fathers were received from 589 families. Data have also been collected from families at a second time point 3 years later (2009). Responses were received from 482 families (464 mothers, 173 fathers and complete mother and father responses from 155 families) representing an overall response rate of 62%.

Sample 4: Predicting and Preventing Adolescent Depression Project: high-risk sample exposed to parent depression.

This sample included 339 children (aged 9 to 17 years) each with a parent diagnosed with recurrent major depression. The Cardiff Predicting and Preventing Adolescent Depression Project is an ongoing three-wave longitudinal study which begun in 2007. One wave of data was available at the time of this thesis being undertaken. The aim of the study was to identify a specific set of variables which predicted the onset of adolescent depression in a high-risk group (the offspring of parents with recurrent depression) and could be reliably utilised in primary care facilities to assist the early identification, treatment and prevention of adolescent depression. Recruitment criteria stipulated that parents (aged 26 - 55 years) had experienced at least two episodes of DSM-IV diagnosed major depression confirmed using the Schedules for Clinical Assessments in Neuropsychiatry (SCAN) diagnostic interview and had a biological child between the ages of 9 and 17 years. Participants were excluded if the parent had a psychotic or bipolar-type diagnosis or a previous history of mania. Families were also excluded if the parent met criteria for mania or hypomania at the time of interview or if the participating child had severe learning disabilities (IQ<50). Participants were interviewed an average of 16 months apart. Data were collected from parents and children via multiple methods including semi-structured diagnostic interviews (Schedules for Clinical Assessment in Neuropsychiatry and parent and child versions of the Child and Adolescent Psychiatric Assessment), self-report questionnaire methods and cognitive assessments. Saliva and blood samples were also collected to provide DNA and cortisol data. In most cases, assessments were conducted in the family homes by a team of two interviewers, all of whom were psychology graduates. The 339 families (317 mothers and 22 fathers, mean age 42.7 years) were recruited from across the United Kingdom, mainly Wales. 265 families were recruited from general practices in South Wales, 64 were recruited from a

previous research sample that had consented to participate in future research and 10 families were volunteers.

Research designs and statistical analyses

The samples used in the present thesis were based on four different research designs. The next section of this chapter will describe each research design along with an explanation of the statistical analyses used.

1) Longitudinal design and analysis

Longitudinal designs with measures collected at multiple time points provide one important means of testing the direction of observed associations between variables which cannot be ascertained using cross-sectional data. Testing the direction of effects is necessary for establishing whether relations between variables represent potentially causal links because statistical associations could be accounted for by reverse causation or by a third unmeasured confounding variable. Important to note, whilst longitudinal designs are useful for examining reverse causation they cannot exclude the effects of unmeasured confounds. Longitudinal data drawn from the SWFS were analysed in the present thesis using Structural Equation Modelling (SEM) techniques. SEM refers to a class of statistical techniques which enable tests of hypothesized relations between variables (Kelloway, 1998). A specific form of SEM; cross-lagged panel analysis, was used in the present thesis. Cross-lagged panel analysis was developed specifically for testing the presence of bidirectional effects using longitudinal data (Figure 1). By estimating the concurrent or baseline association between variables (represented by the double headed arrow connecting the Time 1 variables), paths γ_3 and γ_4 in Figure 1 represent the unique contribution of each Time 1 variable in accounting for variation in each cross-construct Time 2 variable. Paths γ_3 and γ_4 are therefore partial regression coefficients. Cross-lagged

models also provide an estimate of the stability of the relationship between the same variables measured at different time points (paths γ_1 and γ_2 in Figure 1 respectively). By estimating cross-lagged stability, the model allows a quantification of 'within-individual' change in Time 2 outcome variables following exposure to Time 1 variables: Controlling for the stability in depression symptoms by estimating path γ_1 results in an estimate of the change in depression symptoms at Time 2 which is accounted for by the level of parent-child hostility at Time 1 and vice versa (path γ_2). The proportion of variance in each outcome variable which has been explained by the predictor variables is calculated as part of the cross-lagged panel analysis using the R^2 statistic. In terms of goodness-of-fit, the cross-lagged model can be said to perfectly describe the observed data because every pathway depicted in Figure 1 is estimated and hence the model is statistically saturated.

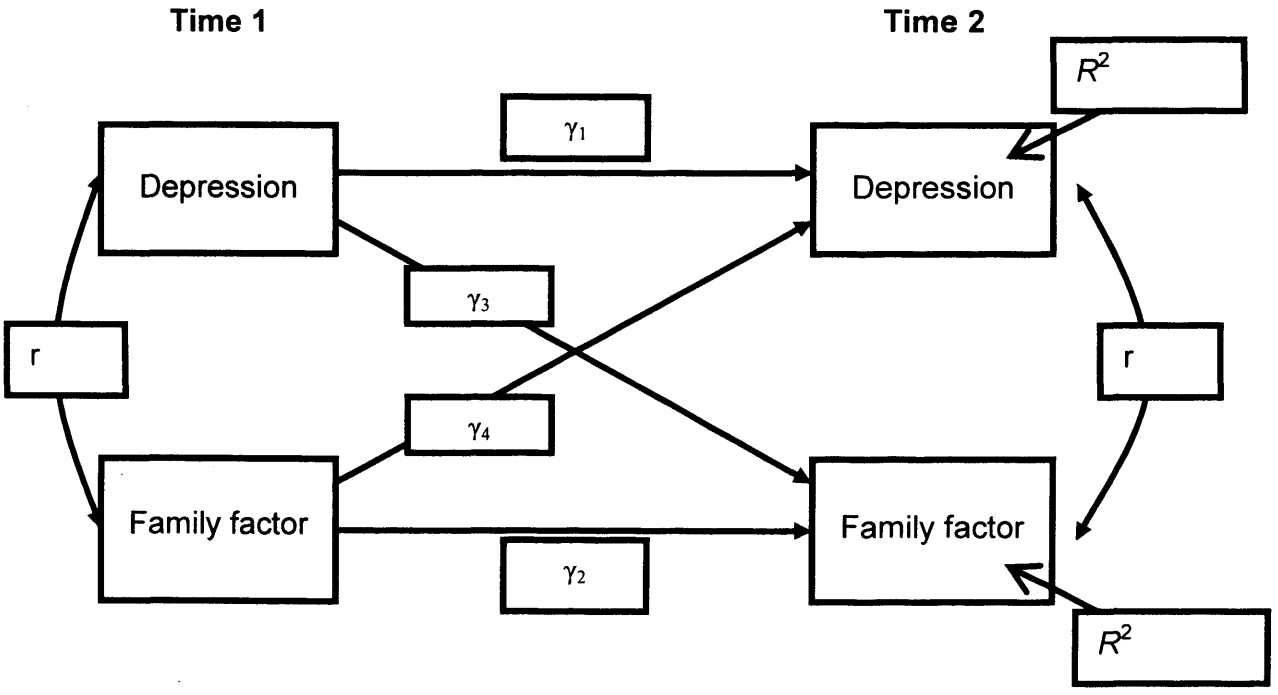

Figure 1. Cross-lagged path model assessing direction of effects

2) Twin design

The use of the twin design in behaviour genetics is made possible by the existence of two types of twin pairs which differ in their degree of genetic relatedness: monozygotic twin pairs (MZ) result from the splitting of the same fertilized egg cell and share 100% of their genetic material whereas dizygotic twin pairs (DZ) form from two individually fertilized egg cells and therefore share, on average, 50% of their genetic material. Behaviour genetic theory attributes the total variation in a trait to heritable and environmental effects. The heritability of a trait is the amount of variation which can be attributed to genetic influences and is divided into either additive or non-additive effects. Additive genetic effects (A) refer to the combined effects of multiple genetic loci. Non-additive genetic effects refer either to dominance (D) - interactions between genes at the same chromosomal loci in the genome, or to epistasis - interactions between genes at different chromosomal loci. Environmental effects are described as either shared or nonshared. The shared environmental effect (C) refers to all non-genetic influences which act to make twins in a pair similar whilst the non-shared environmental effect (E) refers to all non-genetic influences which result in differences between twins and is also referred to as the 'individual specific' environment. Measurement error is also subsumed within this estimate. The rationale underlying the twin design is as follows: If genetic factors influence the origin of a trait, excess similarity between MZ versus DZ twin pairs is expected due to their increased genetic similarity. Additive genetic influences are suggested by a DZ twin correlation which is approximately half the MZ twin correlation (because DZ twins share on average 50% of their additive genetic material) and non-additive genetic influences are indicated by a DZ twin correlation which is less than half the MZ twin correlation. Shared environmental influences are indicated by a DZ twin correlation which is greater than half the MZ twin correlation because it is assumed that the common environment influences MZ and DZ twins to the same extent

therefore increasing the similarity of DZ twins relative to MZ twins (Medland & Hatemi, 2009).

Comparing MZ and DZ twin correlations provides an initial 'broad-brush' estimate of the relative contribution of genetic and environmental factors to the origin of a measured trait. Statistical model-fitting analyses which use a combination of path analysis and SEM have been specifically tailored for the twin design to provide an extension of the basic correlational approach by allowing multivariate analyses of more than one phenotype (e.g. environmental risk factor and psychopathology), the generation of confidence intervals for each parameter estimate and tests of the effects of age and gender on twin variances and covariances. In addition, model fitting allows for 'parameter estimation' rather than simple 'parameter calculation' by quantifying how well a model describes the data (Posthuma & Boomsma, 2005). Model-fitting is therefore a more sophisticated method of providing genetic and environmental estimates of increased accuracy and is widely used in twin analyses to test complex hypotheses and to conduct bivariate or multivariate analyses. Genetic and environmental estimates are modelled as latent variables and their values are estimated using statistical model fitting procedures. Bivariate twin analysis decomposes variation between two measures (e.g. family factors and depression) into genetic, shared and non-shared environmental influences and has been used to test genetic and environmental mediation effects of environmental risk factors (Neale & Cardon, 2002). Environmental risk effects have also been tested in the twin design using MZ twin difference scores (Pike, Reiss, Hetherington, & Plomin, 1996). Since MZ twins share 100% of their genes, when differences between MZ twin in the same pair on a putative environmental risk variable are associated with differences in outcome scores, the link can be unambiguously attributed to the influence of environmental factors (Pike, Reiss, et al., 1996).

3) IVF research design

The IVF design is an alternative genetically sensitive method that permits a separation of genetic and environmental influences using the same principle as an adoption design (the separation of social and biological parentage) but in utero . There are various methods of IVF which result in varying degrees of genetic relatedness between parents and children. Children conceived via IVF may be genetically related to both parents via homologous IVF, genetically related to the mother only via sperm donation, genetically related to the father only via egg donation or genetically related to neither parent via embryo donation. Through gestational surrogacy it is possible for children to be genetically related to both parents but to have their intrauterine environment provided by a genetically unrelated woman. If an association is mediated by environmental and genetic factors then it will be observed in both genetically related and genetically unrelated parent-child pairs since both provide the social rearing environment. However since the association is mediated by genetic in addition to environmental factors, it will be stronger in the genetically related than the genetically unrelated group. If an association is genetically mediated only, it will only be observed in genetically related parent-child pairs, there will be no association between genetically unrelated parent-child pairs because no environmental transmission of risk is involved. If an association is environmentally mediated it will be observed in both genetically related and genetically unrelated parent-child pairs to a similar extent and it should not be significantly stronger in genetically-related parent-child pairs (see Table 1).

Table 1. Use of the IVF design to test environmental and genetic links between variables

	Genetically related parent-child pairs	Genetically unrelated parent-child pairs
Environmentally mediated	✓	✓
Genetically Mediated	✓	✗
Both genetically and environmentally mediated	✓✓	✓

4) GxE designs

GxE can be investigated using several different methodological approaches based on quantitative behavioural genetic methods and molecular genetic research designs. A molecular genetic design was used in the present thesis in order to test the influence of specific gene variants involved in the regulation of biological systems implicated in the aetiology of depression. GxE is assumed to be present in this design if depression symptom levels are significantly higher in individuals who possess the hypothesized risk allele and are also exposed to the environmental risk variable. The interaction term was computed as the product of the genetic and environmental risk variables and was used in a multiple linear regression analyses to predict child and adolescent depression symptoms.

In designing high quality GxE studies there are a number of methodological issues to consider. Firstly, there is the statistical debate of whether interactions within a GxE

framework should be quantified according to an additive or multiplicative mathematical model. This is important because the two methods can yield different conclusions regarding the presence or absence of an interaction. Additive models are based on the premise that in the absence of interaction, risk factors add in their effects whereas multiplicative models suggest that in the absence of interaction, risk factors multiply in their effects (Schwartz, 2006; Zammit, 2008). Models based on the additive assumption such as linear regression are employed when outcome measures are continuous whereas models based on the multiplicative assumption such as logistic regression are employed when outcome measures are categorical (Schwartz, 2006). As stated by Rutter et al., (2009), the majority of biological researchers favour the use of an additive model because this provides a better match to biological concepts.

A second issue in the methodology of GxE studies is whether a genetic main effect is required in order to statistically define the presence of an interaction: According to Risch et al., (2009), the most likely explanation for an interaction without a main effect would be a reversal in the direction of the environment-by-genotype association wherein for example, the risk of depression increases with the number of S alleles in the presence of stressful life events and decreases with the number of S alleles in the absence of stressful life events (Risch et al., 2009). However, Moffitt et al., (2005) draw attention to a paradox relevant to the question of whether a main genetic effect is required: the main drive behind GxE research is to identify why the search for genetic main effects has been relatively unsuccessful. The concept of GxE was referred to as a potential explanation for the lack of significant genetic main effects: genetic effects may not manifest in the absence of environmental risk exposure. As Moffitt et al., (2005) state, if a gene's contribution to a disorder is conditional on the environment, a genetic main effect will not be detected in the absence of the environmental influence.

A third issue in testing GxE refers to the way in which the environmental risk exposure is conceptualized, measured and assessed (Hammen et al., 2010; Monroe & Reid, 2008; Uher & McGuffin, 2010). The methods used to assess environmental risk exposure have often been overshadowed by the multiplicity of issues associated with assessing genetic risk variables (Monroe & Reid, 2008). However variation in the methods used to assess life stress for example may, in part, account for the conflicting results regarding GxE in relation to 5HTTLPR (Uher & McGuffin, 2010). Uher & McGuffin (2010) for example report a systematic relationship between the method used to assess environmental adversity and the results of studies examining interaction with 5HTTLPR: the genetic variant appears to effect objectively occurring adversity rather than self-reported stress exposure. Variation in findings according to the assessment of environmental risk is also evident in studies of GxE between childhood maltreatment and CRHR1: significant effects have been found using measures which rely on the emotional recall of traumatic experiences but not more objective assessments of the frequency of occurrence of childhood abuse (Bradley et al., 2008; Polanczyk et al., 2009). Careful consideration of the measurement of environmental risk exposure is therefore required (Hammen et al., 2010; Moffitt et al., 2005).

Despite the methodological controversies associated with GxE, it has become a major focus of depression related research and when examined using well selected environmental and genetic risk variables and according to strict research criteria (Moffitt et al., 2005), findings hold promise for progressing towards an understanding of how genetic and environmental risk factors work together to increase risk for depression.

Chapter Summary

This thesis aimed to test the presence of environmental effects and GxE in the aetiological pathways to child and adolescent depression symptoms using four distinct but complementary research designs and already available data. Research questions were therefore addressed across multiple samples and designs taking advantage of the strengths of each and supplementing the weaknesses. The three studies constituting the empirical body of this thesis are presented next.

Chapter 5

Parent-child hostility and child depression symptoms: the direction of effects, role of genetic factors and gender

Paper 1: Currently under second review –Lewis, G., Harold, G., Collishaw, S., & Thapar, A. (2011). Parent-child hostility and child depression symptoms: the direction of effects, role of genetic factors and gender. Development and Psychopathology.

Abstract

Past research suggests that parent-child hostility is associated with child and adolescent depression. However, the extent to which associations are explained by environmental mechanisms is unclear due to the potential role of inherited factors and child effects on parents. In testing the presence of environmental effects, combining the varying attributes of different research designs is an important methodological tool. The aim of this study was to use a combination of research designs to critically test whether parent-child hostility confers environmental risk effects for child and adolescent depression. A longitudinal community sample of parents and children was used to test the direction of effects and a genetically sensitive twin sample was used to disentangle environmental from genetic influences. The role of both parent and child gender was also examined. Significant effects were found for daughters only: using cross-lagged panel correlation analysis, bidirectional effects were found for mother-daughter hostility and depression symptoms. A significant pathway was found between daughter depressive symptoms and father-daughter hostility but not vice versa. Genetic analyses revealed a significant environmental effect of mother hostility on symptoms of daughter depression. There was also a genetic contribution to this association. The association between father hostility and daughter depression was accounted for by genetic factors only. Findings suggest that negative mother-daughter relationships have an environmental risk effect on offspring depression that is not explained by inherited factors or reverse causation. Results are discussed in relation to potential intervention implications.

Introduction

Depression is a common mental health problem among youths, estimated to affect between 0.4% and 2.5% of children and 0.7% and 9.8% of adolescents, with lifetime prevalence rising to 25% by 18 years of age (Costello, et al., 2003; Kessler, et al., 2001). Sub-threshold symptoms and clinical diagnoses of depression have been found to impair functioning and predict recurrence rates to a similar extent (Gotlib, et al., 1995; Shankman et al., 2009). Identifying risk factors for child and adolescent depression has become a major clinical and research concern since depression in childhood strongly predicts depression in adulthood and adult depression is predicted to become the second leading cause of disability adjusted life years (DALYs) worldwide by the year 2020 (Murray & Lopez, 1997). This has drawn attention to the importance of understanding the aetiology of child and adolescent depression and in particular, to identifying malleable environmental risk factors which precede the onset of symptoms and can therefore be targeted by preventive interventions designed to reduce future rates of the disorder in adulthood.

Due to limited availability of experimental approaches for testing environmental risk effects on human psychopathology, the varying attributes of the research designs that are available are of vital importance (Kendler & Gardner, 2010; Rutter, et al., 2001; van Os, et al., 2010). A variety of methods exist which permit advancement towards the identification of underlying aetiological pathways and consist of natural quasi-experimental designs and specialized statistical techniques. Combining the particular strengths and offsetting the weaknesses of different research designs in a complementary fashion is therefore an important methodological tool (van Os, et al., 2010). This can be achieved in several ways. Firstly, when convergent results are obtained using different designs (for example; longitudinal, randomized controlled trials, genetically informative studies and quasi-experimental methods) which have

alternative strengths, this adds validity to the inference of a potential underlying causal process. In addition to replicating the same finding across different designs, a second useful method is to combine multiple datasets with complementary features and use them in conjunction to test different questions that cannot be examined when samples are considered separately (Kendler & Gardner, 2010; van Os, et al., 2010). For example, the use of a longitudinal sample to test the direction of effects between variables and the use of a genetically informative sample to test whether associations between the same variables are accounted for by environmental and/or genetic factors. This is also an important methodological strategy.

Depression aetiology: Risk factors and testing effects

The aetiology of depressive symptoms in childhood and adolescence has been attributed to both genetic and environmental factors (Rice & Thapar, 2008) and is likely to occur through interactions between multiple developmental pathways (Cicchetti & Toth, 1998). A range of twin and family studies have reported moderate genetic influences on depression symptoms and disorder with the role of genetic factors increasing in adolescence and the influence of environmental factors greater in childhood (Rice, Harold, & Thapar, 2002; Thapar & McGuffin, 1994). In terms of specific risk factors, the parent-child relationship has been identified as an important predictor of child and adolescent depression with negative relationships associated with symptom onset (Davies & Cummings, 1994) and positive relationships associated with symptom prevention (Davies & Cicchetti, 2004). A specific aspect of the parent-child relationship which has been associated with child depression symptoms is parent-child hostility (Low & Stocker, 2005). A recent meta-analysis found that compared to other dimensions of the parent-child relationship such as parent-child rejection and parental control, parent-child hostility, defined as an absence of warmth and the presence of parental aversive behavior, was most

strongly associated with child depression . However, the extent to which this association is predictive such that hostile parent-child relationships lead to depressive symptoms, remains unclear. Whereas twin studies have consistently shown that depressive symptoms in childhood and adolescence are moderately heritable, establishing true environmentally mediated risk factors has been less straightforward and has been challenged on two accounts (Rutter, et al., 2001). The first refers to the issue of reverse causation and the direction of effects. Specifically, with regard to reverse causation, the association between parent-child relations and negative symptoms could arise because symptoms displayed by children and adolescents result in increased negativity in the parent-child relationship rather than vice versa (Bell, 1968). In order to examine the direction of effects between parent-child hostility and child depression symptoms, longitudinal models which control for initial baseline symptoms, the stability and change in associations across time and which estimate reciprocal effects are required (Rutter et al., 2001).

The second challenge to establishing the presence of potential cause and effect relations stems from the possibility that association between parent-child relations and child depression symptoms could arise because of an unmeasured third variable, confounding that includes inherited factors. A range of genetically sensitive studies report heritable influences on measures of family adversity including parenting and the parent-child relationship (McGue, et al., 2005; Neiderhiser, et al., 1994; Plomin, et al., 1994). These studies have found that measures of family adversity assess in part, genetically influenced characteristics of children and parents (Elkins, McGue, & Iacono, 1997b; McGue, et al., 2005; Neiderhiser, et al., 1999), and can arise as a result of active, evocative as well as passive gene-environment correlation (Plomin, et al., 1994; Price & Jaffee, 2008). Since genetic factors influence the parent-child relationship and children's depression symptoms, it is possible that associations between the two are accounted for by common genetic

influences rather than through environmental processes. In order to investigate the possibility of a genetic confound, genetically sensitive data are required to disentangle the environmental risk effects of family adversity on psychopathology from shared genetic liability. The importance of rigorously testing environmental risk factors for psychopathology is being increasingly highlighted (Rutter, et al., 2001). The first aim of the current study was to use multiple research designs to test the presence of environmental risk effects. Firstly, a longitudinal community sample with a range of well validated measures administered at multiple time points was used to examine the direction of the association between parent-child hostility and child depression symptoms. The longitudinal nature of this design permitted a test of the temporal precedence of the risk factor in relation to the outcome. . However, since samples consisting of genetically related parent-child pairs do not permit a disentanglement of environmental from genetic effects, the possibility that longitudinal associations were accounted for by genetic factors remains. As a complement to the longitudinal analyses, a genetically-sensitive twin sample was also used to test whether the cross-lagged associations observed were accounted for by environmentally mediated effects of parent-child hostility on child depression.

In establishing the aetiology of depression, the role of gender is of considerable importance. A consistently demonstrated female preponderance of approximately 2:1 exists for both depressive disorder and symptoms (Nolen-Hoeksema, 2001; Piccinelli & Wilkinson, 2000). In addition to prevalence rates, the genetic and environmental aetiology of depression has also been found to vary by gender. For example, some evidence suggests gender differences in the heritability of depression, although these findings are inconsistent. With regard to the influences of adversity, evidence suggests that girls may be more sensitive to the effects of the family environment and to interpersonal stressors in general than boys (Bifulco, Brown, Morgan, Ball, & Campbell, 1998; Veijola et al., 1998). In addition, there is evidence of differences in

the effects of the parent-child relationship for boys and girls. For example, sons have been found to elicit less verbal interaction than daughters (Leaper, Anderson, & Sanders, 1998). The second aim of the current study was therefore to test whether the association between parent-child hostility and child depression symptoms varies by gender when examined in both longitudinal and genetically sensitive designs.

The parent-child relationship and child depression symptoms: the direction of effects and the role of child gender

Several prospective longitudinal studies have been conducted to examine the direction of the association between various aspects of the parent-child relationship and child depression. Reciprocal models of influence have been reported for various dimensions of the parent-child relationship and child depression symptoms including parent-child warmth but not harsh punishment (Hipwell, et al., 2008), overall level of perceived parent-child relationship quality including communication, trust and alienation (Branje, et al., 2010) and parent-child rejection (Hale, et al., 2008). Unidirectional models reporting parent effects on children but not vice versa have also been reported (Chen, Liu, & Li, 2000; Stice, Ragan, & Randall, 2004). Rueter, Scaramella, Wallace & Conger (1999) also report a unidirectional parent effects model: increases in parent-adolescent disagreements predicted adolescent internalizing symptoms which in turn predicted first onset depressive disorder, but the reverse pathway from internalizing symptoms to increased parent-adolescent disagreements was not significant.

With regard to the moderating role of parent and child gender, inconsistent results have also been reported. In a four wave longitudinal study, Hale et al., (2008) report a stronger initial cross-sectional association between parental rejection and depression symptoms for girls and at waves 3-4, the association between adolescent

depression and parental rejection was significant only for girls. Soenens, Luyckx, Vansteenkiste, Duriez, and Goosens (2008) found reciprocal influences between paternal psychological control and depressed mood for both sons and daughters whereas for maternal psychological control, a unidirectional model emerged as the best fit for daughters with depressed mood resulting in increased psychological control but not vice versa. Conversely, the study by Branje et al., (2010) reports a bidirectional model for mother relationship quality for daughters and sons whereas perceived relationship quality with fathers was related to depressive symptoms only for boys. Research on the direction of the association between parent-child relations and depressive symptoms is therefore inconsistent and in addition, studies which have examined the role of parent and child gender have produced conflicting results.

Parent-child relationship factors and child depression symptoms: the role of genetic factors

The previous studies examined the direction of the association between parent-child relationship quality and child and adolescent depression symptoms, providing a test of the direction of effects. However, they were not genetically sensitive so could not control for the potential role of inherited factors. Genetic factors have been investigated using multivariate twin designs capable of testing whether putative environmental effects on psychopathology remain after the influence of shared genetic factors has been controlled. Pike, McGuire, Hetherington, Reiss, and Plomin (1996) found that the non-shared environment significantly contributed to the association between maternal but not paternal negativity and depressive symptoms in a sample of sibling pairs aged 10-18 years. There was a substantial genetic contribution to the association for both maternal and paternal negativity. The cross-sectional nature of this study precluded examination of the direction of effects. Using longitudinal twin data, Neiderhiser, Reiss, Hetherington, and Plomin (1999) also

found that genetic factors accounted for a substantial proportion of the association between maternal and paternal conflict-negativity and depressive symptoms (the genetic parameter dropped below significance for maternal conflict-negativity and depression but still accounted for 70% of the association). The non-shared environment contributed approximately 30% to the association for both maternal and paternal conflict-negativity. Whilst a bidirectional model of effects was supported for paternal conflict-negativity and depression symptoms, a unidirectional model in which parental negativity predicted increased depressive symptoms but not vice versa was the most parsimonious for maternal conflict-negativity.

Although longitudinal and genetically sensitive, this study did not explore the potentially important role of child and adolescent gender. Only one study to date has examined the role of genetic and environmental factors in the association between family environment and depression symptoms separately for sons and daughters (Jacobson & Rowe, 1999). The best fitting model was one which allowed parameters to vary by adolescent gender: Genetic factors contributed more substantially to the association between family connectedness and depressed mood for females whilst the influence of the non-shared environment was greater for males. However this study used a very global measure of general family functioning so did not specifically examine the parent-child relationship and did not explore the role of parent gender which the previous studies have highlighted as important.

The Present Study

The present study utilised existing data from two studies based on different samples and research designs. The designs were used in conjunction to test different hypotheses regarding the presence of environmental processes. Data from 1) a longitudinal community study and 2) a cross-sectional community based twin study

were examined to test whether hostile parent-child relationships have an environmental risk effect on symptoms of child depression after accounting for reverse causation and genetic factors. Analyses were conducted separately for parent and child gender. Based on previous genetically sensitive studies, it was hypothesized that the association between mother and father hostility and child depression symptoms would be mediated by both genetic and environmental factors. With regard to the direction of the association, previous findings are inconsistent but have reported both parent-driven and child-driven effects, so bidirectional models of influence were examined. It was also hypothesized that differences would be found according to both parent and adolescent gender, however, predictions on the direction of gender effects were not formulated based on inconsistent results in this area.

Methods

Participants

1) Longitudinal Community Sample. The longitudinal sample used to test the direction of effects was drawn from The South Wales Family Study (SWFS) which comprised 387 parents and children recruited from 12 schools across South Wales. Demographic statistics for this sample have shown that it is representative of families living in England and Wales in terms of family composition, parent education and ethnicity (*Social Trends*, 2004). For the present study, data from waves one (1999) and two (2000) were used. Children were aged 11– 12 years at Time 1 (mean = 11.7 years) and 12 – 13 years at Time 2 (mean = 12.7 years). These time points were selected as children have transitioned from primary to secondary school in the previous year, a period that is congruent with a developmentally related increase in the prevalence of depression symptoms (West, Sweeting, & Young, 2010) and the emergence of a marked gender difference (female preponderance of 2:1) (Nolen-

Hoeksema, 2001). Of the 387 families who provided data at Time 1, 318 (82%) were retained at Time 2. Two families were removed because of uncertainty as to who the children resided with. The final sample comprised 316 families each with one participating child and an approximately even split of boys (n=157) and girls (n=159). The sample comprised single and two-parent families. The majority of children lived in two parent families (80.4%) comprising biological and step-parents. Parents were invited to participate in the study by a letter explaining the project and were further informed of the aims of the research through presentations at scheduled parent-teacher evenings. Parents who provided informed written consent received questionnaires through the mail. Mothers and fathers both received a questionnaire and were asked to complete them independently. Children completed questionnaires during the school day under the supervision of the researchers. Child and parent questionnaires contained a range of measures assessing the quality of family relations, psychological well-being, economic conditions and family demographics.

2) Genetically informative twin study. The genetically sensitive sample used to disentangle environmental from genetic effects derived from a community based register of over 6000 twins (*Cardiff Study of All Wales and North West of England Twins (CaStANET)*) born between 1976 and 1991 in Greater Manchester and South Wales and is demographically representative of the UK population (van den Bree et al., 2007). Zygosity was assigned on the basis of a twin similarity questionnaire which has been shown to be over 90% accurate in distinguishing monozygotic from dizygotic twins (Cohen, Dibble, Grawe, & Pollin, 1975). The present study focused on a subsample of 1214 twin families who provided questionnaire data in 2004 that included items on family relationships (1755 families were approached, 1214 responded; 69% response rate). Longitudinal family relationship data were not available. Questionnaires assessing the family environment along with a range of

outcome measures including depressive symptoms were mailed to parents and twin pairs. Informed consent was obtained along with the questionnaire. It was requested that the mother of the twins complete the parent questionnaire where possible; 93% of questionnaires were completed by mothers. 88 of the 1214 replies consisted of parent responses only so were removed from the current sample. 17 twin pairs were removed due to missing zygosity information and 34 twin pairs were removed because of uncertainty as to who they resided with. The final sample therefore comprised 1075 families with twin pairs (653 dizygotic; 422 monozygotic) aged between 12 and 20 years (mean = 16.12 years).

Measures

Depressive symptoms. In the longitudinal SWFS, depressive symptoms were measured using the Child Depression Inventory (Kovacs, 1985), a 26-item self-report measure of depressive symptomatology which showed good internal consistency in this sample (α = .86 at T1 and α = .89 at T2). In the twin study, depressive symptoms were measured using the Short Mood and Feelings Questionnaire (Angold et al., 1995), a 16-item self-report measure of the presence and severity of DSM-IV based symptoms of major depression which showed good internal consistency in this sample (α = .92).

Parental Hostility. Parental hostility was assessed in both samples using the Iowa Youth and Families Project (IYFP) family interaction rating scales (Melby et al., 1993). Children/adolescents completed two IYFP rating scales; one assessing perceived mother hostility and one assessing perceived father hostility. The scales included 5 items including "how often in the past month has your mum/dad got angry with you, criticized you or your ideas, shouted at you because she was upset with you, got into an argument with you or argued with you whenever you disagreed about

something." Responses ranged from "never" to "always" along a 7 point likert scale. Children were asked to complete each scale for the person who was 'most like a mother/father' to them. Parents also completed the IYFP rating scales reporting on the level of hostility directed towards their children during the past month. The parent hostility scale also contained 5 items such as "how often in the past month did you get angry at him/her." In the SWFS, mothers and fathers completed the IYFP rating scales with reference to the same participating child/adolescent. In the twin sample, mothers provided separate ratings for each twin. Two composite variables which combined parent and child/adolescent reports of hostility in the mother and father relationship were used in the SWFS sample to reduce shared rater effects. In the twin sample, the same composite variable was used to assess mother hostility. However, because an insufficient number of father reports were available, adolescent reports of father hostility were used. Although this did not directly correspond to the SWFS, in the SWFS the same pattern of results was found for a model which used adolescent reports of father hostility. Internal consistency for this measure was good in both samples for mother and father hostility (range α = .80 - .85)

Statistical analysis

Longitudinal analyses. Correlations were computed and cross-lagged path models were fitted to the data using maximum likelihood estimation (Joreskog & Sorbom, 2006). Cross-lagged models assess the direction of the association between two variables over time by controlling for the concurrent association between them at each time point and for the stability of each variable over time. Because the bivariate association at each time point is controlled, the cross-lagged relationships represent the unique contribution of each Time 1 variable in accounting for variation in each cross-construct Time 2 variable. Since this model also takes account of the stability of each variable over time, a statistical estimation of within-

individual change in outcome at Time 2 following exposure to putative risk at Time 1 can be assessed and vice versa (the effect of the outcome variable at Time 1 on the risk variable at Time 2).

Genetic analyses. Correlations between parent hostility and depressive symptoms were computed using the survey commands in STATA 10.0 (StataCorp, 2007) which control for the non-independence of data points caused by the clustering of twin pairs by using the standard error as a measure of dispersion instead of the standard deviation. Univariate structural equation models estimating the influence of additive genetic (A), shared environmental (C) and non-shared environmental (E) effects were first fitted to the raw twin data using Mx (Neale, et al., 1999). The goodness-of-fit of each univariate model was compared to a saturated model using a likelihood-ratio x^2 test obtained by subtracting the minus twice log likelihood (-2LL) of the model from the -2LL of the saturated model. The significance of each parameter (A, C and E) was then tested by comparing nested submodels to the full ACE model using a likelihood-ratio x^2 test. Bivariate models are based on the cross-twin cross-trait correlations and decompose the covariation between two variables into its genetic and environmental components. Bivariate correlated factor models (Figure 1) were fitted to the data to estimate the genetic and environmental influences common to or shared by the variables. This allows an estimation of the extent to which the association between two variables is explained by common genetic (genetic mediation) or common environmental (environmental mediation) factors. A second method for testing the presence of environmental mediation uses monozygotic twin difference scores (Pike, Reiss, et al., 1996). Since monozygotic twins are genetically identical, any difference between their scores on a particular measure provides a direct index of the non-shared environment (this estimate also includes measurement error). Environmental mediation can be tested by correlating the difference scores on

two measures; an outcome and putative risk factor. If the correlation is significant, there is evidence of environmental mediation. Differences between monozygotic twins on the parental hostility measures were therefore computed and correlated with monozygotic twin difference scores on the depression measure including age and sex as covariates where appropriate.

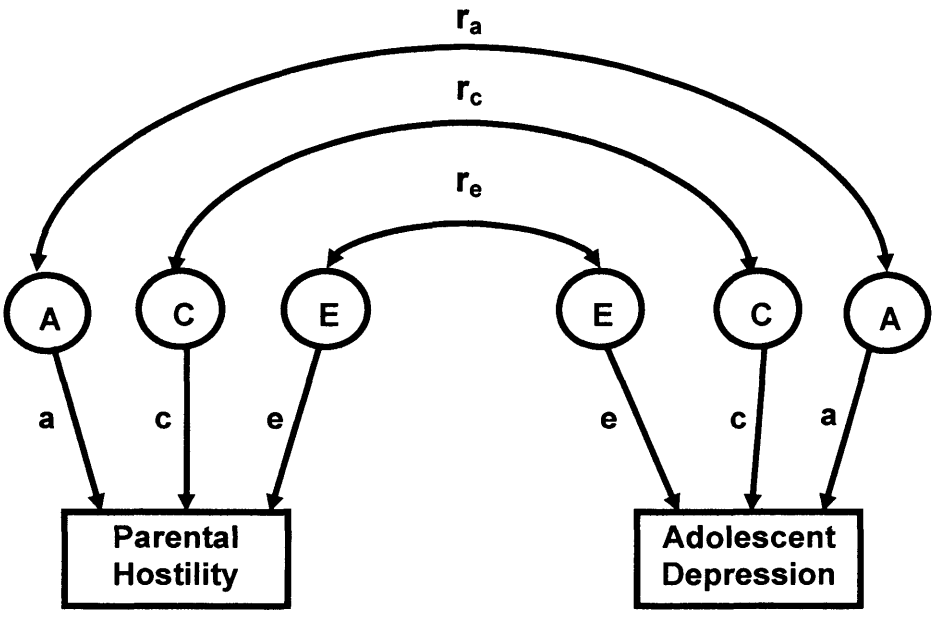

Figure 2. Bivariate correlated factors model. A = additive genetic, C = shared environment, E = non-shared environment. r_a = correlation between additive genetic factors, r_c = correlation between shared environmental factors, r_e = correlation between non-shared environmental factors.

Results

Longitudinal analyses of SWFS community data to test the direction of effects

Longitudinal analyses were used to test the direction of effects. Depression scores were positively skewed at both time points so were square root transformed. Descriptive statistics for all variables are shown in Table 2. Depression scores did not

differ significantly by gender at Time 1 (t (281) = .31, p = .76) or Time 2 (t (294) = .30, p = .76). Mean scores for mother hostility similarly showed no gender difference at Time 1 (t (295) = 1.48, p = .14) or Time 2 (t (295) = .29, p = .77) and no gender differences were found for father hostility at Time 1 (t (259) = .93, p = .35) or Time 2 (t (257) = .31, p = .75). Mother and father hostility scores were normally distributed so were not transformed. Correlations between parental hostility and depression are also presented in Table 2. For boys, there was a significant within-time association between depression symptoms and both mother and father hostility at Time 1 (see Table 2). Across time, mother hostility at Time 1 was not significantly associated with depression at Time 2 but depression symptoms at Time 1 were significantly associated with mother hostility at Time 2 (see Table 2). For girls, there was a significant within-time association between depression symptoms and both mother and father hostility at Time 1 and significant associations across time between depression symptoms at Time 1 and mother and father hostility at Time 2 and vice versa (see Table 2).

Table 2

Descriptive statistics and correlations between parent hostility and child/adolescent depression symptoms within and across time

Correlations among variables: males (n = 157) above diagonal, females (n = 159) below diagonal								
Time1 (1999)	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**Standard Deviation**
1. Depression	-	.34 **	.38 **	.61 **	.33 **	.31 **	8.37	5.57
2. Mother hostility	.46 **	-	.64 **	.14	.56 **	.44 **	26.02	7.46
3. Father hostility	.37 **	.60 **	-	.32 **	.40 **	.61 **	24.91	7.57
Time 2 (2000)								
4. Depression	.61 **	.44 **	.32 **	-	.28 **	.41 **	9.20	7.19
5. Mother hostility	.48 **	.65 **	.49 **	.56 **	-	.59 **	25.45	7.35
6. Father hostility	.41 **	.38 **	.63 **	.40 **	.59 **	-	24.83	7.84
Mean	9.02	24.74	24.05	9.55	25.70	24.51	-	
Standard Deviation	7.55	7.39	7.36	7.99	7.35	8.50		

Cross-lagged path models

Since the cross-lagged model is fully saturated, all goodness-of-fit indices indicated a perfect fit to the data. For sons, the cross-lagged pathway from mother hostility at Time 1 to depression symptoms at Time 2 was not significant (Figure 3a). In addition, the pathway from depression symptoms at Time 1 to mother hostility at Time 2 was not significant (Figure 3a). For daughters, mother hostility at Time 1 significantly predicted increased depression symptoms at Time 2 (Figure 3b) and depression symptoms at Time 1 significantly predicted increased mother hostility at Time 2 (Figure 3b). For father-son hostility, the pathway from father hostility at Time 1 to depression symptoms at Time 2 was not significant (Figure 4a) and the pathway from depression symptoms at Time 1 to father hostility at Time 2 was also not significant (Figure 4a). For daughters, father hostility at Time 1 did not significantly predict increased depression symptoms at Time 2 (Figure 4b) but depression symptoms displayed by daughters at Time 1 significantly predicted increased hostility from fathers at Time 2 (Figure 4b). Stacked model comparisons were used to assess the statistical significance of the difference in pathways across gender: pathways for males and females are fixed to equality and the resulting change in model fit is assessed. Stacked models revealed that the pathway from mother hostility at Time 1 to depression symptoms at Time 2 differed significantly by child gender ($\Delta x^2 = 8.33$, $p < .05$). No other pathways were statistically different across parent or child gender groupings according to the stacked modelling comparisons.

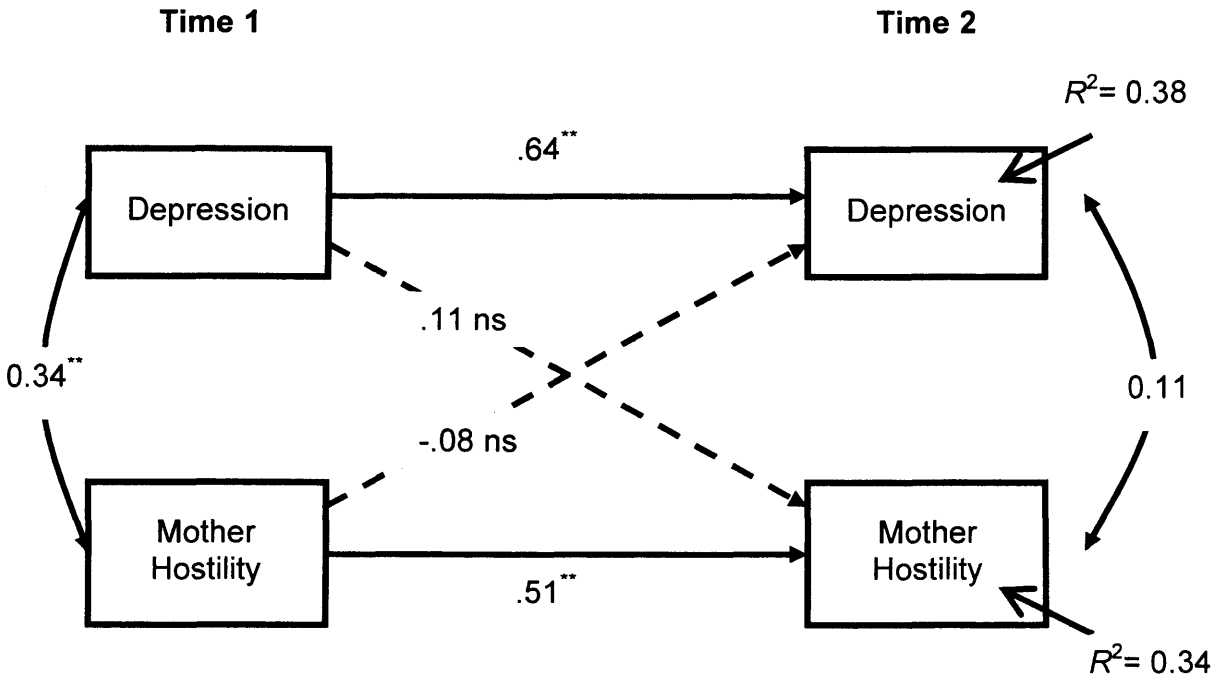

Figure 3 (a). Cross-lagged model for mother-son hostility and depressive symptoms (n = 114).

*p < .05, ** p < .01. + Significantly different to opposite gender pathway.

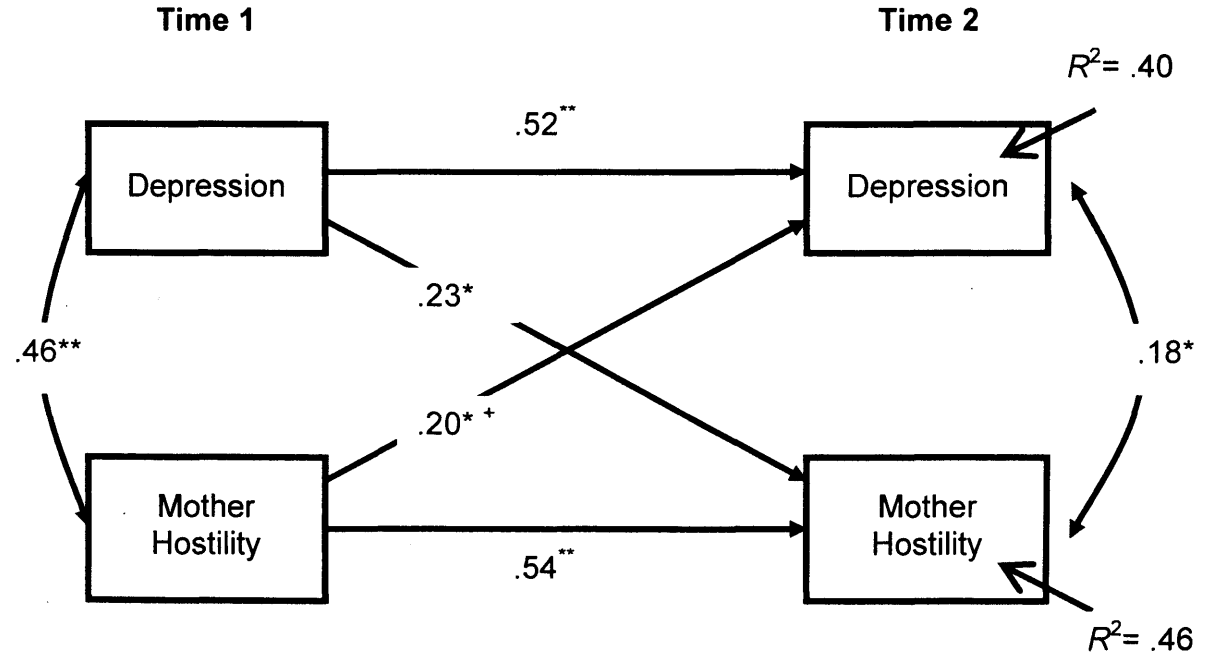

Figure 3 (b). Cross-lagged model for mother-daughter hostility and depressive symptoms (n = 131)

*p < .05, ** p < .01. + Significantly different to opposite gender pathway.

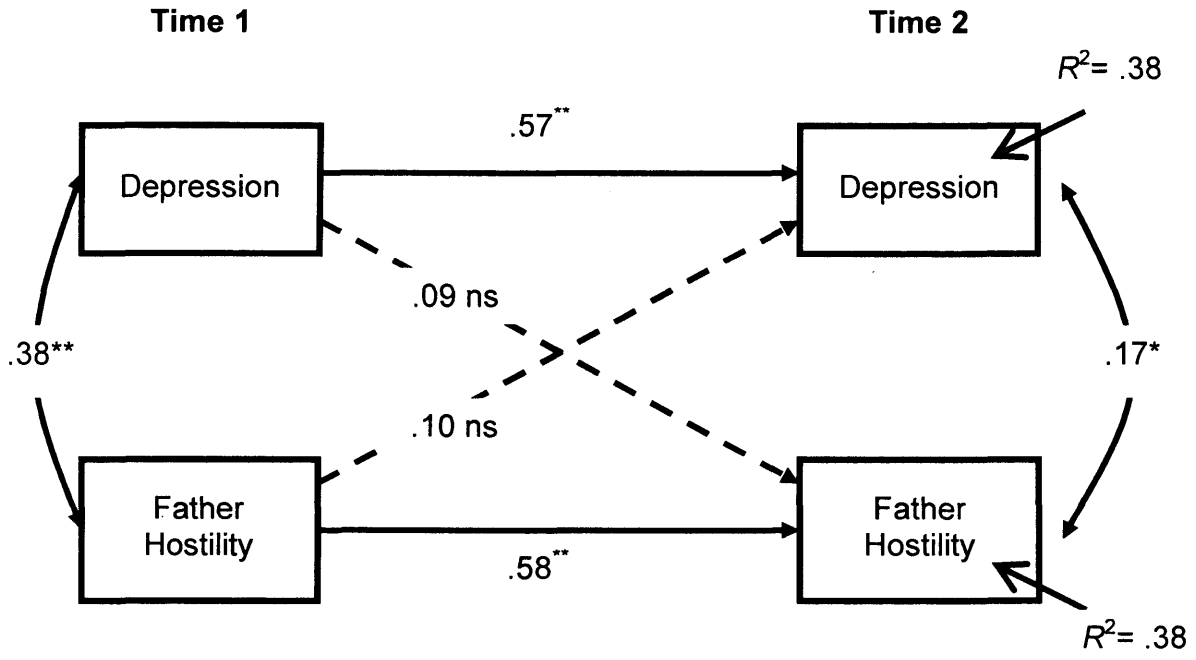

Figure 4 (a). Cross-lagged model for father-son hostility and depressive symptoms (n = 98)

*p < .05, ** p < .01. + Significantly different to opposite gender pathway.

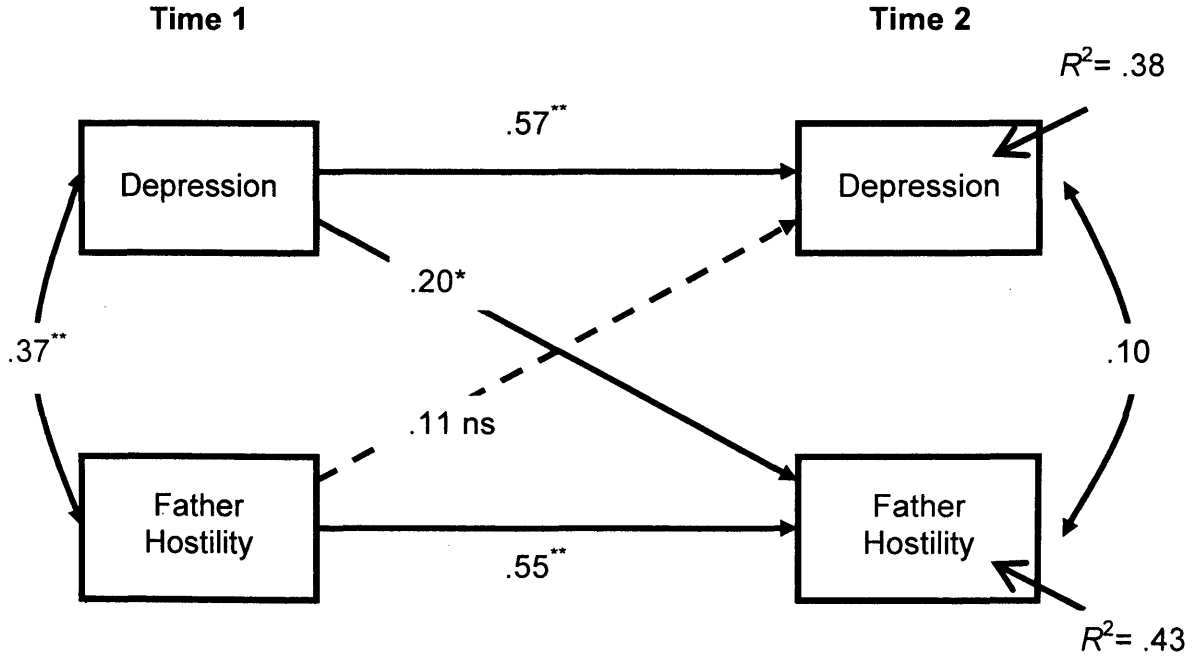

Figure 4 (b). Cross-lagged model for father-daughter hostility and depressive symptoms (n = 119)

*p < .05, ** p < .01. + Significantly different to opposite gender pathway.

Genetically informative twin analyses

Since the longitudinal sample was not genetically informative, a genetically sensitive twin sample was used to test the presence of environmental effects after accounting for genetic influences. The mean depression score for daughters in the CaStANET was 22.62 (SE = .22) and for sons, 20.37 (SE = .31). Due to positive skewness, depression symptom scores were transformed using the transformation Ln (depression+1). Transformed depression symptom scores are used in all subsequent analyses. The mean score for the composite mother hostility variable was 26.75 (SE = .27) for daughters and 26.85 (SE = .30) for sons. Mean scores for father hostility were 13.84 (SE = .21) for daughters and 14.72 (SE = .23) for sons. Mother and father hostility scores (parent, adolescent and composite variables) were normally distributed so were not transformed. Mother-child hostility and depression symptoms were moderately correlated for both daughters ($r = .32$, $p < .01$, $n = 945$) and sons ($r = .30$, $p < .01$, $n = 783$) as were father-daughter ($r = .23$, $p < .01$, $n = 1010$) and father-son ($r = .26$, $p < .00$, $n = 790$) hostility.

Bivariate genetic analyses

Cross-twin cross-trait correlations were computed and are presented in Table 3. Results of the bivariate correlated factors model for mother-daughter hostility and depression are shown in Table 4. The correlation between common genetic factors was significant as was the correlation between non-shared environmental factors. Genetic factors accounted for 75 % of the phenotypic correlation and non-shared environmental factors accounted for 25 % of the phenotypic correlation. Results of the correlated factors analysis for father hostility and depressive symptoms showed evidence of significant genetic mediation only. Bivariate genetic analysis was also conducted for a sub-sample of twins aged 12-16 years and the same pattern of results found. Results of the bivariate correlated factors models for sons are also

presented in Table 4. There was no evidence of significant environmental effects for either mother or father hostility.

Monozygotic twin differences analysis

In order to test whether the patterns of environmental and genetic effects observed in the bivariate genetic analyses for girls could be replicated, a different method of assessing environmental effects in the twin design was also employed: correlating monozygotic twin difference scores. The mean monozygotic twin difference scores were -.54 (sd = 6.07) for mother-daughter hostility, -.20 (sd = 5.61) for father-daughter hostility and .26 (sd = 7.05) for depression symptoms. For sons, mean difference scores were .82 (sd = 4.99) for mother hostility, .10 (sd = 5.49) for father hostility and -.13 (sd = 4.56) for depression. There was a significant correlation between monozygotic twin difference scores for mother-daughter hostility and depression ($r = .20$, $p < .05$, $n = 167$). For father-daughter hostility and depression, the correlation between monozygotic twin difference scores was not significant ($r = .09$, $p < .09$, $n = 176$). Correlations were not significant for sons for either mother ($r = .10$, $p > .05$, $n = 144$) or father ($r = .09$, $p > .05$, $n = 152$) hostility.

Table 3

Cross-twin, cross-trait correlations for mother and father hostility and depressive symptoms

Variables	Cross-twin cross-trait correlations between parent hostility and depression symptoms, daughters only			
	Monozygotic Twin 1-Twin 2		Dizygotic Twin 1-Twin 2	
	Girls	Boys	Girls	Boys
Mother-daughter hostility and depression symptoms	.26**	.37 **	.14	.02
Father-daughter hostility and depression symptoms	.21**	.19 *	.10	.01

Table 4

Parameter estimates, correlated factors analysis and goodness-of-fit statistics for accepted bivariate models, girls on top line, boys below

Model	Parental hostility			Depression symptoms			Correlated factors			Goodness-of-fit statistics		
	A	C	E	A	C	E	r_a	r_c	r_e	-2ll	df	AIC
Mother hostility and depression symptoms	.48 (.35-.66) .65 (.37-.62)	.29 (.12-.40) -	.23 (.19-.29) .35 (.12-.40)	.47 (.36-.57) .51 (.37-.59)	-	.53 (.43-.64) .49 (.46-.67)	.54* (.37-.75) .65* (.40-.65)	-	.24* (.11-.37) .08 (.09-.25)	64.81	53	-41.19
Father hostility and depression symptoms	.65 (.57-.71) .67 (.55-.76)	-	.35 (.23-.43) .33 (.25-.44)	.46 (.35-.55) .50 (.37-.58)	-	.54 (.45-.65) .50 (.48-.67)	.36* (.22-.50) .41* (.24-.47)	-	.09 (-.04-.09) .06 (.02-.10)	68.25	58	-47.75

Note: *Significant genetic and environmental correlations

Discussion

The aim of this study was to use a combination of research designs with complementary attributes to critically test whether hostile parent-child relationships confer predictive environmental risk effects for symptoms of depression in children and adolescents. The combination of a longitudinal community and a genetically informative community based twin design permitted different tests of the presence of environmental processes by allowing an examination of 1) the direction of effects and 2) the role of shared inherited factors. This allowed multiple questions pertaining to the assessment of environmental risk effects to be addressed which could not have been achieved using a single study design. A further aim was to investigate the role of both parent and child gender since this is aetiologically relevant to depression and, it is important that studies assessing environmental links also test moderators of effects.

The longitudinal research study was first used to test the hypothesis of bidirectional effects but since this sample was not genetically sensitive, a twin sample was then used to test the presence of environmental links after genetic influences had been controlled. In the longitudinal research study, significant cross-lagged associations were found for daughters only. Reciprocal influences across time were found between mother-daughter hostility and child and adolescent depressive symptoms; mother-daughter hostility predicted increased depressive symptoms and depressive symptoms displayed by daughters predicted increased mother-daughter hostility supporting a bidirectional model of effects. Since associations were not accounted for by reverse causation and there was a significant cross-lagged path from mother hostility to child and adolescent depression symptoms, results suggest that the experience of maternal hostility temporally precedes the development of depression symptoms. A unidirectional model was found for father-daughter hostility; depressive

symptoms displayed by daughters were predicted increased father-daughter hostility but not vice versa. Since there was no predictive pathway from father hostility to child and adolescent depression symptoms, results suggest that previously observed associations are likely due to reverse causation. For mother-daughter hostility, genetic analyses revealed the presence of significant environmental effects after the influence of genetic factors had also been estimated. A significant contribution of genetic factors was also detected. Although the environmental correlation was of a smaller magnitude than the genetic correlation, it was significant and was supported by the results of the monozygotic twin differences analysis. This is in contrast to a recent study using the same samples which found that parent-child hostility had no environmental effect on child ADHD symptoms (Lifford, Harold, & Thapar, 2009). This may be because heritability estimates are consistently found to be higher for ADHD versus depression symptoms and environmental influences on this developmental phenotype may therefore be more difficult to detect in bivariate twin designs. In addition, ADHD is known to be a more neurological disorder than depression and to be less susceptible to environmental influences (Lifford et al., 2009). In contrast, for sons, no evidence of environmental effects were found in genetic analyses.

Taken together, results underpin the importance of using different designs to critically test environmental risk effects. Specifically, they suggest that although negative mother-child relations are associated with both ADHD and depression symptoms, when critically examined, the risk effect appears to be in relation to depression. The implication is that targeting maternal hostility might be more helpful in alleviating depression symptoms than in treating ADHD symptoms. The finding of genetic mediation only for father-daughter hostility and depressive symptoms could suggest evocative gene-environment correlation whereby genetically influenced symptoms of depression displayed by daughters elicit increased parent-child hostility from fathers.

These findings are important because the majority of previous research on the parent-child relationship and child depression symptoms has either focused on the direction of effects or on the role of genetic factors and has not used multiple research designs. In addition, previous studies have not sufficiently examined parent and child gender. The methodology used in this study has allowed us to demonstrate that differences exist between the roles of mother and father hostility for sons and daughters highlighting the importance of examining both parent and child gender. The lack of significant cross-lagged pathways for sons may be because at 11-13 years of age, girls are more socially and relationship oriented than boys and are therefore more sensitive to the effects of the family environment (Maccoby, 1990). Similarly, Veijola et al., (1998) found that disturbances in the mother-child relationship predicted later depression in females but not in males and that disturbances in the father-child relationship were not predictive of later depression in sons or daughters. Alternatively, rather than being less affected by the parent-child relationship than girls, boys' distress in response to parent-child hostility may be manifested in a different way. For example there is evidence to suggest that in response to family stressors, girls may be more likely to display internalizing symptoms such as depression whereas boys may be more likely to display externalizing symptoms such as conduct problems (Jacobvitz, Hazen, Curran, & Hitchens, 2004). One study for example found that in response to maternal depression, boys were more likely to display externalising symptoms whereas girls were more likely to display internalising symptoms (Essex, Klein, Cho, & Kraemer, 2003).

The reciprocal association between mother-daughter hostility and depressive symptoms reported in this study is consistent with Hipwell et al., (2008) and Branje et al., (2008) but inconsistent with a study by Soenens et al., (2008) which reported a unidirectional association between maternal psychological control and depressed

mood in adolescent females. However psychological control may have a different relationship with depressed mood than maternal hostility. The unidirectional model reported for father-daughter hostility and depressive symptoms is consistent with a number of studies which have reported unidirectional child driven models (Chen et al., 2000; Stice et al., 2004) and also with the study by Branje et al., (2010) which found that perceived relationship quality to mothers but not fathers predicted symptoms of depression in girls. The finding that mother but not father hostility predicted increased depression symptoms in daughters may result from more time spent with the mother as the primary caregiver (in the majority of families) versus the father. A study by Tucker et al., (2003) for example found that mothers spend more time with daughters than with sons and that fathers spend more time with sons than with daughters. There is evidence to suggest that daughters may have a closer relationship with mothers than fathers making hostile maternal behavior more pertinent to their adjustment (Raley & Bianchi, 2006; Russell & Saebel, 1997). Closer mother-daughter versus father-daughter relationships may result from 'same-gender' parent effects wherein common interests are more easily established between same-gender parents and children (Lundberg, 2006; Raley & Bianchi, 2006). For example, Leaper et al., (1998) found that mothers spent more time interacting with daughters than sons and were more likely to use language that expressed praise, approval, agreement, acknowledgement or collaboration.

The bivariate genetic analyses showed genetic and environmental contributions to the association between mother-daughter hostility and depression and genetic effects only on the association between father-daughter hostility and depression. This is consistent with Pike et al., (1996) in suggesting that maternal negativity is associated with depressive symptoms through genetic and non-shared environmental factors. In the present study, 75% of the phenotypic correlation between mother-daughter hostility and depression symptoms was attributable to

genetic factors and 25% to non-shared environmental factors. These estimates are similar to those reported by Pike et al., (1996) and Neiderhiser et al., (1999) who both found that approximately 70% of the association between maternal negativity and depression symptoms was due to genetic factors with the remainder attributable to environmental factors. Pike et al., (1996) found that the non-shared environment did not significantly influence the association between paternal negativity and depression which is consistent with the present study. The finding of an environmental effect of mother-daughter hostility after genetic influences had been controlled is significant in showing that hostility in the mother-daughter relationship has likely true risk effects on depression symptoms in girls, and is not simply the result of shared genetic factors influencing both the relationship and psychopathology.

Limitations

Results of the present study should be considered in light of certain limitations. Although a major strength of this study was the combination of different research designs which allowed different questions relating to environmental effects to be addressed, there are also limitations associated with this approach. The use of two different samples meant that variations in measurement occurred across datasets. In the CaStANET, the Short Mood and Feelings questionnaire was used to assess depressive symptoms whereas in the SWFS, depressive symptoms were assessed using the Child Depression Inventory. However these measures have been shown to correlate highly (Angold et al., 1995) and are both well-validated assessments of depressive symptomatology. Depression symptoms were concurrently associated with parent-child hostility regardless of measure and it has also been noted that consistent findings across different measures of the same psychopathology construct can be indicative of greater confidence in the underlying results (Rutter, et al., 2001). The fact that self-report measures were used in the present study is also a limitation in that multiple informants and methods would be preferable. However, we were

restricted by the pre-existing availability of data. All self-report measures were reliable and valid. An important additional limitation in the present study is that the samples differed in age (mean age in SWFS = 11.7 years at T1 and 12.7 years at T2; mean age in CaStANET = 16.1 years). This is an important consideration given evidence that developmental pathways to depression vary according to age. For example, genetic influences have been found to be more influential in adolescence whereas the effects of the shared environment are more important in childhood (Scourfield, et al., 2003). In addition, age was included as a covariate in the genetic analyses. However, this does not rule out the influence of developmental shifts in rates of depression and parent-child relations as well as aetiological influences which could have caused results to differ across samples. Furthermore, we acknowledge that the use of age as a covariate does not necessarily control for the difference in age across samples. Further tests showed that when genetic analyses were conducted on a more closely matched age range of twins (12-16 years), the same pattern of genetic and environmental effects was found (available on request). Another limitation is that a composite variable which combined parent and adolescent reports was used to assess mother and father hostility in the longitudinal sample whereas adolescent reports of father hostility were used in the twin sample as father reports were not available. However, cross-lagged models resulted in equivalent findings for parent and child models in the longitudinal sample so this should not have affected results. Furthermore, we ran longitudinal models using father reports of father hostility and the same pattern of effects was found (available on request). The twin design used in the present study does not control for the effects of passive gene-environment correlation (Price & Jaffee, 2008), however, the fact that the origin of a family environmental risk factor may be under genetic influence does not rule out the possibility that risk effects are conferred via an entirely environmental process (Rutter et al., 2001).

Conclusions

Although the association between hostile parent-child relationships and child and adolescent depression symptoms is well established, there has been relatively little research employed to critically test whether the link represents a predictive environmental risk effect. Using complementary longitudinal and genetically sensitive research designs, we found that the association between mother-daughter hostility and depressive symptoms was bidirectional and mediated by both genetic and non-shared environmental factors. A different pattern of effects emerged for father-daughter hostility and depressive symptoms: A unidirectional model was supported with depressive symptoms resulting in increased father-daughter hostility but not vice versa. There was no evidence of environmental mediation of this association; effects were found to be genetically mediated only. Although the present study found no evidence for an effect of father-daughter hostility on child depressive symptoms, this finding should be considered within the context of other studies demonstrating the importance of the father-child relationship (Jaffee, Caspi, Moffitt, & Taylor, 2003). In the present study, for boys, no evidence of predictive or environmental links was found. Again, this should not necessarily be interpreted as evidence that the parent-child relationship is unimportant for boys but rather, that effects of parent-child hostility are likely to be stronger for girls in the context of assessing impacts on depressive symptoms. The finding that environmental risk effects were significantly stronger for girls is important because, aiming to identify the presence of environmental effects is a primary aim, however, more important is identifying the specific influences which confer risk, under what circumstances and for whom (Rutter, 2007b; Rutter, et al., 2001). The identification of mother hostility as a likely environmental risk factor for female depressive symptoms is important as it suggests that interventions aimed at improving hostile mother-daughter relations may benefit female depressive symptoms. Findings that certain environmental influences

differentially affect particular subgroups (i.e. girls versus boys) are essential to informing the design of targeted preventive intervention strategies aimed at specific populations which have been found to be of greater effectiveness than either universal or selected interventions (Gladstone & Beardslee, 2009).

Chapter 6

Investigating environmental links between parent depression and child anxiety/depressive symptoms using an assisted conception design

Paper 2: Lewis, G., Rice, F., Harold, G., Collishaw, S., & Thapar, A. (2011). Investigating environmental links between parent depression and child anxiety/depressive symptoms using an assisted conception design. ***Journal of the American Academy of Child and Adolescent Psychiatry, 50(5), 451-459.***

Abstract

Links between maternal and offspring depression symptoms could arise from inherited factors, direct environmental exposure or shared adversity. We used a novel genetically sensitive design to test the extent of environmental links between maternal depression symptoms and child anxiety/depression symptoms, accounting for inherited effects, shared adversity and child age and gender. 852 families with a child born via assisted conception provided questionnaire data. Mothers and fathers were genetically related or unrelated to the child depending on conception method. Parental depression symptoms were assessed using the Hospital Anxiety and Depression scale. Child anxiety/depression symptoms were assessed using the Short Mood and Feelings questionnaire and 6 items tapping generalised anxiety disorder symptoms. Associations between maternal and child symptoms were examined separately for genetically unrelated and related mother-child pairs adjusting for three measures of shared adversity; negative life events, family income and socioeconomic status. Analyses were then run separately for boys and girls, for children and adolescents and the role of paternal depression symptoms was also examined. Significant associations between parent and child symptoms were found for genetically unrelated mother- ($r=.32$, $p<.001$) and father-child pairs ($r=.17$, $p<.05$) and genetically related mother- ($r=.31$, $p<.001$) and father-child pairs ($r=.23$, $p<.001$) and were not explained by the shared adversity measures. Environmental links were present for both children and adolescents and were stronger for girls. The transmission of depression symptoms is due, in part, to environmental processes independent of inherited effects and is not accounted for by shared adversity measures. Girls may be more sensitive to the negative effects of maternal depression symptoms than boys via environmental processes.

Introduction

Children of depressed parents are two-three times more likely than the offspring of non-depressed controls to exhibit elevated levels of depression and anxiety symptoms and depressive disorder (Weissman, Wickramaratne, et al., 2006). Despite consistent evidence that depression is familial, few studies have tested the processes underlying intergenerational transmission of symptoms by teasing apart competing explanations. First, exposure to parent depression symptoms may have a direct environmental effect on children. Second, links between parent and child symptoms could arise through inherited factors. Finally, shared exposure to adversities such as bereavement, divorce or poverty may increase risk for depression in both parents and children, accounting for observed transmission effects. A greater understanding of the pathways involved in intergenerational transmission can help in the identification of modifiable risk factors for child/adolescent depression symptoms that could be targeted in preventive and therapeutic interventions.

Few studies have used genetically sensitive designs to estimate the influence of inherited factors and separate environmental from genetic effects (Eley, et al., 1998; Rice, Harold, & Thapar, 2005; Silberg, et al., 2010; Tully, et al., 2008). One study did not assess maternal depression directly (Eley, et al., 1998) and another reports inconsistent findings depending on the informant used (Rice, et al., 2005). Two studies have directly tested environmental transmission of maternal depression (Silberg, et al., 2010; Tully, et al., 2008). Tully and colleagues (Tully, et al., 2008) report significant associations between maternal major depression and child major depression in adopted and non-adopted families with greater associations in non-adopted mother-child pairs suggesting environmental and genetic effects. Using a Children of Twins design, Silberg and colleagues (Silberg, et al., 2010) found that the intergenerational transmission of maternal depression symptoms was due to family

environmental factors but report no genetic influence. Findings suggest that parent depression symptoms may exert environmental risk effects on child depression symptoms. However, previous studies have not ruled out the possibility that specific measures of shared environmental adversity (affecting both parents and offspring) contribute to this environmental link or accounts for the association between maternal depression and child symptoms.

Further research on the mechanisms underlying intergenerational transmission of depression is required. In addition to the role of shared adversity, such research should also consider the effects of child gender and age which have not been examined using genetically sensitive designs. There is evidence that different factors may be involved in the aetiology and intergenerational transmission of depression for boys and girls (Silberg, et al., 1999). A number of studies have found that the association between maternal and offspring depression is stronger in daughters than in sons (Cortes, et al., 2006; Fergusson, et al., 1995). However, other studies do not report significant gender differences (Bureau, et al., 2009). The aetiology of child and adolescent depression may also vary according to the developmental stage of children. A consistent finding from twin research is that genetic influences on depression symptoms are greater in adolescents whilst shared environment is more influential in children (Thapar & McGuffin, 1994). This suggests that non-inherited factors could play a more prominent role in the intergenerational transmission of depression symptoms for younger children. Finally, there is consistent evidence that anxiety and depression symptoms have shared genetic liability and that, in pre-pubertal children, this liability more commonly manifests as anxiety (Kendler, Gardner, & Lichtenstein, 2008). This suggests the need to examine both anxiety and depression in the offspring of depressed parents.

The present study, based on a sample of children conceived by assisted reproduction, aimed to test whether there are environmentally-mediated links between symptoms of maternal depression and child anxiety/depression, and whether they are accounted for by exposure to three measures of shared adversity – negative life events, family income and socio-economic status. We also investigated the effects of child age and gender. The sample allows tests of environmental and genetic transmission using principles akin to the classic behavioural genetic adoption design: comparisons of associations between genetically unrelated and related parent-child pairs. Associations of parent and child symptoms in genetically unrelated dyads indicates the presence of environmental transmission, implicating the role of the shared environment (C) whilst evidence for genetic effects would be shown if associations are greater in related versus unrelated pairs (G). A recent study using this sample examined depression and conduct symptoms and found preliminary evidence of environmental links (Harold et al., 2010). However, this study focused on mediation of the association by parent-child warmth and hostility and did not rule out the possibility that alternative influences such as shared adversity may account for effects or that associations may differ according to child age and gender. It was hypothesized in the present study that maternal depression symptoms, in addition to showing inherited transmission, would have direct environmental links, not accounted for by shared adversity, with symptoms of depression and anxiety in children and that the environmental association would be stronger in girls and pre-adolescent children under the age of 11 years.

Method

Sample

The sample consisted of families recruited from 19 fertility clinics across the UK and one in the US who had undergone In Vitro Fertilization (IVF) with children born between 1994 and 2002. 22 clinics were approached and 19 agreed to participate,

representing a clinic response rate of 86%. Children included in this report were conceived via one of 4 IVF methods: homologous (genetically related to both parents), sperm donation (genetically related to mother only), egg donation (genetically related to father only), or embryo donation (genetically related to neither parent). All gamete donors were unrelated to parents. One child from each family participated. In the case of multiple births, parents were asked to report on the first born child. Data were collected by postal questionnaire from mothers and fathers on two occasions (T1; 2006 and T2; 2009). Questionnaires included a range of measures assessing details of the pregnancy, child psychopathology, the family environment and parental psychopathology.

1158 families were sent questionnaires at T1. Responses were received from 852 families; 74% response rate). 781 families agreed to be contacted about further studies and these families were mailed follow-up questionnaires at T2. At T2, responses were received from 482 families; 62% response rate. Participants who dropped out at T2 did not differ from those who remained in the study on maternal depression symptoms at T1 (mean retained in study = 4.24 (sd=3.04), mean dropped out of study = 4.46 (sd=3.10), t (848) = 1.04, p = .30) or child anxiety/depression; mother rated (mean retained in study = 4.68 (sd=3.96), mean dropped out of study = 4.50 (sd=4.57), t (847) = .61, p = .55) and father rated (mean retained in study = 4.34 (sd=4.29), mean dropped out of study = 3.93 (sd=4.29), t (568) = 1.15, p = .25). Attrition was not associated with genetic relatedness to the child (x^2 (1) = .94, p = .33). The effects of potential biases caused by sample attrition were investigated by running T1 analyses on the subgroup retained across waves; the same pattern of results was found (available on request).

Comparisons with UK national norms have shown that this sample does not differ from the general population in terms of psychological adjustment with minimal

differences also found between the conception groups (Shelton et al., 2009). The present study used data from families in which the mother had responded (n=852, 431 boys, 421 girls at T1, n=459, 231 boys, 228 girls at T2). At T1 the numbers in each conception group were: 436 homologous, 208 sperm donation, 173 egg donation and 35 embryo donation and at T2: 254 homologous, 99 sperm donation, 91 egg donation and 15 embryo donation. The genetically unrelated group consisted of 208 mother-child pairs (egg, embryo donation) and the genetically related group consisted of 644 mother-child pairs (homologous, sperm donation). Children were aged 4 – 10 years (mean = 6.3 years) at T1 and 7 – 13 years (mean = 9.9 years) at T2. Mothers were aged 27- 61 years (mean = 41.44 years) and fathers were aged 28 -76 years (mean = 44.3 years) at T1. The majority of children (91%) resided with both parents.

Measures

Maternal Depression symptoms. Maternal depression symptoms were assessed using the 7-item self-report depression subscale of the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983). This subscale showed good internal consistency in this sample (α = .75 at T1 and α = .70 at T2) and is well validated (Bjelland, Dahl, Haug, & Neckelmann, 2002). Scores on the subscale range from 0-21 with higher scores representing greater depression symptoms. A recommended clinical cut point of greater than 8 has been validated in clinical and community samples (Bjelland, et al., 2002).

Child anxiety and depression symptoms. Child depression symptoms were assessed using parent ratings (mothers and fathers separately) on the Short Mood and Feelings Questionnaire (SMFQ). Child self-reports were not obtained because of the children's young age and also for ethical reasons (children might not be aware of parental IVF treatment). The SMFQ is a 13-item self-report measure of the

presence and severity of DSM-IV depression symptoms which showed good internal consistency in this sample (α = .73 for mothers and .79 for fathers at T1 and α = .75 for mothers at T2) and is a well validated measure in both clinical and community samples (Thapar & McGuffin, 1998). Total scores range from 0-26 with higher scores representing greater depression symptoms and the recommended clinical cut point is greater than 8 (Thapar & McGuffin, 1998). Child anxiety symptoms were assessed using parent reports on six DSM-IV items for generalized anxiety disorder over the past 3 months (worries, restless, easily tired, irritable, difficulty concentrating, problems sleeping). The anxiety scale showed good internal consistency in this sample (α = .73 for mothers and .67 for fathers at T1 and α = .76 for mothers at T2). Anxiety scores ranged from 0-12 with higher scores representing greater anxiety symptoms. A combined anxiety/depression scale was created by summing SMFQ and DSM-IV anxiety items and showed good internal consistency (α = .83 for mothers and .85 for fathers at T1 and α = .87 for mothers at T2). Scores on the composite measure ranged from 0-38 with higher scores representing more anxiety/depression symptoms.

Negative Life Events. A 35-item Life Events Checklist (Johnson & McCutcheon, 1980) completed by mothers measured the occurrence of a range of significant events in the child's life during the past year such as 'death of a parent, grandparent or sibling.' Fourteen independent negative life events likely to affect both mother and child were used.

Family income. Mothers and fathers were each asked to rate the approximate gross family income on a 7-point scale ranging from <10,000 a year to >60,000 a year.

Family socio-occupational class. Families were classified according to the occupation of the main earner (Office for National Statistics, 2000).

Statistical Analyses

The sample was divided into two groups; genetically unrelated mother-child pairs (egg and embryo donation) and genetically related mother-child pairs (homologous IVF and sperm donation). Association between maternal depression symptoms and child anxiety/depression symptoms was calculated separately in each group. The principle of the IVF design is consistent with the classic behavioural genetic adoption design: The presence of an association in unrelated mother-child pairs would provide evidence of environmental transmission since inherited links are removed therefore indicating the role of the shared environment (C). If an association is seen only in related mother-child pairs, this indicates inherited transmission suggesting that parent-child links are due to genetic factors (G). When association is seen in both groups but is stronger in the related group, this indicates both genetic and environmental links (G+C). Associations were calculated using the Pearson product-moment correlation coefficient (Pearson's r) since variables were continuous and normally distributed after transformation. The difference in the magnitude of correlation coefficients across groups was tested using Fishers' R to Z transformation. This test converts Pearson's r to the normally distributed variable Z enabling a comparison of the strength of associations. Associations were then re-tested in each group controlling for differences in maternal age, child age, and family structure (two parent or single parent family).

To test whether associations differed according to child age, analyses were conducted separately at T1 (mean child age = 6.3 years) and T2 (mean child age = 9.9 years). The sample was then split at T2 into children (aged 11 years and younger) and adolescents (aged 11 years and over). All analyses were conducted

using both mother and father ratings of child symptoms to control for shared rater effects; however, father ratings were only available at T1 due to low paternal response at T2. Associations between maternal depression symptoms and child anxiety/depression symptoms (mother and father rated) were then tested adjusting for three measures of shared adversity: independent stressful life events, family income and family social occupational class. The role of shared adversity was examined at T1 due to the larger available sample size.

The role of child gender was tested next. Mother-child associations were examined separately for boys and girls at both time points (using mother and father ratings at T1 and mother ratings only at T2) to test whether the effects of gender varied by child age.

Finally, the role of paternal depression symptoms was examined. First, associations between maternal depression symptoms and child anxiety/depression were tested controlling for paternal depression symptoms to rule out the possibility that father psychopathology could have been driving mother-child associations. Second, the association between paternal depression symptoms and child anxiety/depression was tested separately in genetically related (homologous IVF, egg donation) and unrelated (sperm and embryo donation) father-child pairs controlling for shared adversity.

Results

Parent and child symptoms and demographic characteristics

Children were significantly younger in the mother unrelated group (egg and embryo donation) (mean age T1 unrelated group = 6.00 years; mean age T1 related group = 6.35 years, $t\ (850) = 3.5$, $p < .001$). A greater percentage of children lived with their mother only in the unrelated (10%) versus the related group (5%) and this difference

was statistically significant (x^2 (1) = 5.1, p < .05). Mothers in the unrelated group were significantly older; mean maternal age in the unrelated group was 44.91 years, mean age in the related group was 40.31 years, (t (849) = 12.79, p < .001). There were more multiple births in the unrelated versus the related group (24% versus 21%) but this difference was not statistically significant (x^2 (1) = .76, p > .05).

Mean maternal depression symptoms did not differ according to relatedness at T1 (mean unrelated = 4.63, SD = 3.06, range = 0 – 15, n = 208, mean related = 4.24, SD = 3.06, range = 0 – 18, n = 642, t (848) = 1.59, p = .11) or T2 (mean unrelated = 4.58, SD = 3.49, range = 0 – 18, n = 106, mean related = 4.04, SD = 3.19, range = 0 – 21, n = 353, t (457) =1.48, p=.14). Mean paternal depression symptoms did not differ at T1 (mean unrelated = 3.86, SD = 2.70, range = 0 – 14, n = 167, mean related = 3.87, SD = 3.12, range = 0 – 21, n = 410, t (575) = 0.4, p = .97). Mothers reported significantly higher mean depression symptoms than fathers (t (576) = 5.64, p < .01). Parent depression symptoms were positively skewed so were square root transformed. Child anxiety/depression symptoms also showed no significant differences across groups according to mother ratings (mean unrelated = 4.04, SD = 3.54, range = 0 – 19, n = 208, mean related = 4.78, SD = 4.44. range = 0 – 19, n = 641, t (847) = 2.18, p=.06 at T1; mean unrelated = 5.18, SD = 4.21, range = 0 – 21, n = 105, mean related = 4.91, SD = 4.82, range = 0 – 30, n = 352, t (455) = .522, p = .60 at T2) and father ratings (mean unrelated = 3.66, SD = 3.79, range = 0 – 20, n = 133, mean related = 4.32, SD = 4.37, range = 0 – 32, n = 437, t(568) = 1.58, p=.12). 15% of mothers and 9% of fathers scored above the clinical cut-point on the HADS at T1 and 14% of mothers at T2. 5% of children scored above the clinical cut-point on the SMFQ at T1 according to both mother and father ratings and 7% at T2 according to mother ratings.

Testing for inherited and environmental links between maternal depression symptoms and child anxiety/depression.

The association between maternal depression symptoms and child anxiety/depression was significant in both groups for mother rated (unrelated: r=.32, p < .001, n=207, related: r=.31, p < .001, n=639) and father rated (unrelated: r=.26, p < .01, n=132, related: r=.22, p < .001, n=436) child symptoms at T1. Correlation coefficients did not differ across related and unrelated groups according to Fishers' R to Z for mother ratings (Z=.28. p=.39) or father ratings (Z=.31. p=.38) of child symptoms. Correlation coefficients did not differ across rater in unrelated (Z=.66. p=.25) and related (Z=1.56. p=.06) groups. To control for known group differences, maternal age, child age and family structure were then entered as covariates at T1. Associations remained significant for mother (unrelated; r=.33, p < .001, n=207; related; r=.31, p < .001, n=638) and father (unrelated; r=.26, p < .01, n=132, related; r=.22, p < .001, n=436) ratings of child symptoms.

Testing the contribution of shared adversity

The association at T1 was examined controlling for negative life events likely to affect mother and child, family income and family social occupational class (Table 5). Associations remained significant and unattenuated after controlling for each adversity separately across groups for mother and father rated child symptoms. When all three variables were entered into a multiple partial correlation analysis with maternal depression symptoms, associations for mother and father rated child symptoms remained significant in both groups. Associations remained the same in genetically unrelated and related groups, after controlling for shared adversity, when child/adolescent anxiety and depression symptoms were considered separately (Table 6).

Table 5. Testing environmental transmission controlling for shared adversity

	Genetically related		Genetically unrelated	
	Partial correlation coefficient	**N**	**Partial correlation coefficient**	**N**
Independent negative life events				
Mother rated child symptoms	.30 ***	637	.30 ***	206
Father rated child symptoms	.23 ***	434	.26 **	131
Family income				
Mother rated child symptoms	.29 ***	614	.33 ***	202
Father rated child symptoms	.21 ***	431	.23 **	131
Family socio-occupational class				
	.31 ***	622	.31 ***	197
Mother rated child symptoms	.23 ***	431	.25 **	131
Father rated child symptoms				
Multiple partial correlation				
Mother rated child symptoms	.30 ***	596	.30 ***	191
Father rated child symptoms	.22 ***	416	.22 **	129

*p<.05, **p<.01, ***p<.001

Table 6. Testing environmental transmission separately for child anxiety and depression symptoms. Mother rated child symptoms above, father rated below. Mother only at T2.

	Genetically related		Genetically unrelated	
	Correlation coefficient	**N**	**Correlation coefficient**	**N**
Child depression symptoms T1	.25***	639	.29***	207
	.18***	439	.32***	133
Child anxiety symptoms T1	.31***	638	.31***	207
	.23***	439	.16***	133
Child depression symptoms T2	.29***	340	.40***	99
Child anxiety symptoms T2	.27***	347	.40***	100

*p<.05, **p<.01, ***p<.001

Age effects

The analyses at T2 when children were aged 7-13 years showed the same pattern of effects as T1 (Table 7). Correlation coefficients did not differ according to Fishers' R to Z (Z=1.04, p=.15). Maternal depression symptoms also predicted child anxiety/depression in both the unrelated and related groups in children under and over 11 years.

Table 7. Associations between maternal depression symptoms and child anxiety/depression symptoms tested separately by child age

Maternal depressive symptoms and child anxiety/depression				
	Relatedness Group			
	Genetically related		**Genetically unrelated**	
	Correlation coefficient	**N**	**Correlation coefficient**	**N**
T1 (age 4-10 years)	.31 ***	639	.32 ***	207
T2 (age 7-13 years)	.32 ***	352	.44 ***	105
Sample split by age at T2	**Genetically related**		**Genetically unrelated**	
	Correlation coefficient	**N**	**Correlation coefficient**	**N**
Over 11 years T2	.34 ***	134	.40 *	33
Under 11 years T2	.32 ***	218	.46 ***	72

*p<.05, **p<.01, ***p<.001

Child gender

Associations tested separately for child gender are displayed in Table 8. Significant association between maternal depression symptoms and mother rated child anxiety/depression was found for girls in unrelated and related groups at T1.

Correlations did not differ according to Fishers' R to Z (Z=.31, p=.38). When father rated data were used, the same pattern was evidenced for girls in unrelated and related groups and correlations did not differ according to Fishers' R to Z (Z=.1.21, p=.11). At T2, for girls, the association remained significant in unrelated and related groups for mother rated child symptoms and correlations were not significantly different (Z=.83, p=.20). For boys, at T1 the association was significant in the related but not the unrelated group and this pattern was replicated with father reported child symptoms (related: r=.21, p < .001, n=210, unrelated: r=.10, p = .46, n=59). However, the magnitude of associations did not differ significantly between genetically related and unrelated groups, for either mother (Z=.82, p=.38) or father (Z=.75, p=.23) ratings. At T2, the association for boys was significant in both related and unrelated groups. Comparisons of correlations for boys and girls in the unrelated group at T1 were significant for both mother (Z=1.71, P<.05) and father (Z=1.73, P<.05) ratings with a larger effect observed for girls. The difference between boys and girls in the unrelated group at T2 approached significance (z=1.54, p=.06). Gender and age results remained the same when analyses were rerun including the previously used covariates and shared adversities.

Table 8. Associations between maternal depression symptoms and child anxiety/depression symptoms tested separately by child gender

Maternal depressive symptoms and child anxiety/depression				
Gender effects	**Relatedness Group**			
	Genetically related		**Genetically unrelated**	
	Correlation coefficient	**N**	**Correlation coefficient**	**N**
Boys T1				
Mother rated child symptoms	.28 ***	329	.18	99
Father rated child symptoms	.21 ***	210	.10	59
Girls T1				
Mother rated child symptoms	.37 ***	309	.40 ***	107
Father rated child symptoms	.24 ***	225	.39 ***	107
Boys T2	.26 ***	185	.25 **	43
Girls T2	.41 ***	165	.51 ***	60

*p<.05, **p<.01, ***p<.001

The role of paternal depression symptoms

The association between maternal depression symptoms and child anxiety/depression was tested controlling for the role of paternal depression symptoms. Associations remained significant for mother ratings of child symptoms (unrelated: r=.31, p < .001, n=133, related: r=.28, p < .001, n=441) and father ratings (unrelated: r=.17, p < .05, n=131, related: r=.16, p < .001, n=434). The association between paternal depression symptoms and child depression/anxiety was significant in the unrelated (r=.17, p<.05, n=166) and related (r=.23, p<.001, n=408) groups using mother rated child symptoms at T1. Correlation coefficients did not differ according to Fishers' R to Z (z=.67, p=.25) and remained significant after controlling for the same covariates and shared adversities.

Discussion

The importance of genetically sensitive designs in identifying environmental risk factors is well recognised. The aim of this study was to test the extent to which environmental links between maternal depression symptoms and child symptoms demonstrated in previous reports were explained by measures of shared adversity and by child age and gender.

Results are consistent with an environmental link between maternal depression symptoms and child anxiety/depression. Associations were observed regardless of whether mothers and children were genetically related or not. There are now converging findings across different genetically sensitive designs of environmental links between maternal and offspring depression (Harold, et al., 2010; Silberg, et al., 2010; Tully, et al., 2008). Our findings therefore consolidate evidence that intergenerational transmission of depression symptoms is due at least in part to environmental mechanisms and does not arise entirely from shared genetic liability. The presence of an association in the unrelated group also rules out passive gene-environment correlation which is absent in genetically unrelated mother-child pairs. We accounted for three measures of shared adversity; negative life events, family income and family socioeconomic status and for depression in fathers. Adjusted associations remained significant in genetically unrelated and related parent-child pairs. This favours the suggestion that non-inherited links between mother and child depression may arise from direct environmental effects and are not attributable to the extraneous variables measured in this study. This is consistent with a study which found that shared stressful life events and social support networks did not contribute to links between maternal and child depression (Rice, et al., 2005). Although the covariates, family income, family social occupational class and life events may be genetically influenced and meaningful genetic variance may therefore be

unsystematically removed, the pattern of genetic and environmental transmission was the same whether or not the covariates were included.

Child age is an important factor to consider since depression aetiology has been found to differ developmentally with greater genetic influences in adolescence (Scourfield, et al., 2003). Significant environmental links were found when children were 4-10 years and 7-13 years. At T2 significant environmental transmission effects were found in both younger children (7-11 years) and those in early adolescence (11-13 years). Significant environmental transmission effects were found in both age groups suggesting that associations were not due to the young age of the sample and that environmental links also operate in children entering early adolescence. This concurs with previous genetically sensitive studies on transmission effects which looked at older samples (Silberg et al., 2010; Tully et al., 2008).

Tully and colleagues (Tully, et al., 2008) found evidence for the role of genetic factors. Although genetic influences cannot be ruled out in the present study, we found no clear support for a genetic contribution to transmission apart from in boys (see later); maternal-child associations were not stronger in the genetically related group. Interestingly, the study by Silberg and colleagues (Silberg, et al., 2010) also reports no significant genetic influence. However, in contrast to the study by Tully and colleagues (Tully, et al., 2008), our findings and those of Silberg and colleagues (Silberg, et al., 2010) relied on symptoms rather than diagnoses of depression so it is possible that inherited transmission is more pronounced for maternal depression symptoms of greater severity. Indeed, a study of adolescent female twins found that the contribution of shared environmental factors differed according to the phenotype examined with shared environmental effects observed for a broad depressive phenotype but not for major depressive disorder (Glowinski, et al., 2003). Alternatively, it may be that the heritable phenotype manifests in a different way, for

example, as overall levels of psychopathology in children rather than as depression symptoms per se. In contrast to these previous studies, we examined a composite of depression/anxiety scores. However, the pattern of maternal-child associations was found to be the same for child depression scores alone. Another consideration is that none of the designs (adoption, children of twins, IVF) are able to account for genetic heterogeneity between depression in childhood and depression in adulthood. If different genes are responsible for depression in childhood versus adulthood (Kendler, et al., 2008), this may have the effect of reducing the genetic transmission estimate. This is an important direction for future research.

Based on a range of studies suggesting that maternal depression may have stronger adverse effects for girls, we examined whether patterns of intergenerational transmission differed by gender. The significant association in genetically unrelated mother-daughter pairs indicates an environmental effect. For mother-son pairs, the association was only significant in the genetically related group suggesting that environmental transmission was less strong for boys. These findings are in agreement with numerous non-genetic studies which report stronger associations between maternal depression and child depression in girls (Cortes, et al., 2006; Fergusson, et al., 1995) and suggest that for girls this effect may occur through environmental pathways because the association was of a similar magnitude in the genetically unrelated and related groups. This concurs with research suggesting that girls are more sensitive to stressful life events in general, especially those of an interpersonal nature. The significant association between maternal depression symptoms and child anxiety/depression in related but not unrelated groups for boys does not conclusively rule out environmental transmission for boys as the difference in the magnitude of correlations was not large. Indeed, environmental transmission was seen for boys in analyses conducted at T2. Prior non-genetic studies of the association between maternal and child depression have reported stronger

associations for girls only at puberty which coincides with the emergence of the gender difference in depression prevalence (Cortes, et al., 2006). However, the present study suggests that from a younger age, girls may already possess an increased vulnerability to maternal depression exposure. A recent study attests to this view (Silk, Shaw, Skuban, Oland, & Kovacs, 2006).

The results of this study must be interpreted in light of certain limitations. We cannot rule out the possibility that other unmeasured shared adversities contribute to the intergenerational link. The association between maternal depression symptoms and child mood cannot be assumed to be unidirectional. A lack of power in the longitudinal analyses when the sample is split by conception group and gender precluded an investigation of reverse causation. Despite evidence that the association is likely to be bidirectional, research suggests that it is unlikely to be accounted for by child effects on parent symptoms alone. A recent prospective longitudinal study of parental depressive symptoms and child temperament found significant parent to child effects but little support for child to parent effects (Hanington, Ramchandani, & Stein, 2010). Like other longitudinal studies, attrition affected sample retention especially for father participation. There was no evidence that this biased the results (those who dropped out of the study did not differ to those who were retained on maternal and child depression symptom scores), but it did limit the scope and power available for some T2 analyses. Significant environmental transmission but a lack of differences between the related and unrelated groups suggesting no genetic transmission could also be accounted for by the use of self-report questionnaire measures which are subject to greater error than interview measures. The present study focused on symptom ratings and further studies focusing on clinical diagnoses of parent depression and on clinical outcomes in children would be of value. However, focus on symptoms is still useful to an understanding of clinical diagnoses because symptoms and disorder show similar

correlates (e.g. increased rates in postpubertal girls) and similar patterns of association with risk factors (e.g. life events, family adversity).

Another issue is the use of an age-based cut-off to approximate the effects of pre- and post-puberty as this is not an exact measure of pubertal development. However pubertal data were not collected so this was the best available approximation. Finally, IVF may result in epigenetic modifications that lead to offspring born with imprinting disorders (DeBaun, Niemitz, & Feinberg, 2003). In practice, however, this sample has been shown to be comparable to the normal population in terms of emotional and behavioural adjustment. This does not rule out the possibility that epigenetic modifications have occurred though this would presumably have the effect of introducing greater phenotypic differences between parents and children in this sample compared to naturally conceived offspring and parents.

Conclusions

The findings have clinical implications for the design of preventive interventions: concluding that a putative causal risk factor such as maternal depression symptom exposure confers its effect via an environmental mechanism suggests that this factor can be targeted in attempts to prevent and treat child and adolescent depression. In support of this, evidence suggests that changes in current maternal depression status have corresponding effects on child outcome (Weissman, Pilowsky, et al., 2006). The environmental effect of maternal depression appears to be stronger in girls than boys suggesting that depression in mothers carries a particular risk effect for daughters implying that interventions targeted at treating parental depression may have a larger impact on girls as supported by a recent meta-analysis of preventive interventions (Stice, Shaw, Bohon, Marti, & Rohde, 2009). In addition we found that parent depression has an environmental link with anxiety/depression symptoms from childhood through to early adolescence. We found little support for the role of genetic

factors on the transmission of depression symptoms apart from for boys and this concurs with a prior genetically sensitive study using a Children of Twins model (Silberg, et al., 2010). This does not negate the role of child and adult specific genetic factors in the aetiology of depression symptoms which is a well established and consistent finding. It is likely that both the aetiology and transmission of depression is due to a complex interplay between genetic and environmental factors.

Chapter 7

Maternal Depression and Child and Adolescent Depression Symptoms: An Exploratory Test for Moderation by CRHR1, FKBP5 and NR3C1 Gene Variants

Paper 3: Maternal Depression and Child and Adolescent Depression Symptoms: An Exploratory Test for Moderation by *CRHR1, FKBP5* and *NR3C1* Gene Variants. Lewis G, Collishaw S, Harold G, Rice F, Thapar A. *Behavior Genetics*. Jul 26. [Epub ahead of print]

Maternal depression, mother-child hostility and child and adolescent depression symptoms: testing gene-environment interaction

This thesis has examined the role of family processes in the aetiology of child and adolescent depression symptoms using different research designs to test environmental risk effects of two specific family variables - parent-child hostility (chapter 5), and parent depression (chapter 6). Results from chapters 5 and 6 suggest that both of these family risk factors have environmentally mediated links with depression symptoms in children and adolescents. In addition to environmental processes the aetiological pathways to depression are known to involve genetic susceptibility, however, main effects of specific genes have not been consistently identified. An important aim in addition to identifying the specific environments which confer risk is therefore to test for interaction with specific gene variants (GxE) (Karg, et al., 2011). In examining GxE, it is first necessary to establish whether the candidate psychosocial risk factors confer direct environmental effects not accounted for solely by genetic influences. Following the identification of environmental risk effects in chapters 5 and 6, in the next chapter I test whether the associations found between each family factor – maternal depression and mother-child hostility with child and adolescent depression symptoms are moderated by gene variants previously implicated as contributing to the stress response. The main content of the chapter is an original paper (currently under 2nd review) that focuses on genetic interaction with recurrent maternal depression. For the purpose of this thesis, since environmental risk effects were also found for mother-child hostility, I have conducted additional exploratory GxE analyses on this psychosocial risk factor. These analyses were not included in the submitted paper to retain focus and also, because the dataset that included the necessary molecular genetic and hostility data was small.

Abstract

This study investigated whether the association between recurrent maternal depression and offspring depression symptoms was moderated by a selection of gene variants shown to be involved in the regulation of the stress response. The sample consisted of 271 children (aged 9 to 16 years) whose mothers had experienced at least two episodes of DSM-IV major depression and 165 controls (aged 12 to 16 years) drawn from a population-based twin register. 7 single nucleotide polymorphisms (SNPs) from three genes were genotyped in children. The genes tested were the *Corticotrophin Receptor Type 1* gene (*CRHR1*), the gene coding for the FK506 binding protein 51 (*FKBP5*) and the *glucocorticoid receptor gene* (*NR3c1*) gene along with a haplotype formed by the SNPs in *CRHR1*. A significant association was found between recurrent maternal depression and depression symptoms in offspring. However, none of the SNPs were associated with offspring depression symptoms and associations did not differ according to the presence of recurrent maternal depression. Findings suggest that previously reported interaction between *CRHR1* in relation to childhood maltreatment and child depression does not extend to another stressor; recurrent maternal depression. Similarly, variation in *FKBP5* and *NR3c1* does not appear to predict child and adolescent depression symptoms in the presence of recurrent maternal depression. However, caution is required due to a relatively small sample size.

Introduction

Depression is a complex and multifactorial disorder, likely to result from the effects of numerous interacting pathways involving both genetic and environmental risk (Lau & Eley, 2010). Molecular genetic studies have so far had limited success in consistently identifying susceptibility genes for depression (Cichon et al., 2009; Green et al., 2010; Muglia, et al., 2010; Shi, et al., 2010). This has led to increasing interest in the potential contribution of gene-environment interaction (GxE) (Caspi, et al., 2003). GxE can be defined as genetically influenced sensitivity to the effects of stress and adversity and offers an alternative mechanism through which genetic susceptibility to depression may manifest (Rutter & Silberg, 2002). Twin and family studies suggest that gene-environment interaction is an important contributor to depression risk (Kendler et al., 1995; Rice, et al., 2006; Silberg, et al., 1999). This has now led to increasing interest in examining GxE at a molecular level (Caspi et al., 2002; Karg, et al., 2011).

To be considered suitable candidates for GxE studies, environmental risk variables must show environmentally mediated risk effects on the outcome measure that are not accounted for purely by genetic factors (Moffitt, Caspi, & Rutter, 2005). However, environmental risks such as life events and family adversity that have been associated with depression have also been found to be genetically influenced (Kendler, Thornton, et al., 2001; Plomin, et al., 1994). Thus, genetically informative studies and other types of quasi-experimental designs have been important for testing whether associated risk factors have environmentally mediated effects (Kendler, Thornton, et al., 2001; Rutter, 2007b). A consistently established risk factor for depression in children and adolescents is early and chronic adversity including exposure to parent depression (Weissman, Wickramaratne, et al., 2006). Children of depressed parents are 2-3 times more likely than children of non-depressed controls to display a range of negative outcomes including depression symptoms and disorder

and these effects have been shown to persist across time and to predict functioning in adolescence and adulthood (Weissman, Wickramaratne, et al., 2006; Weissman, Wolk, Goldstein, et al., 1999)

There is now a considerable amount of evidence to suggest that the effects of exposure to maternal depression are conferred via "environmental" risk processes to predict increased risk of child and adolescent depression. A range of recent studies using genetically sensitive and treatment designs have suggested that intergenerational transmission of depression from mothers to children is likely to involve environmental as well as inherited processes (Harold, et al., 2010; Lewis, Rice, Harold, Collishaw, & Thapar, 2011; Pilowsky, et al., 2008; Silberg, et al., 2010; Tully, et al., 2008; Weissman, Pilowsky, et al., 2006). A series of treatment studies report that the remission of maternal depression following treatment with antidepressants resulted in concomitant improvements in children's symptoms suggesting the presence of an environmental process (Weissman et al., 2006; Pilowsky et al., 2008). However, treatment studies cannot rule out the potential contribution of genetic factors. Several studies have directly tested environmental transmission of maternal depression using genetically sensitive designs (Harold, et al., 2010; Lewis, et al., 2011; Silberg, et al., 2010; Tully, et al., 2008). Tully and colleagues (Tully, et al., 2008) report significant associations between maternal major depression and child major depression in adopted and non-adopted families with greater associations in non-adopted mother-child pairs suggesting both environmental and genetic effects. Other genetically sensitive studies have supported the role of an environmental process in the transmission of risk but have not found evidence for genetic effects. Using a Children of Twins design, Silberg and colleagues found that the intergenerational transmission of maternal depression symptoms was due to family environmental factors but report no genetic influence (Silberg, et al., 2010). Using a sample of children conceived via In Vitro Fertilization

(IVF), Harold et al., (2010) and Lewis et al., (2011) report a significant association between maternal and child depression in genetically unrelated and related mother-child pairs with no difference in the magnitude of associations across groups. A range of studies therefore suggest maternal depression as an environmental risk factor for child depression and consequently, as a potential candidate for GxE studies (Moffitt, et al., 2005).

In selecting the candidate genes for GxE studies, in addition to demonstrating some evidence of association with depression, it is also necessary that the GxE is biologically plausible. There should therefore be some evidence that the selected genes and putative environmental risk variables affect the same biological systems that are also implicated in pathogenesis of the disorder (Moffitt et al., 2005). This is an important requirement in selecting gene variants. A main biological system potentially involved in the pathogenesis of depression is the Hypothalamic-Pituitary-Adrenal (HPA) axis. The HPA axis is known to regulate the biological response to stress and dysregulation of this system has therefore been implicated as a potential biological mechanism underlying the aetiology of depression (Holsboer, 2000).

HPA axis gene variants have been investigated in several GxE studies in relation to depression. Gene variants thought to influence activity of the corticotropin-releasing hormone receptor (*CRHR1*) have been investigated in several GxE studies assessing the effects of early child abuse and maltreatment in predicting child and adolescent depression. Based on prior evidence that child abuse and trauma have effects on the stress response, Bradley et al., (2008) used an association study design to examine whether the effects of child abuse on adult depression symptoms were moderated by gene variants in the *CRHR1* gene. In a predominantly African American sample (97.4% African American), Bradley et al., (2008) found that after correcting for multiple testing, two single nucleotide polymorphisms (SNPs) and one

haplotype in the *CRHR1* gene significantly moderated the association between sexual, physical and emotional abuse in childhood and adult depression symptoms. 5 SNPs showed significant interaction before correcting for multiple testing. In the same study but using a second, smaller sample (87.7% Caucasian), Bradley et al., (2008) also tested whether the same SNPs conferred a protective effect against the development of major depression in the presence of moderate to severe child abuse. A significant overrepresentation of the SNPs and haplotype was found in women who had experienced moderate to severe child abuse but had not developed major depression. Both SNPs and the haplotype were associated with a protective effect. The significant GxE effect for the haplotype has been replicated (Polanczyk, et al., 2009; Ressler, et al., 2009). However, in the study by Polanczyk et al., (2009) significant GxE was found only when maltreatment was assessed using the same emotional recall measure employed by Bradley et al., (2008). When maltreatment was assessed in a separate sample using a different measure, the same GxE effect was not significant.

In addition to the *CRHR1* gene, variants in the glucocorticoid receptor gene (*NR3c1*) and a gene coding for a co-chaperone protein called FK506 binding protein 51 (*FKBP5*) have been associated with depression and a range of depression-related phenotypes, although findings are inconsistent. Polymorphisms in the *FKBP5* gene have been found to associate with differential upregulation of the protein following activation of the glucocorticoid receptor, differences in glucocorticoid receptor sensitivity and differences in stress hormone regulation (Binder et al., 2008). Alleles associated with enhanced expression of *FKBP5* following activation of the glucocorticoid receptor lead to an increase in the resistance of the receptor and therefore to decreased efficiency of the negative feedback system. This results in a prolongation of the stress hormone response (Binder et al., 2009). Variants in the *FKBP5* gene have been associated with increased recurrence of depression

episodes and with faster response to antidepressant treatment although they were not significantly associated with disease status (Binder, et al., 2008). Lekman et al., (2008) found that a variant of the *FKBP5* gene was associated with disease status after correction for multiple testing and that a different variant was associated with both remission and response. However, only the association with remission remained after correction for multiple testing. A recent study also found that two variants in the *FKBP5* gene were significantly associated with unipolar depression diagnosis (Zobel et al., 2010). The variants were also associated with right hippocampal volume and response to antidepressant treatment which are putative biological markers of depression susceptibility. Brent et al., (2010) found that two variants (one which concurred with the finding by Zobel et al., 2010) were associated with suicidal events in an adolescent sample. In addition to depression, polymorphisms in the *FKBP5* gene have been associated with recovery from psychosocial stress (Ising et al., 2008). A variant in the glucocorticoid receptor gene (*Bcl1*) also altered cortisol response to stress in the same study. Individuals with the *Bcl1*polymorphism have been found to show an increased sensitivity to glucocorticoids in response to the dexamethasone suppression test (van Rossum et al., 2006). The *Bcl1* polymorphism has also been found to occur more frequently in groups of depressed cases versus controls (van Rossum, et al., 2006; Zobel et al., 2008). These results suggest that individuals carrying these variants might be at risk of displaying chronically elevated stress hormone levels after repeated stress which may constitute a risk factor for stress-related disorders including depression.

An important question is whether findings of interaction between gene variants which have shown links with depression extend to other known "psychosocial" risk factors. The aim of the present study was to examine whether previously studied gene variants implicated in the pathogenesis of depression interact with the presence of recurrent maternal depression to predict symptoms of depression in children and

adolescents. It was hypothesized that previously implicated SNPs in *CRHR1* would moderate the effect of maternal depression on child and adolescent depression symptoms. Investigations of *FKBP5* and the *BcI1* polymorphism in the *NR3c1* gene were exploratory in nature because these gene variants have been examined in case-control association studies but not GxE studies. However based on prior associations with depression, it was hypothesized that SNPs in these genes would also moderate the effect of maternal depression on child depression symptoms.

Methods

Sample

Participants in the current study were mothers and children drawn from two larger samples: a high-risk sample of the offspring of mothers with lifetime diagnoses of recurrent major depression (at least 2 episodes of DSM-IV major depression) and a subsample of a population based twin register: the Cardiff study of all Wales and North West of England Twins (CaStANET) (van den Bree, et al., 2007). Two groups of children were therefore created: one consisting of children who had been exposed to maternal depression and the other consisting of of children whose mothers had not been diagnosed with major depression which constituted a population based control group.

High-risk/ "exposed" sample: 271 mothers and children (109 boys and 162 girls aged 9.00 – 17.00 years, mean age = 12.36 years) who participated in the Predicting and Preventing Adolescent Depression Project (overall n = 340, see Figure 1) were included in the current study. Each child resided with at least one biological parent with a history of recurrent depression (at least two episodes of DSM-IV major depression) confirmed using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) diagnostic interview. The sample had been recruited from primary care settings.

Control/ "unexposed" sample: 165 mothers and children (55 boys and 110 girls aged 12.25 – 16.67 years, mean age = 14.35 years) were drawn from a population-based twin register (CaStANET). A random sample of 548 twin pairs was mailed to obtain DNA samples via saliva mouth wash kits in 2008 and 360 individuals replied. These individuals showed similar demographic characteristics to the overall twin sample. For the purposes of this study (see Figure 1), one individual from each twin pair (selected randomly) was included and twin controls unexposed to current maternal depression were selected using a cut-off score of <8 on maternal ratings of the Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983); a 7-item scale assessing mothers' symptoms over the last 3 months. This cut-off point has been found to show appropriate levels of sensitivity and specificity and to discriminate depressed from non depressed cases (Bjelland, et al., 2002; Herrmann, 1997; Kuijpers et al., 2003; Zigmond & Snaith, 1983). The HADS has been validated against the SCAN in the sample of depressed mothers used in the present study and showed a sensitivity of 81.97% and a specificity of 75.23% and correctly identified 76.73% of depression cases.

Measures

Child depression symptoms. Child and adolescent depression symptoms were assessed in both samples using self-reports with the Short Mood and Feelings Questionnaire (SMFQ) (Angold, et al., 1995). The SMFQ is a 13-item self-report measure of the presence and severity of DSM-IV based symptoms of major depression. Participants reported on their depression symptoms over the past 3 months according to whether each item was 'not true,' 'sometimes true,' or 'true.' Responses were coded 0, 1 and 2 respectively and possible total scores ranged from 0-26. The SMFQ showed good internal consistency in the high-risk (α = .88) and control (α=.92) samples.

DNA extraction and genotyping

In both samples saliva was collected using Oragene self-collection kits. DNA was extracted and quantified using Picogreen. All SNPs were commercially genotyped by K Biosciences (www.kbiosciences.co.uk). In the *CRHR1* gene, 3 SNPs were genotyped: rs7209436, rs242924 and rs110404. A common TAT haplotype formed by these 3 SNPs was also examined (SNPs are in LD with each other). Haplotype analysis was performed using PLINK (Purcell et al., 2007). In the *FKBP5* gene, 3 SNPs were tested: rs1360780, rs4713916 and rs3800373. One SNP in the *NR3c1* gene was tested: rs41423247. Association analysis was based on risk genotypes highlighted by previous studies.

Statistical Analyses

Analyses were conducted separately for each SNP. SNPs were coded under an additive model and analysed by genotype rather than allele to maintain consistency with prior studies (Bradley et al., 2008; Polanczyk et al., 2009). First, the association between maternal depression and child and adolescent depression symptoms was tested using linear regression. Second, we tested whether genotype frequencies varied according to whether children and adolescents belonged to the exposed versus the unexposed sample. Third, the association between each SNP and child and adolescent depression symptoms was tested using linear regression. Finally, interaction analyses were conducted using multiple linear regression. The maternal depression variable (coded as 0 for the unexposed group and 1 for the exposed group), genotype (coded under an additive model; 0, 1 and 2 with the highest score representing the risk genotype) and an interaction term computed from the maternal depression and genotype variables were simultaneously entered into a multiple linear regression model to predict child and adolescent depression symptoms. All analyses were conducted in the high-risk and population samples separately and also in the

"combined sample" which included both the exposed and the unexposed groups (n=436).

Results

Children and adolescents were significantly older in the unexposed control sample (mean control sample = 14.35 years, mean high-risk sample = 12.36 years, $t(434)=11.91$, $p<.001$). There were significantly more girls than boys in both samples (control sample; $\chi^2(1)=16.78$, $p<.01$; high-risk sample $\chi^2(1)=8.89$, $p<.01$). In the combined sample, child depression scores ranged from 0-25 with a mean of 5.20 (sd=5.55). In the combined sample, child depression scores were significantly higher for females (mean males=4.19, sd=4.90, n=164, mean females=5.81, sd=5.84, n=271, $t(434)=2.96$, $p<.01$). Child depression scores did not differ by gender when the high-risk and control samples were tested separately. Child depression scores were positively skewed so were natural log transformed and transformed scores were used in all subsequent analyses. Child depression scores were not correlated with child age in either sample separately or in the combined sample.

Association between maternal depression and child and adolescent depression symptoms

Child depression scores were significantly higher in the high-risk sample ($t(434)=4.19$, $p<.001$, n=434). There was a significant association between exposure group (high-risk, control) and child and adolescent depression symptoms overall ($\beta=.21$, $p<.001$, n=434) and for boys ($\beta=.19$, $p<.01$, n=163) and girls ($\beta=.24$, $p<.001$, n=271) separately.

Association between child genotype and child and adolescent depression symptoms

Genotype frequencies for each SNP are presented in Table 9 separately for the combined, high-risk and control samples. All SNPs in both samples were in Hardy-Weinberg equilibrium (HWE). There were no significant differences in the frequency of each genotype between the high-risk and control samples. Mean child and adolescent depression scores according to genotype are shown in Table 10. Using linear regression analyses, there was no evidence of association between child and adolescent depression scores and the SNP genotypes and haplotype (Table 11).

Table 9. Genotype frequencies for each SNP

Gene and SNP Accession Number	Genotype frequencies (%)			Difference test (high-risk versus population sample)	
	Combined	High-risk	Control	X^2 (df)	p value
CRHR1					
rs7209436					
CC	*36.7*	*35.7*	*38.4*	.41 (2)	.82
TT	16.2	16.9	15.1		
CT	47.1	47.4	46.5		
rs242924					
GG	*34.9*	*34.2*	*36.0*	.25 (2)	.88
TT	17.8	18.4	16.8		
TG	47.3	47.4	47.2		
rs110402					
GG	*35.4*	*34.4*	*37.1*	.74 (2)	.69
AA	17.2	18.3	15.2		
AG	47.3	47.4	47.7		
Number of TAT haplotype copies					
0	36.7	35.6	38.7	1.0 (2)	.61
1	47.7	47.5	48.0		
2	15.6	16.9	13.3		
FKBP5					
rs1360780					
TT	9.6	10.7	7.7	2.6 (2)	.28
CC	49.5	51.1	46.8		
CT	40.9	38.2	45.5		
rs3800373					
GG	9.3	10.2	7.8	1.7 (2)	.42
GT	36.8	34.6	40.5		
TT	53.9	55.3	51.6		
rs4713916					
AA	10.2	12.5	6.2	4.1 (2)	.13
GG	50.9	49.8	52.7		
GA	38.9	37.7	41.1		
NR3c1					
rs41423247					
GG	37.6	35.0	42.1	2.5 (2)	.28
CC	13.4	13.2	13.8		
GC	49.0	51.9	44.1		

*Hypothesized risk allele for each SNP is based on previous studies and is presented in bold and italics

Table 10. Mean child and adolescent depression scores according to genotype

Gene and SNP Accession Number	**Mean, sd, child/adolescent depression scores**	
	High-risk	**Control**
CRHR1		
rs7209436		
CC	5.74, 5.65 (n=95)	3.80, 4.55 (n=60)
TT	6.58, 5.60 (n=45)	4.61, 2.09 (n=23)
CT	5.78, 5.95 (n=126)	4.77, 5.47 (n=71)
rs242924		
GG	5.85, 5.65 (n=91)	3.93, 4.52 (n=57)
TT	6.98, 6.25 (n=49)	3.08, 5.89 (n=26)
TG	5.57, 5.66 (n=126)	4.29, 5.06 (n=73)
rs110402		
GG	5.64, 5.46 (n=90)	3.82, 4.46 (n=55)
AA	7.15, 6.29 (n=48)	3.41, 6.32 (n=22)
AG	5.39, 5.60 (n=124)	4.49, 5.12 (n=69)
Number of haplotype copies		
0	5.49, 5.44 (n=93)	3.75. 4.40 (n=57)
1	5.71, 5.94 (n=124)	4.87, 5.52 (n=69)
2	6.61, 5.66 (n=44)	2.26, 5.02 (n=19)
FKBP5		
rs1360780		
TT	6.39, 5.84 (n=28)	3.00, 2.37 (n=12)
CC	5.84, 5.85 (n=134)	3.81, 4.81 (n=70)
CT	5.53, 5.35 (n=100)	4.48, 5.61 (n=69)
rs3800373		
GG	7.07, 5.56 (n=27)	3.50, 2.75 (n=12)
GT	5.59, 5.47 (n=92)	4.42, 5.70 (n=60)
TT	5.88, 6.00 (n=147)	3.71, 4.74 (n=76)
rs4713916		
AA	5.36, 4.83 (n=33)	3.78, 3.31 (n=9)
GG	5.96, 6.06 (n=132)	3.63, 4.63 (n=73)
GA	5.90, 5.54 (n=100)	4.85, 5.90 (n=59)
NR3c1		
rs41423247		
GG	6.20, 6.05 (n=93)	4.24, 4.90 (n=62)
CC	6.11, 5.99 (n=35)	2.71, 3.58 (n=21)
GC	5.58, 5.40 (n=138)	4.34, 5.58 (n=64)

*Hypothesized risk allele for each SNP is based on previous studies and is presented in bold and italics

Table 11. Association between each SNP and child and adolescent depression symptoms

Gene and SNP Accession Number	Combined	High-risk	Control
CRHR1			
rs7209436	β= -.00, p=.94, n=424	β= -.04, p=.48, n=265	β= -.09, p=.24, n=158
rs242924	β= -.01, p=.87, n=426	β= -.05, p=.47, n=265	β= .08, p=.34, n=160
rs110404	β= -.04, p=.46, n=412	β= -.06, p=.30, n=261	β= .04, p=.62, n=150
FKBP5			
rs1360780	β= .00, p=.99, n=417	β= -.00, p=.98, n=261	β= .01, p=.91, n=155
rs3800373	β= .03, p=.49, n=418	β= .04, p=.54, n=265	β= .03, p=.68, n=152
rs4713916	β= .02, p=.75, n=410	β= -.02, p=.72,n=264	β= .06, p=.47, n=145
NR3c1			
rs41423247	β= .02, p=.71, n=417	β= .00, p=1.0, n=265	β= .08, p=.32, n=151

Testing interaction between child genotype, maternal depression and child and adolescent depression symptoms

Results of the interaction analyses are presented in Table 12 separately for each SNP. None of the SNPs were found to significantly interact with maternal depression in the prediction of child and adolescent depression symptoms.

Table 12. Interaction between each SNP and maternal depression

Interaction analyses		
Multiple regression model for each SNP	**Standardized Beta Coefficient**	**p value**
***CRHR1* rs7209436**		
Genetic polymorphism	.09	.28
Maternal depression	.22	<.001
Interaction term	-.10 (n=424)	.19
***CRHR1* rs242924**		
Genetic polymorphism	.07	.37
Maternal depression	.22	<.001
Interaction term	-.09 (n=426)	.24
***CRHR1* rs110404**		
Genetic polymorphism	.04	.64
Maternal depression	.20	<.001
Interaction term	-.08 (n=412)	.31
***CRHR1* TAT haplotype copies**		
Number of haplotype copies	-.06	.44
Maternal depression	.20	<.001
Interaction term	.10 (n=410)	.24
***FKBP5* rs1360780**		
Genetic polymorphism	.008	.92
Maternal depression	.20	<.001
Interaction term	-.01 (n=417)	.95
***FKBP5* rs3800373**		
Genetic polymorphism	.03	.72
Maternal depression	.21	<.001
Interaction term	.01 (n=418)	.94
***FKBP5* rs4713916**		
Genetic polymorphism	.06	.49
Maternal depression	.20	<.001
Interaction term	-.07 (n=410)	.44
***NR3c1* rs41423247**		
Genetic polymorphism	.07	.35
Maternal depression	.24	<.001
Interaction term	-.06 (n=417)	.47

Testing for differences according to child age, gender and current maternal depression diagnosis

Since the samples differed according to child age with the unexposed sample being significantly older, interaction models were run including a subsample of children exposed to maternal depression aged 12-17 years (n=170) to provide an age match to the control sample. Results (Table 13) did not differ with non-significant interactions found for all SNPs. Due to the previous finding of environmental risk

effects of maternal depression being stronger for girls, GxE analyses were tested using girls only (n=272). No significant interaction effects were found (Table 14). Finally, results were re-run selecting only those children in the exposed sample whose mothers met DSM-IV diagnostic criteria for major depressive disorder at the time of the assessment of child depression symptoms (i.e. children whose mothers were currently depressed; n=52). The pattern of results did not differ (Table 15).

Table 13. Interaction between each SNP and maternal depression for adolescents aged 12-17 years

Interaction analyses		
Multiple regression model for each SNP	**Standardized Beta Coefficient**	**p value**
***CRHR1* rs7209436**		
Genetic polymorphism	.07	.34
Maternal depression	.24	<.001
Interaction term	-.11 (n=329)	.13
***CRHR1* rs242924**		
Genetic polymorphism	.06	.44
Maternal depression	.24	<.001
Interaction term	-.11 (n=329)	.14
***CRHR1* rs110404**		
Genetic polymorphism	.03	.73
Maternal depression	.23	<.001
Interaction term	-.10 (n=320)	.18
***CRHR1* TAT haplotype copies**		
Number of haplotype copies	-.05	.52
Maternal depression	.22	<.001
Interaction term	.12 (n=318)	.17
***FKBP5* rs1360780**		
Genetic polymorphism	.003	.97
Maternal depression	.22	<.01
Interaction term	-.001 (n=323)	.99
***FKBP5* rs3800373**		
Genetic polymorphism	.03	.77
Maternal depression	.22	<.01
Interaction term	.02 (n=323)	.85
***FKBP5* rs4713916**		
Genetic polymorphism	.06	.53
Maternal depression	.26	<.001
Interaction term	-.08 (n=316)	.46
***NR3c1* rs41423247**		
Genetic polymorphism	.06	.42
Maternal depression	.23	<.001
Interaction term	-.12 (n=323)	.12

Table 14. Interaction between each SNP and maternal depression for girls only

Interaction analyses		
Multiple regression model for each SNP	**Standardized Beta Coefficient**	**p value**
***CRHR1* rs7209436**		
Genetic polymorphism	.05	.58
Maternal depression	.25	<.001
Interaction term	-.09 (n=266)	.34
***CRHR1* rs242924**		
Genetic polymorphism	.04	.66
Maternal depression	.25	<.001
Interaction term	-.08 (n=267)	.39
***CRHR1* rs110404**		
Genetic polymorphism	.003	.98
Maternal depression	.23	<.001
Interaction term	-.07 (n=257)	.48
***CRHR1* TAT haplotype copies**		
Number of haplotype copies	-.01	.90
Maternal depression	.23	<.001
Interaction term	.07 (n=256)	.46
***FKBP5* rs1360780**		
Genetic polymorphism	.08	.46
Maternal depression	.24	<.01
Interaction term	-.02 (n=260)	.87
***FKBP5* rs3800373**		
Genetic polymorphism	.07	.46
Maternal depression	.25	<.01
Interaction term	-.008 (n=263)	.94
***FKBP5* rs4713916**		
Genetic polymorphism	.06	.61
Maternal depression	.23	<.01
Interaction term	-.08 (n=255)	.48
***NR3c1* rs41423247**		
Genetic polymorphism	.08	.38
Maternal depression	.28	<.01
Interaction term	-.09 (n=260)	.41

Table 15. Interaction between each SNP and diagnosed maternal depression

Interaction analyses		
Multiple regression model for each SNP	**Standardized Beta Coefficient**	**p value**
***CRHR1* rs7209436**		
Genetic polymorphism	.10	.21
Maternal depression	.22	<.01
Interaction term	-.08 (n=201)	.31
***CRHR1* rs242924**		
Genetic polymorphism	.09	.22
Maternal depression	.22	<.001
Interaction term	-.06 (n=203)	.44
***CRHR1* rs110404**		
Genetic polymorphism	.07	.42
Maternal depression	.22	<.001
Interaction term	-.05 (n=193)	.57
***CRHR1* TAT haplotype copies**		
Number of haplotype copies	-.08	.34
Maternal depression	.22	<.01
Interaction term	.07 (n=192)	.40
***FKBP5* rs1360780**		
Genetic polymorphism	.001	.99
Maternal depression	.20	<.05
Interaction term	.02 (n=197)	.87
***FKBP5* rs3800373**		
Genetic polymorphism	.02	.85
Maternal depression	.22	<.05
Interaction term	.01 (n=195)	.90
***FKBP5* rs4713916**		
Genetic polymorphism	.04	.54
Maternal depression	.28	<.001
Interaction term	-.13 (n=188)	.22
***NR3c1* rs41423247**		
Genetic polymorphism	.05	.57
Maternal depression	.23	<.001
Interaction term	.06 (n=194)	.42

Discussion

The aim of this study was to test whether the association between maternal depression and child and adolescent depression symptoms was moderated by specific gene variants that have evidenced prior links with depression and have also been shown to affect the biological response to stress. In line with a large body of previous literature, there was a significant association between recurrent maternal depression and child and adolescent depression symptoms (Brennan, Le Brocque, & Hammen, 2003; Connell & Goodman, 2002; Weissman, Wickramaratne, et al., 2006). However, none of the genetic polymorphisms investigated showed a significant association with child and adolescent depression symptoms. In addition, there was no evidence of any gene variant interacting with recurrent maternal depression to predict child and adolescent depression symptoms.

The three polymorphisms in the *CRHR1* gene have previously been found to interact with early exposure to child abuse in predicting adult depression symptoms. We investigated the same SNPs individually and a haplotype formed by these three SNPs which has been found to confer a protective effect on depression symptoms in response to early childhood abuse (Bradley et al., 2008; Polanczyk et al., 2009). Results of the present study suggest that whilst a replicated effect of the *CRHR1* gene in moderating the association between child abuse and adult depression has previously been demonstrated, this may not extend to another major stressor - recurrent maternal depression. However similar to our findings, one previous study failed to detect significant GxE between the same variants in the *CRHR1* gene and child abuse when utilising a different measure which unlike the one used by Bradley and colleagues, was not reliant on the recall of emotional memories (Polanczyk et al., 2009). This suggests that the individual recall of negative emotions in relation to stressors or the exact type of measure might be important when investigating GxE. The adversity measure (recurrent maternal depression) in the present study is not

based on the child's perception or emotional recall of family experiences, it is purely an assessment of exposure versus non-exposure. It may also be the case that the construct of exposure to recurrent maternal depression needs to be disaggregated into more proximal environmental risk components such as negative family relationships (Pilowsky et al., 2006) or "current" exposure. However, when the exposure variable was limited to those mothers with a current clinical depression diagnosis, this did not alter results.

As other GxE findings have not been consistent, the current study further highlights that the types of measures and environmental risks are important sources of variation in GxE studies (Rutter, et al., 2009). This view is further supported by a recent meta-analysis of data on a gene variant in the serotonin transporter gene (*5HTTLPR*) interacting with stress to increase risk of depression (Karg et al., 2011). In contrast to the previous meta-analysis (Risch, et al., 2009), this study found that *5HTTLPR* moderated the association between stress and depression and highlighted the fact that both the assessment method and the nature of the stressor were critical. Effects were strongest when the stressors were defined as childhood maltreatment and specific medical conditions. GxE was weakest in relation to stressful life events although interview assessments of life events appeared to be superior to questionnaires.

In addition to variants in the *CRHR1* gene which have previously been found to interact with stress exposure to predict depression, a secondary aim was to investigate GxE between maternal depression and three polymorphisms in the *FKBP5* gene which is known to regulate the function of the glucocorticoid receptor. We also tested whether a known polymorphism in the glucocorticoid receptor gene interacted with maternal depression symptoms to predict child and adolescent

depression symptoms. We found no evidence of significant GxE for any of these variants.

There are several potential reasons for the lack of significant GxE in the present study. The GxE may not apply to the gene variants, psychosocial stressor and outcome measure tested or may not be applicable to the particular age group examined. There is also the important possibility that our results may reflect a true finding. Alternatively it may be that our results represent false negatives and the sample size in the present study was too small for detecting interaction effects. Tests of interactions tend to have considerably less power than tests of main effects (Mcclelland & Judd, 1993) and this is an important issue with regard to testing GxE (Flint & Munafo, 2008). Also, given that maternal depression is common, potentially more so in mothers of twins (Thorpe, Golding, Macgillivray, & Greenwood, 1991), it is possible that some of the control mothers had previous episodes of depression but did not currently have high HAD scores and this would further reduce power. It is also possible that the gene variants investigated, although selected *a priori* on the basis of previous research findings, are not causally relevant and index other presumably functional gene variants. However, testing additional variants in these genes would result in a greater multiple testing burden and require much larger samples. An important direction for future research would therefore be to investigate functional variants in other genes known to be involved in the regulation of biological systems implicated in the pathogenesis of depression.

Heterogeneity in findings according to gender and age have been consistently reported across GxE depression research (Cichon, et al., 2009; Eley, et al., 2004). Also, life events, maternal depression and family adversity appear to have a stronger risk effect on depression in girls (Cortes, et al., 2006; Jenkins & Curwen, 2008; Lewis, et al., 2011; Silberg, et al., 1999). The heritability of depression also appears

to change with age (Scourfield, et al., 2003; Thapar & McGuffin, 1994) with cross-generational studies suggesting there may be aetiological heterogeneity between depression in young people and symptoms later in life (Silberg, et al., 2010). With regard to the interaction between *5HTTLPR* and life events in predicting depression, inconsistent results have been reported for adolescent males and stronger associations have been suggested for females (Uher & McGuffin et al., 2010). With regard to the *CRHR1* gene, one prior case-control study examining the association between *CRHR1* and cortisol responses to the dexamethasone/CRH test found a significant association only in men (Heim et al., 2008). However, the GxE has been observed in a sample of women only (Polanczyk et al., 2009). Secondary exploratory analyses in our study showed no main genetic or interaction effects when children and adolescents and boys and girls were examined separately, however, sample sizes became very small. A useful direction for future research on GxE and depression would therefore be to use larger samples to stratify according to both age and gender provided there is an *a priori* hypothesis for doing so given the risks of multiple testing.

Another source of variability across studies is the definition of outcomes. The present study focused on depression symptoms in children rather than diagnoses. It may be that findings differ for outcomes of greater severity. However, Bradley et al., (2008) examined diagnoses as well as symptoms of depression and found significant GxE for both and Polanczyk et al., (2009) used diagnoses of major and recurrent depression. A limitation of investigating younger populations is that the offspring in the present study have not yet passed through the main developmental risk period for depression (Birmaher, et al., 2004). It may also be the case that an outcome measure more closely related to HPA axis functioning such as cortisol levels or responses to the dexamethasone/CRH test may be more suitable. Since these measures are heritable (Holsboer, 2000) and likely to be more proximal indicators of

HPA axis reactivity, some might argue that they constitute better outcome measures and this is an interesting direction for future research. There are certainly many who consider that these types of "intermediate phenotypes" may be more useful than psychiatric outcomes in genetic studies although there is still a need to eventually relate these findings to psychopathology. Also, outcomes need to be reasonably easy to measure given the need for replication and large sample sizes. All investigators are agreed that large samples that are well characterized on phenotype outcome and environmental risk measures are desirable.

One contentious issue with regard to GxE studies relates to the statistical debate of whether interactions in a GxE framework should be quantified according to an additive or multiplicative mathematical model (Risch, et al., 2009; Rutter, et al., 2009; Zammit & Owen, 2006). The present study used a model based on the additive assumption since the outcome measure was continuous and data were therefore analysed using linear regression. As stated by Rutter et al., (2009), the majority of biological researchers favour the use of an additive model because this provides a better match to biological concepts. Another issue pertaining to the methodology of GxE studies where opinions are divided is whether a genetic main effect is required in order to statistically define the presence of an interaction: According to a recent meta-analysis by Risch et al., (2009), the most likely explanation for an interaction without a main effect would be a reversal in the direction of the environment-by-genotype association where for example, the risk of depression increases with the number of S alleles in the presence of stressful life events and decreases with the number of S alleles in the absence of stressful life events (Risch et al., 2009). However, Moffitt draws attention to a paradox relevant to the question (Moffitt et al., 2005): the main drive behind GxE research is to identify why the search for genetic main effects has been relatively unsuccessful. The concept of GxE was referred to as

a potential explanation for the lack of significant genetic main effects - genetic effects may not manifest in the absence of environmental risk exposure.

Conclusion

Recurrent maternal depression does not appear to interact with gene variants thought to be involved in the regulation of the HPA axis and predict symptoms of depression in children and adolescents in this sample. However, caution is required due to the relatively small sample size.

Appendix to Chapter 7

Mother-child hostility and child and adolescent depression symptoms: an exploratory test for moderation by specific gene variants.

As discussed in Chapters 3 and 5, negative parent-child relations involving conflict, hostility and rejection are associated with child and adolescent depression symptoms (Cummings & Davies, 1996; Harold & Conger, 1997). Findings from Chapter 5 add to previous literature suggesting that mother-child hostility (Lewis, et al., 2011) and parent-child conflict/negativity (Neiderhiser, et al., 1999; Pike, McGuire, et al., 1996) have environmentally mediated effects on child and adolescent depression symptoms. Negative parent-child relationships can therefore be considered suitable environmental risk factors in GxE studies. Previous twin studies suggest that negative family relationships interact with inherited liability to increase risk of depression in children and adolescents (Lau & Eley, 2008; Rice, et al., 2006). As previously described, for GxE to be implicated it must also be shown that the psychosocial risk variable affects biological systems implicated in the pathogenesis of the disorder (Moffitt, et al., 2005). For example, in response to psychosocial stress tests, women who have experienced early childhood abuse evidence significantly greater adrenocorticotropic hormone (ACTH) responses and reduced cortisol reactivity (Heim, Newport, Bonsall, Miller, & Nemeroff, 2001). Similarly, the experience of childhood trauma has been found to result in long-term hyper-reactivity of the CRH system (Heim & Nemeroff, 2001; Tarullo & Gunnar, 2006). A similar pattern of findings is evident for less extreme forms of negative parent-child relationships. Repetti et al., (2002) report that children from families characterized by conflict, non-nurturant parenting behaviour and neglect, evidence disruptions in HPA axis functioning whilst in a sample of children from families characterized by negative interactions and low positivity, Flinn & England (1997) found abnormal cortisol profiles which were also associated with reduced immunity and illness. Finally,

Chorpita & Barlow, (1998) report that low levels of familial warmth and high levels of restrictive, controlling parenting were associated with disruptions in the HPA axis response to stress, increased corticotrophin releasing hormone and hyper-cortisolism. Studies therefore suggest that negative parent-child relationships induce changes in the HPA system which may in turn result in increased sensitivity to subsequently experienced stressors, predisposing to the development of mood related disorders such as depression.

The following additional analyses were undertaken in view of findings from Chapter 5 wherein environmental effects independent of genetic influences were demonstrated for mother-child hostility. The aim was not to undertake a definitive GxE study on mother-child hostility as such data were not available. Rather, these analyses are presented as a complement to the preceding chapters and to begin to explore whether the selected gene variants implicated in HPA axis regulation interact with perceptions of mother-child hostility to predict symptoms of depression in children and adolescents. Due to evidence from Chapter 5 that mother-child hostility confers stronger environmental risk effects for girls, interaction analyses were also tested on girls only.

Methods

Sample

Participants in the CaStANET population-based twin study who had provided both questionnaire data and DNA were included. Data on perceptions of mother-child hostility were not available from the high-risk sample. The sample was the control sample described in the preceding paper.

Measures

Child depression symptoms. Child and adolescent depression symptoms were assessed as discussed in Chapter 5 using self-reports with the Short Mood and Feelings Questionnaire (Angold, et al., 1995). Internal consistency in this sample was good (α = .88).

Mother-child hostility. Mother-child hostility was assessed using maternal reports on the Iowa Youth and Families Project (IYFP) rating Scales (Melby, et al., 1993) as described in Chapter 5. Internal consistency in this sample was good (α = .90).

Genotyping and statistics

The genotyping and statistical methods have already been described. Analyses were based on three *CRHR1* SNPs and a common TAT haplotype formed by these 3 SNPs. Three SNPs in the *FKBP5* gene and one SNP in the *NR3c1*gene were also tested: rs41423247.

Results

The mean mother-child hostility score was 13.74 (sd = 6.3). Mother-child hostility scores were positively skewed so were square root transformed and transformed scores were used in all subsequent analyses. Genotype frequencies for each SNP are shown in Table 16.

Table 16. Genotype frequencies for each SNP

Gene and SNP Accession Number	Genotype frequencies (%)
CRHR1	
rs7209436	
CC	*38.4*
TT	15.1
CT	46.5
rs242924	
GG	*36.0*
TT	16.8
TG	47.2
rs110402	
GG	*37.1*
AA	15.2
AG	47.7
Number of TAT haplotype copies	
0	36.7
1	47.7
2	15.6
FKBP5	
rs1360780	
TT	7.7
CC	49.8
CT	45.5
rs3800373	
GG	7.8
GT	40.5
TT	51.6
rs4713916	
AA	6.2
GG	52.7
GA	41.1
NR3c1	
rs41423247	
GG	42.1
CC	13.8
GC	44.1

*Hypothesized risk allele for each SNP in bold and italics

Association between mother-child hostility and child and adolescent depression symptoms

There was a significant association between mother-child hostility and child and adolescent depression symptoms in the overall sample (β=.35, $p<.01$, n=165) and for boys (β=.43, $p<.01$, n=51) and girls (β=.30, $p<.01$, n=111) separately.

Association between genotype and child and adolescent depression

Mean mother-child hostility scores according to genotype for each SNP are shown in Table 17. As previously described, there was no main effect of any SNP on child and adolescent depression symptoms.

Table 17. Mean mother-child hostility scores according to genotype

Gene and SNP Accession Number	Mean and sd for mother-child hostility
CRHR1	
rs7209436	
CC	13.72, 6.53 (n=58)
TT	13.27, 6.06 (n=22)
CT	14.12, 5.75 (n=67)
rs242924	
GG	13.64, 6.52 (n=55)
TT	13.68, 5.89 (n=25)
TG	13.96, 5.81 (n=69)
rs110402	
GG	13.53, 6.41 (n=53)
AA	13.62, 5.72 (n=21)
AG	14.11, 5.81 (n=65)
Number of haplotype copies	
0	13.75 6.42 (n=55)
1	14.11 5.76 (n=65)
2	13.11 5.89 (n=18)
FKBP5	
rs1360780	
TT	13.18, 4.34 (n=11)
CC	13.39, 6.22 (n=67)
CT	14.58, 6.12 (n=66)
rs3800373	
GG	13.18, 4.38 (n=11)
GT	14.33, 6.23 (n=57)
TT	13.58, 6.21 (n=73)
rs4713916	
AA	12.33 3.24 (n=9)
GG	13.24 5.86 (n=70)
GA	14.60 6.26 (n=55)
NR3c1	
rs41423247	
GG	13.95, 7.12 (n=58)
CC	14.42, 6.22 (n=19)
GC	13.60, 4.91 (n=63)

* Hypothesized risk allele for each SNP in bold and italics

Testing interaction analyses

Results of the interaction analyses for mother-child hostility are presented in Table 18 separately for each SNP. None of the SNPs were found to significantly interact with mother-child hostility in the prediction of child and adolescent depression symptoms. When girls only were selected, there was still no significant evidence of GxE (Table 19).

Table 18. Interaction between each SNP and mother hostility

Interaction analyses		
Multiple regression model for each SNP	**Standardized Beta Coefficient**	**p value**
***CRHR1* rs7209436**		
Genetic polymorphism	.06	.63
Mother-child hostility	.11	<.05
Interaction term	-.00 (n=146)	.84
***CRHR1* rs242924**		
Genetic polymorphism	.09	.47
Mother-child hostility	.12	<.01
Interaction term	-.02 (n=148)	.62
***CRHR1* rs110404**		
Genetic polymorphism	.06	.20
Mother-child hostility	.16	<.01
Interaction term	-.04 (n=138)	.24
***CRHR1* TAT haplotype copies**		
Number of haplotype copies	-.15	.24
Mother-child hostility	.10	<.05
Interaction term	-.04 (n=137)	.32
***FKBP5* rs1360780**		
Genetic polymorphism	-.10	.50
Mother-child hostility	.09	<.01
Interaction term	.02 (n=143)	.56
***FKBP5* rs3800373**		
Genetic polymorphism	-.16	.29
Mother-child hostility	.08	<.01
Interaction term	.04 (n=140)	.28
***FKBP5* rs4713916**		
Genetic polymorphism	-.04	.82
Mother-child hostility	.10	<.01
Interaction term	.01 (n=133)	.77
***NR3c1* rs41423247**		
Genetic polymorphism	.08	.51
Mother-child hostility	.12	<.05
Interaction term	-.02 (n=139)	.64

Table 19. Interaction between each SNP and mother hostility for girls only

Interaction analyses		
Multiple regression model for each SNP	**Standardized Beta Coefficient**	**p value**
***CRHR1* rs7209436**		
Genetic polymorphism	.05	.63
Mother-child hostility	.29	<.01
Interaction term	-.05 (n=96)	.62
***CRHR1* rs242924**		
Genetic polymorphism	.04	.68
Mother-child hostility	.30	<.01
Interaction term	-.05 (n=97)	.64
***CRHR1* rs110404**		
Genetic polymorphism	-.21	.23
Mother-child hostility	.17	<.05
Interaction term	-.06 (n=91)	.22
***CRHR1* TAT haplotype copies**		
Number of haplotype copies	-.01	.93
Mother-child hostility	.30	<.01
Interaction term	.13 (n=90)	.21
***FKBP5* rs1360780**		
Genetic polymorphism	.02	.83
Mother-child hostility	.28	<.01
Interaction term	.05 (n=94)	.60
***FKBP5* rs3800373**		
Genetic polymorphism	-.17	.38
Mother-child hostility	.27	<.01
Interaction term	.04 (n=93)	.72
***FKBP5* rs4713916**		
Genetic polymorphism	.001	.99
Mother-child hostility	.29	<.01
Interaction term	.03 (n=87)	.77
***NR3c1* rs41423247**		
Genetic polymorphism	-.01	.95
Mother-child hostility	.26	<.01
Interaction term	.06 (n=92)	.59

Discussion

In line with previous literature (McLeod et al., 2007; Harold & Conger, 1997), and as shown in Chapter 5 in the larger twin data set, there was a significant association between mother-child hostility and child and adolescent depression symptoms. However, none of the genetic polymorphisms showed a significant association with child and adolescent depression symptoms. In addition, there was no evidence of any gene variant interacting with mother-child hostility to predict child and adolescent symptoms. No interaction effects were observed when girls were examined separately. Results of these further exploratory analyses suggest that whilst a replicated effect of the *CRHR1* gene in moderating the association between child abuse and adult depression has previously been demonstrated, this may not extend to milder forms of family psychosocial stress such as mother-child hostility. However, as described in the preceding paper, there are many caveats to this conclusion.

Conclusion

An exploratory analysis showed that mother-child hostility does not appear to interact with the previously tested HPA axis gene variants in predicting symptoms of depression in children and adolescents in the present sample.

Chapter 8

Overall Discussion

Depression is a complex disorder that is heterogeneous in manifestation and multifactorial in origin. The wide range of negative outcomes in conjunction with a persistent lifecourse trajectory make the prevention of symptoms when they emerge in childhood and adolescence a vital area for scientific research and clinical practice. This requires an understanding of multiple causal pathways, the diverse range of processes likely to operate for different individuals and the complex interplay between risk and protective factors (Cicchetti & Gunnar, 2008). This thesis investigated the role of family risk factors in the aetiology of child and adolescent depression symptoms, testing both environmental and genetic processes. In understanding the aetiological pathways to depression, identifying risk factors that confer effects through environmental processes is especially important since the environment is potentially malleable and therefore amenable to intervention (Kendler & Gardner, 2010; Rutter et al., 2001). The first aim of this thesis was to test environmental effects of two specific family risk factors for child and adolescent depression – parent-child hostility and parent depression, using different yet complementary quasi-experimental research designs. Since depression is due to the co-action and interaction of both environmental and heritable effects but main genetic have not been consistently identified, I next tested whether each of these family risk factors interacted with specific gene variants to predict child and adolescent depression symptoms. The potential role of parent and child gender was tested across studies since prior research has suggested that this may be an important predictor of variation in environmental and GxE effects that has not been extensively tested using quasi-experimental research designs.

Summary of findings

This thesis was divided into three empirical chapters which each addressed different research questions relating to the putative environmental effects of specific family risk factors on child and adolescent depression symptoms. Study 1 focused on the association between parent-child hostility and child and adolescent depression symptoms using two distinct yet complementary research designs. A longitudinal community sample was first used to test the direction of effects and a genetically sensitive twin sample was then employed to test whether environmental processes contributed to the association after accounting for the influence of genetic factors. Associations were tested separately for mothers and fathers and sons and daughters. Cross-lagged panel analyses in the longitudinal sample revealed bidirectional associations between mother-daughter hostility and child and adolescent depression symptoms. In contrast, daughter depression symptoms were found to predict father hostility but not vice versa. Significant cross-lagged effects were not found for sons in response to either mother or father hostility suggesting the likely presence of gender differences in responses to parent-child hostility. Using the genetically sensitive sample, the association between mother-daughter hostility and depression symptoms was found to be mediated by environmental processes independent of genetic influences. A significant genetic component to the association was also identified. The association between father-daughter hostility and child and adolescent depression symptoms was found to be genetically mediated only with no evidence of environmental effects. For boys, no evidence of environmental mediation was found; associations were accounted for by genetic factors only. Results from study 1 therefore identify maternal hostility as a likely environmental risk factor for depression symptoms in girls and highlight the importance of considering heterogeneity in the effects of family risk variables according to both parent and child gender.

In study 2, significant environmental links were found in the intergenerational transmission of parent depression symptoms using an alternative genetically sensitive design based on a sample of children conceived via IVF. Significant environmental effects were found in the intergenerational transmission of both maternal and paternal depression symptoms and for both sons and daughters. No evidence of genetic effects on the intergenerational transmission of depression symptoms was detected and like results for parent-child hostility, environmental links were found to be significantly stronger for girls.

Following the establishment of mother-daughter hostility and parent depression as likely environmental risk factors for child and adolescent depression, study 3 tested gene-environment interaction between these family factors and several gene variants involved in regulation of the HPA axis. Although environmental effects of mother hostility were only detected for girls, gene-environment interaction was investigated for both males and females based on prior evidence that some of the gene variants (i.e. in the *CRHR1* gene) evidence risk effects only for males (Heim et al., 2009). Comparable with results from study 2 (but not using a genetically sensitive design), a significant association of both mother-child hostility and maternal depression with child and adolescent depression symptoms was detected. However, none of the gene variants investigated showed a significant association with child and adolescent depression symptoms and there was no evidence of interaction with either psychosocial risk factor. There was therefore no supporting evidence for the presence of GxE in relation to the specific gene variants and environmental measures investigated. Using different but complementary quasi-experimental research designs, this thesis has demonstrated environmental risk effects of two specific aspects of the family environment - mother-child hostility and parent

depression, and identifies both parent and child gender as important predictors of variation in environmental effects.

Integration of findings across studies

The three studies conducted in this thesis investigated the putative environmental effects of family processes in the aetiology of child and adolescent depression. The studies were unique in that different family risk factors were examined using different designs but are also complementary in that a set of integrative conclusions pertaining to the aetiology of child and adolescent depression symptoms can be drawn regarding the presence of environmental effects, the role of genetic factors and gender differences.

Environmental risk effects

Evidence of environmental effects was detected for two different family risk variables using alternative genetically sensitive designs. The fact that converging findings were detected across designs based on different principles (twin and IVF 'adoption' design), provides convincing evidence that family stressors contribute to the aetiological pathways to child and adolescent depression symptoms through environmental as well as genetic risk processes. In conjunction with other genetically sensitive studies that have examined alternative family factors such as parent-child conflict/negativity and global family functioning (Jacobson & Rowe, 1999; Neiderhiser, et al., 1999; Pike, McGuire, et al., 1996), findings therefore suggest that a range of specific, measured aspects of the family environment contribute to the development of child and adolescent depression symptoms. This reaffirms the role of family stressors as an important source of environmental risk in the aetiological pathways to child and adolescent depression symptoms and adds to a range of non-genetic studies which also support the importance of the role of the family (Collins,

Maccoby, Steinberg, Hetherington, & Bornstein, 2000; Cummings & Davies, 1996; Hammen, et al., 2004; Harold, Shelton, Goeke-Morey, & Cummings, 2004; Restifo & Bogels, 2009).

This thesis did not test mediating mechanisms which may account for the environmental effects of each family factor on child and adolescent depression symptoms or importantly, the influence of other environmental risk factors which may be related to the occurrence of maternal hostility and parental depression such as socioeconomic status and stressful life events. These aspects of the wider psychosocial network may be important precursors of parent depression and parent hostility and may therefore contribute to the overall aetiological pathways. In attempting to account for the effects of parent depression, exposure to parents' depressed mood may act as a psychosocial stressor and therefore predispose to the development of child and adolescent depression (Brennan, et al., 2003; Bureau, et al., 2009). This may involve processes of social learning or emotional contagion (Downey & Coyne, 1990; Joiner & Katz, 1999).

Hostile parenting behaviour by mothers may also constitute a direct source of psychosocial stress for children which results in symptoms of depression (Bureau et al., 2009; Low & Stocker, 2005). This would suggest a proximal link between exposure to family stressors and symptoms of depression in children. Additionally, parents' depressed mood may be linked to children's adjustment indirectly by disrupting their ability to parent effectively and reducing the quality of the parent-child relationship (Foster, Webster, et al., 2008; Kim-Cohen, Caspi, Rutter, Tomas, & Moffitt, 2006). This possibility has received a substantial amount of support (Cummings, Keller, & Davies, 2005). Numerous studies report associations between maternal depression and the parent-child relationship (Downey & Coyne, 1990) and similar associations have also been reported for fathers (Wilson & Durbin, 2010). In

addition to conferring a direct influence and affecting the parent-child relationship, it is also possible that exposure to parent depression and maternal hostility confers risk via the creation of maladaptive cognitive processes in children such as perceptions of threat and self-blame (Cummings, et al., 2005). Also, adolescents whose parents are diagnosed with major depression have been found to be more sensitive to the depressogenic effects of stressful life events and girls in particular have been found to be more sensitive to these effects than boys (Bouma, Ormel, Verhulst, & Oldehinkel, 2008).

A recently conducted study by our own research group used the IVF design to test whether the association between parent and child depression symptoms was mediated by parent-child warmth and hostility. Significant mediation was found in genetically related parent-child pairs only. The same family factors did not mediate the association in genetically unrelated parent-child pairs which suggested the presence of passive rGE in this group (Harold et al., 2010). That is, parent-child hostility did not appear to represent an environmentally mediated link between parent and child depression. It is therefore likely that alternative measures of the family environment may mediate the association between parent and child depression in genetically unrelated (hence via environmental mechanisms) parent-child pairs or that different ways of assessing warmth and hostility may be required to better tap mediation effects. One potential mediator may be "expressed emotion" which has been linked with child and adolescent depression symptoms in genetically sensitive studies (Gibb, Uhrlass, Grassia, Benas, & McGeary, 2009). Expressed emotion can be measured in a variety of ways that confer advantage over self-report questionnaire methods. For example, 5-minute speech samples and observational methods have been used to rate levels of parental warmth and negativity towards children (Caspi et al., 2004). This draws attention to the advantages associated with multi-source, multi-method assessments.

Gender differences

In addition to environmental risk effects, a common finding which emerged across the studies in this thesis was the presence of gender differences. Stronger environmental effects were observed for girls in response to both maternal hostility and maternal depression symptoms. A range of non-genetic studies have suggested that girls are more susceptible to the negative effects of interpersonal stress (Bifulco, et al., 1998; Cortes, et al., 2006; Jenkins & Curwen, 2008; Veijola, et al., 1998). Results of this thesis provide support for these findings using a genetically sensitive design which allowed the influence of genetic factors to be controlled. These findings therefore extend existing work by suggesting that increased female susceptibility to family stressors is likely to arise through environmental processes and may emerge relatively early in development since stronger effects were observed in response to maternal depression when children were aged 4-10 years.

Stronger environmental effects for girls were demonstrated in two separate studies using different research designs and different measures of the family environment. This adds additional strength to the conclusion that girls are more susceptible to the effects of family related stressors via environmental processes. In seeking to identify specific environmental processes which may account for this effect, it is possible that girls may be more susceptible for socialization reasons such as increased sensitivity to the family environment due to a heightened need for affiliation (Cyranowski et al., 2000). Alternatively, it may be that boys are equally as affected but manifest their response patterns in a different way, for example, by externalising rather than internalising (Essex, et al., 2003). Gender differences in rumination have also been implicated as a potential process underlying the female preponderance in depression prevalence (Nolen-Hoeksema, Larson, & Grayson, 1999). Rumination has been suggested as an underlying mechanism in the aetiology of depression, particularly

among adolescent girls (Stone, Hankin, Gibb, & Abela, 2011). Stone et al., (2011) found that the tendency to co-ruminate – frequently discuss and rehash problems with peers, mediated the gender difference in depression onset.

It may be also be that interpersonal, family related stress needs to surpass a certain threshold in order for boys to be equally as affected as girls. The measures used in the present thesis tap variation in the normative range of family functioning. For example arguments, criticism and anger rather than maltreatment and symptom level maternal depression rather than clinical diagnoses (major depression diagnoses were assessed in study 3 but no gender differences were found). In support of this, males and females who have experienced childhood maltreatment including sexual and physical abuse have been found to evidence comparable outcomes when assessed in adolescence including similar rates of depression (Batten, Aslan, Maciejewski, & Mazure, 2004; Maikovich-Fong & Jaffee, 2010; Shaffer, Yates, & Egeland, 2009).

The finding that girls may be more reactive to family stressors than boys also raises the question of whether boys are more resilient to interpersonal stress or have different coping styles which confer a protective effect. There is some evidence that adolescent boys tend to use problem-focused coping styles which involve active strategies designed to reduce and avoid distress whereas adolescent girls tend to rely more on emotion focused coping and fixate on negative aspects of situations (Kort-Butler, 2009). Problem-focused coping has been associated with lessening depressed mood whereas emotion focused coping has been associated with depression symptoms (Kort-Butler, 2009).

The role of genetic factors

Using a bivariate genetic design which decomposes twin pair covariance into heritable and environmental components, study 1 of this thesis found moderate but significant genetic influences on both parent-child hostility and child and adolescent depression symptoms. Genetic influences on measures of the family environment are likely to indicate the presence of passive rGE and have been reported for a range of variables (Kendler & Baker, 2007; McGue, et al., 2005; Plomin, et al., 1994). Genetic influences on depression symptoms suggest that a heritable component is involved in aetiology which is a well established finding across twin studies (Rice, et al., 2002). In addition, a significant genetic component to the association between both mother- and father-child hostility and child and adolescent depression symptoms was detected. In contrast, no evidence of genetic links in the intergenerational transmission of depression symptoms was detected using the IVF design in study 2. The only indication of a possible genetic influence in the IVF study was for boys at Time 1. Associations were observably larger in genetically related than unrelated mother-son pairs, however, this difference was not statistically significant so the presence of genetic effects could not be confirmed.

The lack of a significant genetic influence on the intergenerational transmission of depression symptoms adds to existing findings from an adoption design wherein associations between maternal depression and child depression were larger in genetically related than unrelated dyads but differences were not statistically significant (Tully, et al., 2008). A COT design also failed to find significant genetic influences on the intergenerational transmission of depression symptoms (Silberg, et al., 2010). Across studies, findings therefore suggest that the intergenerational transmission of depression symptoms is primarily due to environmental factors. The fact that genetic factors have been detected for child-specific and adult-specific

depression but not for intergenerational transmission suggests that the genetic factors which influence child and adolescent depression do not persist across the lifecourse. For example, Silberg et al., (2010) found that child-specific genetic effects were unrelated to the genes for adult depression suggesting that genetic effects on depression symptoms are specific to the child and adolescent developmental period.

Although evidence for GxE was not supported in the current thesis, it would be informative to test alternative gene variants and also, to disaggregate the maternal depression construct into components of severity, chronicity and timing. It is likely that GxE is a promising area of future research with regard to understanding the aetiology of child and adolescent depression symptoms (Caspi, et al., 2010; Karg, et al., 2011; Nugent, Tyrka, Carpenter, & Price, 2011). For example, a recent meta-analysis of 54 studies on the association between the 5HTTLPR variant in the serotonin transporter gene found significant evidence for interaction with childhood maltreatment, specific medical conditions and stressful life events in the prediction of depression (Karg et al., 2011). The 5HTTLPR variant was not examined in the present thesis due to the unavailability of commercial genotyping and a lack of in-house genotyping resources due to cost restrictions. However, based on recent findings, this variant represents an interesting direction for future research.

Implications of findings and integration with other research

The identification of risk factors that are potentially related to depression symptoms through environmental pathways is useful in implicating potential intervention targets for treatment programs aimed at reducing prevalence rates and alleviating depression symptoms for children and adolescents. Optimal treatment practices for child and adolescent depression are controversial following inconsistent guidelines (Birmaher et al., 2007). In addition, interventions often have low success rates or

confer only short-term effects (Thapar, Collishaw, Potter, & Thapar, 2010). Intervention strategies can be classified according to whether they are prevention or treatment based (Gillham, Shatte, & Freres, 2000). Special attention has been paid to the development of preventive interventions aimed at high-risk populations since these confer a greater long-term benefit than therapeutic interventions which treat symptoms that have already emerged (Thapar, et al., 2010). A recent review of preventive intervention programs based on cognitive behavioural therapy, interpersonal therapy and family-based prevention strategies found that highest success rates were associated with evidence-based, targeted and indicated programs and that future intervention studies should attend to moderators of intervention effects and aim to enhance the family environment (Gladstone & Beardslee, 2009). Importantly, results from the present thesis pertain to the design of selective prevention programs by suggesting that interventions could usefully be targeted at children experiencing maternal hostility or parent depression. Also, evidence of bidirectional effects in the association between mother-daughter hostility and depression symptoms suggests that interventions targeting this environmental risk factor should include both parents and children.

Evidence that girls are more strongly affected by maternal hostility and parent depression suggest that treatment programs targeting these risk factors may be better aimed at girls whereas boys may benefit from alternatively focused interventions which target, for example, problem-focused coping strategies (Kort-Butler, 2009). In support of this, prior evidence suggests that certain treatment programs have stronger effects for samples comprised predominantly of females and that gender is an important moderator of treatment effects (Stice, et al., 2004). In a meta-analysis of 32 prevention programs for child and adolescent depression which comprised a mixture of cognitive behavioural, interpersonal and school based therapies, Stice et al., (2004) found higher post-test and follow-up success rates

when samples contained more females than males. This replicated a previous study (Horowitz & Garber, 2006) which also found gender to moderate the effects of depression treatment programs based on a range of methods including school based therapy and cognitive behavioural therapy. Gender differences in socialization, affiliation needs and cognitive rumination may account for these effects (Cyranowski et al., 2000; Stone et al., 2011).

Research has demonstrated that interventions for child and adolescent depression which target the family environment have had mixed success (Henken, 2007). However, results of the present thesis suggest that intervention effects may be underestimated in samples comprising both boys and girls. Interpersonal therapy which focuses on social roles and interactions has been found to be an effective treatment option for child and adolescent depression in three randomised control trials (Mufson et al., 2004) and is considered a worthwhile psychological treatment alongside cognitive behavioural therapy (Thapar et al., 2010). This adds to the evidence that family relationships have predictive environmental links with child depression. A recent study examined the association between family support (defined according to levels of cohesion, expressiveness and conflict) and depressive symptoms over the course of a 23-year longitudinal follow-up study (Kamen, Cosgrove, McKellar, & Cronkite, 2011). The addition of family support significantly improved the fit of the model in predicting depression symptoms, highlighting the importance of considering family relationships as a contextual factor when treating depression. In addition, higher levels of family support were related to a more rapid decrease in depression symptoms over the course of the follow-up period and there was a significant interaction with gender; women with supportive family relationships reported the most rapid recovery.

Treating parent depression has been found to result in improvements in behavioural and emotional problems in children (Pilowsky, et al., 2008; Weissman, Pilowsky, et al., 2006) however, some studies have failed to find significant effects (Gunlicks & Weissman, 2008). In addition, the presence of parent depression has been found to moderate prevention effects with children and adolescents whose parents are currently depressed showing less improvement in response to cognitive behavioural therapy than depressed adolescents without a depressed parent (Garber et al., 2009). Results from this thesis suggest that interventions which treat parent depression in order to observe child improvements should include both mothers and fathers since both maternal and paternal depression were found to confer environmental risk effects. However, since other genetically sensitive studies have not found environmental effects for paternal depression (Tully et al., 2008), this conclusion requires replication. A recent study (Compas et al., 2010) examined potential mediators of a family group cognitive-behavioural preventive intervention for the families of parents with a history of major depressive disorder. The program focused on improving the effectiveness of parenting skills and teaching adolescents secondary control coping skills and had been found to have significant prevention effects in a prior study (Compas et al., 2009). Increases in the adolescents' use of coping skills along with increases in positive parenting were found to mediate the effects of the intervention in reducing symptoms of anxiety, depression and externalising behaviours in adolescents (Compas et al., 2010). This highlights the importance of testing mediating mechanisms as well as outcomes in treatment and intervention studies to identify precisely how symptom improvement is achieved and also, to test likely causal hypotheses regarding the role of psychosocial risk factors. For example, if improvements in mother-daughter hostility are found to ameliorate the association between maternal and child depression, this would provide further evidence that maternal hostility confers a potentially causal environmental risk effect.

In addition to informing the design of preventive interventions and providing a basis for intervention studies, the identification of potentially causal psychosocial risk factors has implications for GxE studies and for potential treatment strategies based on GxE research which is an emerging area of study (Brody, Beach, Philibert, Chen, & Murry, 2009; Cicchetti & Gunnar, 2008; Leve, Harold, Ge, Neiderhiser, & Patterson, 2010; McCrory, De Brito, & Viding, 2010). Protective influences are hypothesized to have their greatest effects on youth at highest risk (Rutter, 1985). When this risk involves genetic susceptibility, targeting environmental factors known to moderate genetic effects should reduce the negative impact for genetically at risk individuals. For example in a randomized prevention trial, Brody et al., (2009) found that when individuals at genetic risk for depression by virtue of possession of the risk allele of 5HTTLPR participated in a family based intervention program they evidenced significant reductions in problem behaviour relative to a control group. Adolescents at genetic risk who did not receive the intervention initiated risk behaviour at twice the rate of genetically at risk youths in the intervention program and adolescents not at genetic risk in either condition. The comparison between genetically at-risk individuals in the intervention versus the control conditions suggests that the environmental gains attained reduced the impact of the genetic risk factor.

It is likely that the most successful intervention programs for child and adolescent depression will adopt a family-wide approach, taking account of both parents and children and recognizing that children are at risk before they manifest negative symptoms for example, when they are exposed to environmental risk factors such as parent depression and negative parent-child relationships. It is also likely that intervention effectiveness may one day be enhanced by identifying groups who are most likely to respond and this may involve including genetic information on both

parents and children and by using biomarkers such as cortisol reactivity as predictors of treatment response (Dozier et al., 2006).

Strengths

A main strength of this thesis was the use of different quasi-experimental research designs to attempt to advance beyond statistical association and test the processes underlying family risk effects on child and adolescent depression symptoms. The use of different natural experiments which permitted a disentanglement of variables that ordinarily go together allowed competing hypotheses to be pitted against each other (for example the extent of environmental relative to genetic risk) and the use of a longitudinal research design allowed tests of temporal precedence which is also a useful quasi-experimental method for establishing causality (Rutter, 2009). Anotherstrength was the focus on specific family risk variables. Examining specific, measured psychosocial risk factors is important because the main aim of research in this area is not to identify whether an "overall" environmental risk effect exists, but to identify the specific environments which confer risk, the processes underlying their effects and the particular subgroups for whom risk effects may be strongest. This is also important to the development of intervention strategies. Since targeted interventions aimed at specific risk factors and subgroups have been shown to be most effective, it is essential that studies testing environmental risk effects focus on specific aspects of the environment rather than, for example, on global constructs of general family functioning so that interventions can be targeted accordingly. A further strength was the examination of both parent and child gender - factors which may affect the presence and strength of environmental risk effects. The identification of moderating factors is an important issue for the development of specifically targeted interventions aimed at particular subgroups and may be relevant to explaining why certain interventions are effective whereas others are not and why environmental

effects may be detected for certain aspects of the family environment but not others. Prior longitudinal and genetically sensitive research in this area has not tended to examine the role of gender or has done so for either parents or children separately.

In addition to establishing environmental effects, this thesis tested whether psychosocial risk factors link with biological processes – the influence of genetic factors likely to be involved in the regulation of the stress response. The fact that both environmental and genetic risk factors along with gene-environment interaction were examined is important because, although the identification of environmental risk factors is necessary for pinpointing malleable intervention targets, understanding the genetic contribution to depression is important to advancing our understanding of the complex and multifactorial aetiological pathways to depression.

Limitations

Results from the present thesis must be considered in the context of certain limitations. First, although the designs used had various features which allowed advancement beyond evidence of statistical association, they remain quasi-experimental. Conclusions of a potential cause and effect relationship can therefore be inferred, however, it is necessary to consider that experimental methods such as randomised control trials are required to advance further towards the demonstration of causal effects. Second, a range of limitations arose from the combination of multiple different datasets. These are detailed in the individual discussion sections of the separate studies comprising this thesis. For example, the differences in age ranges and measures across datasets resulted in certain discrepancies that may have affected the comparability of the samples.

Third is the use of symptom level measurements of depression as opposed to clinical diagnoses. Diagnoses of depression were used in study 3 but only for mothers; child and adolescent depression symptoms were again assessed using continuous questionnaire based methods. Findings of environmental effects cannot therefore be said to pertain directly to child and adolescent depression diagnoses since it is possible that effects vary according to severity. The thesis also relied on the use of self-report data throughout. Self-report data is liable to a range of biases including inaccuracy due to retrospective recall and dependence on the reporters' current mood state. The latter is especially pertinent when assessing depression symptoms due to negative affectivity bias which may affect information recall. Multi-source, multi-informant measures in conjunction with observational assessments offer a range of important advantages. This is especially relevant to the assessment of the parent-child relationship wherein observational measures may confer distinct advantage.

A further limitation refers to the fact that many of the children sampled had not yet passed through the main developmental risk period for the emergence of depression symptoms and were still pre-pubertal. This means that many of the children who will go on to experience depression in adolescence and early adulthood were yet to have experienced the onset of symptoms. This may have reduced the power to detect associations with putative risk factors.

Finally, an important limitation of this thesis stemmed from the decision to focus on two specific family environmental risk factors for child and adolescent depression. This meant that the potential contributory role of other environmental influences which may be important steps on the aetiological pathways to depression were not examined. Such influences may be more distal risk factors that are part of the wider social network and are associated with the occurrence of parent depression and

parent-child hostility such as socioeconomic status and stressful life events. It is important to consider the fact the parent depression and parent-child hostility do not occur in a "vacuum" and do not confer risk effects for child and adolescent depression in isolation but are part of a wider and more complex network of psychosocial risk factors which act in concert to increase risk of depression in children and adolescents. One of the most important features of the aetiology of depression is that it is multifactorial so although it is of use to test the effects of specific individual risk factors, it is important to acknowledge that results should be interpreted in terms of how they contribute to more complex and integrative aetiological process models.

Future Directions

This thesis has demonstrated that two specific aspects of the family environment contribute to child and adolescent depression through environmental risk processes. However, there are many other aspects and dimensions of the family environment which may confer risk for child and adolescent depression that have not been investigated using quasi-experimental research designs whilst also considering the role of parent and child gender. In order to gain a more holistic understanding of family risk effects for child and adolescent depression, it would be informative to use longitudinal and genetically sensitive research designs to test the "environmental" influence of a range of other dimensions of the parent-child relationship and family environment that have not yet been examined. These would include for example parent-child rejection, withdrawal, and neglect and inter-parental conflict which all have established associations with child and adolescent depression (McLeod et al., 2007) but wherein the extent of causal environmental links is unclear. Using the same quasi-experimental framework, it would also be informative to test 'positive' aspects of the parent-child relationship in order to identify potentially protective

environmental effects. These would include parent-child warmth and support which have been associated with child and adolescent depression (McLeod et al., 2007) but have not been examined in a genetically sensitive framework testing environmental effects, the direction of associations and the role of parent and child gender.

Another direction for future research following the results of this thesis would be to use alternative statistical methods. For example, cross-lagged panel analysis is only one method of testing the direction of effects. There are a range of alternative longitudinal statistical methods available for approximating cause and effect inferences. The statistical field of longitudinal data analysis is continually evolving with the emergence of techniques such as generalized estimating equation modelling, regression discontinuity analyses, latent growth curve analysis and autoregressive path models. Importantly, these methods can be used in conjunction in order to maximize the benefits of each. In testing bidirectional effects in the association between parental monitoring and adolescent delinquent behaviour for example, Laird, Pettit, Dodge, & Bates (2003), first used latent growth curve modelling to examine rates of correlated change in parental monitoring and adolescent delinquency and then conducted the more traditional cross-lagged panel analysis method to test for bidirectional associations at specific time points (Laird, et al., 2003; Pettit & Arsiwalla, 2008), emphasizing the benefits of combining not only multiple research strategies and datasets, but also different statistical methods. When used in conjunction with large, well characterized, genetically sensitive samples with a corpus of available phenotypic data, such statistical methods allow ever closer advancement towards a quasi-experimental assessment of cause and effect relations. It would be informative to build upon the 2-wave longitudinal model tested in the current thesis by using 3-wave longitudinal data to test a meditational model of effects. Latent growth curve modelling is an attractive alternative to other longitudinal analysis methods when 3-wave data are available, allowing change in

individual trajectories according to underlying processes to be examined (Vujeva & Furman, 2011). Such a model could test whether children's cognitions or inferential styles mediate the association between maternal hostility and depression symptoms and do so separately by parent and child gender.

Importantly, parent-child hostility and parent depression do not confer isolated risk effects for child and adolescent depression but rather, are likely to be part of a larger psychosocial network comprising multiple interlinked variables. It is likely that the effects of distal factors such as negative life events and socioeconomic status are mediated by more proximal influences such as parent psychopathology and the parent-child relationship (Lau, Rijsdijk, Gregory, McGuffin, & Eley, 2007). One of the most pertinent features of the aetiology of depression is that it is multifactorial. It would therefore be informative to test a wider psychosocial process model which incorporates not only factors that may mediate the effects of parent-child hostility and parent depression but also, distal factors which may be related to the occurrence of both of these risk factors. This would therefore permit a comprehensive test of a more complex and multifaceted psychosocial process model of environmental risk effects. Such a model should also include both biological and genetic risk factors and test aetiological pathways separately for parent and child gender. In a comprehensive study, Lau et al., (2007) tested an integrated multilevel model which included social, genetic and cognitive risk factors within a genetically sensitive framework. It would be useful to add to these integrative findings by testing a similar model which incorporated different distal and proximal influences such as maternal hostility since this thesis suggests this as an environmental risk factor. It would also be informative to test such a model over three waves of data using for example, latent growth curve analysis in order to establish the direction of effects and also, to investigate mothers and fathers and sons and daughters separately.

This thesis has shown, using a range of genetically sensitive and longitudinal research designs, that the aetiological pathways to child and adolescent depression symptoms involve risk factors that confer their effects, in part, through environmental processes. However, there are a wide range of family factors and research methods yet available to further examine environmental risk effects in the aetiological pathways to child and adolescent depression. This offers exciting prospects for future research which should work towards the establishment of integrative and multifactorial process models involving distal and proximal risk factors and incorporating both environmental and genetic risk pathways.

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Appendices: Appendix 1: The Child Depression Inventory

1. _____ I am sad once in a while
   _____ I am sad many times
   _____ I am sad all the time

2. _____ Nothing will ever work out for me
   _____ I am not sure if things will work out for me
   _____ Things will work out okay for me

3. _____ I do most things okay
   _____ I do many things okay
   _____ I do everything wrong

4. _____ I have fun with most things
   _____ I have fun with some things
   _____ Nothing is fun at all

5. _____ I am bad all the time
   _____ I am bad many times
   _____ I am bad once in a while

6. _____ I think about bad things happening to me once in a while
   _____ I worry that bad things will happen to me
   _____ I am sure that terrible things will happen to me

7. _____ I hate myself
   _____ I do not like myself
   _____ I like myself

8. _____ All bad things are my fault
   _____ Many bad things are my fault
   _____ Bad things are usually not my fault

9. _____ I feel like crying everyday
   _____ I feel like crying many days
   _____ I feel like crying once in a while

10. _____ Things bother me all the time
    _____ Things bother me many times
    _____ Things bother me once in a while

11. _____ I like being with people
    _____ I do not like being with people many times
    _____ I do not want to be with people at all

12. _____ I cannot make up my mind about things
    _____ It is hard to make up my mind about things
    _____ I make up my mind about things easily

13. _____ I look okay
    _____ There are some bad things about my looks
    _____ I look ugly

14. _____ I have to push myself all the time to do my school work
_____ I have to push myself many times to do my school work
_____ Doing school work is not a big problem

15. _____ I have trouble sleeping every night
_____ I have trouble sleeping many nights
_____ I sleep pretty well

16. _____ I am tired once in a while
_____ I am tired many days
_____ I am tired all the time

17. _____ Most days I do not feel like eating
_____ Many days I do not feel like eating
_____ I eat pretty well

18. _____ I do not worry about aches and pains
_____ I worry about aches and pains
_____ I worry about aches and pains all the time

19. _____ I do not feel alone
_____ I feel alone many times
_____ I feel alone all the time

20. _____ I never have fun at school
_____ I have fun at school only once in a while
_____ I have fun at school many times

21. _____ I have many friends
_____ I have friends but I wish I had more
_____ I do not have any friends

22. _____ My school work is alright
_____ My school work is not as good as before
_____ I do very badly in subjects I used to be good in

23. _____ I can never be as good as other kids
_____ I can be as good as other kids if I want to
_____ I am just as good as other kids

24. _____ Nobody really loves me
_____ I am not sure if anybody loves me
_____ I am sure that somebody loves me

25. _____ I usually do what I am told
_____ I do not do what I am told most times
_____ I never do what I am told

26. _____ I get along with other people
_____ I get into fights many times
_____ I get into fights all the time

Appendix 2: The Short Mood and Feelings Questionnaire, child report

	Not true	Sometimes	True
1. I felt miserable or unhappy	0	1	2
2. I didn't enjoy anything at all	0	1	2
3. I felt so tired I just sat around and did nothing	0	1	2
4. I was very restless	0	1	2
5. I felt I was no good any more	0	1	2
6. I cried a lot	0	1	2
7. I found it hard to think properly or concentrate	0	1	2
8. I hated myself	0	1	2
9. I thought I was a bad person	0	1	2
10. I felt lonely	0	1	2
11. I thought nobody loved me	0	1	2
12. I thought I could never be as good as the other kids	0	1	2
13. I did everything wrong	0	1	2

Appendix 3: The Short Mood and Feelings Questionnaire, parent report

	Not True	Somewhat True	Certainly True
1. She/he felt miserable or unhappy.....	0	1	2
2. She/he didn't enjoy anything at all.....	0	1	2
3. She/he felt so tired she/he sat around and did nothing.....	0	1	2
4. She/he was very restless.....	0	1	2
5. She/he felt she/he was no good any more.....	0	1	2
6. She/he cried a lot.....	0	1	2
7. She/he found it hard to think properly or concentrate.....	0	1	2
8. She/he hated himself/herself.....	0	1	2
9. She/he felt she/he was a bad person.....	0	1	2
10. She/he felt lonely.....	0	1	2
11. She/he felt nobody loved him/her.....	0	1	2
12. She/he thought she/he could never be as good as other kids/young people	0	1	2
13. She/he felt she/he did everything wrong.....	0	1	2

Appendix 4: DSM-IV anxiety items

	Not True	Sometimes True	Very True
1. Worries	0	1	2
2. Restless, feels on edge	0	1	2
3. Easily tired	0	1	2
4. Irritable	0	1	2
5. Difficulty concentrating, mind goes blank	0	1	2
6. Problems sleeping, difficulty falling or staying asleep, or restless unsatisfying sleep	0	1	2

Appendix 5: Iowa Youth and Families Project Rating Scales, child report

	Never	Almost never	Not too often	About half	Fairly often	Almost always	Always
1. Let you know he/she really cares about you	1	2	3	4	5	6	7
2. Get angry at you	1	2	3	4	5	6	7
3. Criticise you or your ideas	1	2	3	4	5	6	7
4. Shout at you because she was upset with you	1	2	3	4	5	6	7
5. Act loving and affectionate toward you	1	2	3	4	5	6	7
6. Get into an argument with you	1	2	3	4	5	6	7
7. Let you know that he/she appreciates you, your ideas or the things you do	1	2	3	4	5	6	7
8. Help you do something that was important to you	1	2	3	4	5	6	7
9. Argue with you whenever you disagreed about something	1	2	3	4	5	6	7
10. Act supportive and understanding toward you	1	2	3	4	5	6	7

***Items which form the hostility subscale highlighted**

Appendix 6: Iowa Youth and Families Project Rating Scales, parent report

	Never	Almost never	Not too often	About half	Fairly often	Almost always	Always
1. Let him/her know you really care about him/her	1	2	3	4	5	6	7
2. Get angry at him/her	1	2	3	4	5	6	7
3. Criticise his/her ideas	1	2	3	4	5	6	7
4. Shout at him/her because you were upset with him/her	1	2	3	4	5	6	7
5. Act loving and affectionate towards him/her	1	2	3	4	5	6	7
6. Let him/her know that you appreciate his or her ideas or the things he/she does	1	2	3	4	5	6	7
7. Help him/her do something that was important to him/her	1	2	3	4	5	6	7
8. Argue with him/her when you disagreed about something	1	2	3	4	5	6	7
9. Act supportive or understanding towards him/her	1	2	3	4	5	6	7

***Items which form the hostility subscale highlighted**

Appendix 7: Life Events Questionnaire

	YES	NO	Very unpleasant	A bit unpleasant	No effect	A bit pleasant	Very pleasant
1. Moving to a new neighbourhood	1	0	A	B	C	D	E
2. Birth of a new brother, sister or adopted or fostered child	1	0	A	B	C	D	E
3. A new stepbrother or stepsister	1	0	A	B	C	D	E
4. Changing to a new school	1	0	A	B	C	D	E
5. Serious illness or injury in a parent, brother or sister	1	0	A	B	C	D	E
6. Parents divorced or separated	1	0	A	B	C	D	E
7. Increased quarrelling between parents	1	0	A	B	C	D	E
8. Death of parent, brother or sister	1	0	A	B	C	D	E
9. Death of grandparent	1	0	A	B	C	D	E
10. Death of a close friend	1	0	A	B	C	D	E
11. Father/stepfather away from home more often	1	0	A	B	C	D	E
12. Mother/stepmother away from home more often	1	0	A	B	C	D	E
13. Brother or sister (or step-brother/step-sister) leaving home	1	0	A	B	C	D	E
14 Serious illness or injury in a close friend	1	0	A	B	C	D	E
15 Parent getting into trouble with the police	1	0	A	B	C	D	E
16 Mother or stepmother going back to work or starting work for the	1	0	A	B	C	D	E

first time							
17. Entry into the home of a new partner for mother or father	1	0	A	B	C	D	E
18. Parent going to prison	1	0	A	B	C	D	E
19. Special recognition for good school work	1	0	A	B	C	D	E
20. Joining a new club	1	0	A	B	C	D	E
21. Doing badly in an exam	1	0	A	B	C	D	E
22. Parent being less interested or loving towards child	1	0	A	B	C	D	E
23. Parent nagging or picking on child more	1	0	A	B	C	D	E
24. Serious illness or injury to this child	1	0	A	B	C	D	E
25. Failing to be picked for a school or club team, band or orchestra	1	0	A	B	C	D	E
26. Doing badly in school work	1	0	A	B	C	D	E
27. Being picked for a school or club team, band or orchestra	1	0	A	B	C	D	E
28. Special prize or recognition for doing well in an activity (like sports, music or art)	1	0	A	B	C	D	E
29. A close friend moves a long way away	1	0	A	B	C	D	E
30. Losing a close friend through arguments or being dropped	1	0	A	B	C	D	E
31. Death of a pet	1	0	A	B	C	D	E
32. Mother losing job	1	0	A	B	C	D	E
33. Father losing job	1	0	A	B	C	D	E
34. Bullying by another child/ children	1	0	A	B	C	D	E
35. Any event that we have not mentioned but that you think may be important	1	0	A	B	C	D	E

*Items used in Chapter 6 Life Events Scale highlighted

Appendix 8: Hospital Anxiety and Depression scale

1. **I feel tense or wound up** ☐ most of the time ☐ a lot of the time ☐ time to time, occasionally ☐ not at all	8. **Worrying thoughts go through my mind** ☐ a great deal of the time ☐ a lot of the time ☐ from time to time but not too often ☐ only occasionally
2. **I still enjoy the things I used to enjoy** ☐ definitely as much ☐ not quite as much ☐ only a little ☐ hardly at all	9. **I feel cheerful** ☐ not at all ☐ not often ☐ sometimes ☐ most of the time
3. **I get a sort of frightened feeling something awful is about to happen** ☐ very definitely and quite badly ☐ yes, but not too badly ☐ a little but it does not worry me ☐ not at all	10. **I can sit at ease and feel relaxed** ☐ definitely ☐ usually ☐ not often ☐ not at all
4. **I feel as if I am slowed down** ☐ nearly all the time ☐ very often ☐ sometimes ☐ not at all	11. **I feel restless and I often have to be on the move** ☐ very much indeed ☐ quite a lot ☐ not very much ☐ not at all
5. **I get a sort of frightened feeling like butterflies in the stomach** ☐ not at all ☐ occasionally ☐ quite often ☐ very often	12. **I look forward with enjoyment to things** ☐ very often indeed ☐ quite often ☐ not very often ☐ not at all
6. **I have lost interest in my appearance** ☐ definitely ☐ I don't take so much care as I should ☐ I may not take quite so much care ☐ I take just as much care as ever	13. **I get sudden feelings of panic** ☐ very often indeed ☐ quite often ☐ not very often ☐ not at all
7. **I can laugh and see the funny side of things** ☐ as much as I always could ☐ not quite so much now ☐ definitely not so much now ☐ not at all	14. **I can enjoy a good book or radio or TV programme** ☐ often ☐ sometimes ☐ not often ☐ very seldom

*Items forming depression subscale highlighted

Appendix 9: Family income

8. Approximate gross family income (please tick)			
Up to £10,000		£40,000 - £50,000	
£10,000 - £20,000		£50,000 - £60,000	
£20,000 - £30,000		£60,000 +	
£30,000 - £40,000			