Epileptic seizures and epilepsy in young people with 22q11.2 deletion syndrome: prevalence and links with other neurodevelopmental disorders

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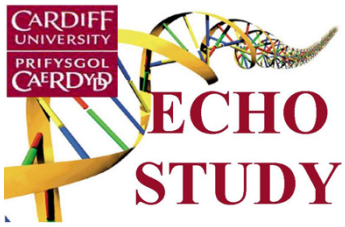

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Acknowledgements

I would like to thank my primary supervisor, Professor Marianne van den Bree, for her excellent advice and for her patience and support. I would also like to thank my secondary supervisors, Professor David Linden and Professor Michael Owen for their guidance during this PhD. I have thoroughly enjoyed learning from all of my supervisors.

I would like to thank Dr Rhys Thomas, who at the beginning of my PhD helped to introduce me to the concepts and terminology involved in epilepsy research. Dr Thomas also provided advice for the design of the measures to be used during the 'second stage' of the systematic assessment of epileptic seizures and epilepsy in young people with 22q11.2DS, described in this thesis. I would also like to thank Dr Sarah Knott, who provided guidance when I was preparing the Unusual Spell Interview. I would like to thank Dr Khalid Hamandi, who kindly gave up his time to review all of the data from the second stage of assessment and provided the diagnoses of epileptic seizures and epilepsy. Dr Hamandi also reviewed the EEG traces in this thesis, along with Dr Gareth Payne. I would like to thank both of these individuals for their support in helping me to learn how to read EEG traces for background abnormalities and epileptiform discharges. I must also thank the staff in the Clinical Neurophysiology Department at the University Hospital of Wales for helping me to understand how to conduct and interpret EEG assessments. I also wish to thank Professor Phillip Smith and Professor Michael Kerr, who at the beginning of my PhD allowed me to shadow their epilepsy clinics and learn more about how this condition is diagnosed and managed.

Special thanks must go to Hayley Moulding and Dr Ullrich Bartsch, who helped me to design and conduct the 24-hour pilot and case-control EEG studies described in this thesis. I would also like to thank all staff members and PhD students involved with the ECHO study. Special thanks must go to Hayley Moss, whose advice and support helped me through some of the more difficult periods of the PhD.

I would like to thank my family; my mother Barbara and my father Colin, my cousin, Paul, my grandmother, Vera, and my great-aunt, Isabel, for their incredible support during these past few years. I am especially thankful to my partner Emily, who experienced all of the highs and lows of a PhD with me and was always there to support me.

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Contributions

I carried out all of the literature reviews in this thesis, conducted all analyses and wrote all of this thesis.

With regard to the first stage of the systematic assessment of epileptic seizures and epilepsy in young people with 22q11.2DS, the Epilepsy Screen Questionnaire was already used within the ECHO study when I began my PhD. I helped with the data entry process for this measure. I derived all of the ESQ summary variables and conducted all analyses using the ESQ presented this thesis, and interpreted the results.

With regard to the second stage of assessment, I prepared an application for ethical approval for this study along with a fellow PhD student (Hayley Moulding, Cardiff University). I amended the original version of the Unusual Spell Interview for use in this thesis and designed the Unusual Spell Diary, with input from consultant epileptologists (Dr Khalid Hamandi and Dr Rhys Thomas, Cardiff University). I co-led participant recruitment for this second stage of assessment along with Hayley Moulding. I conducted all of the unusual spell interviews described in this thesis, collected all of the relevant medical records, administered all of the unusual spells diaries and collected the majority of the data pertaining to a family history of seizures and epilepsy. The 24-hour ambulatory EEG study with deletion carriers and control siblings was conducted in the family home, requiring travel to different parts of the UK. I conducted 27/44 (61.7%) of these visits along with undergraduate placement students, Hayley Moulding and Dr Ullrich Bartsch (University of Bristol). I screened all of the EEG traces from deletion carriers and controls. An epileptologist (Dr Khalid Hamandi) then reviewed all of the EEG traces that I identified as potentially containing an abnormality. A consultant neurophysiologist, Dr Gareth Payne, reviewed the EEG data from four deletion carriers, to check waveforms that the epileptologist was unsure about. I prepared each of the 'case-report forms' (CRFs, i.e. a summary of all the data from the first and second stages of the epilepsy assessment) for review by the epileptologist. The epileptologist then reviewed all of the CRFs and provided diagnoses of epileptic seizures and epilepsy. I entered, processed and statistically analysed the data from all of the measures used during the second stage of assessment and interpreted the results.

I prepared an application for ethical approval of the pilot 24-hour EEG study with children and adolescents from the general population, alongside Hayley Moulding. I conducted all of the EEG assessments in this pilot study alongside Hayley Moulding and Dr Ullrich Bartsch

Data relating to cognition, psychiatric disorders, sleep disturbance, autism spectrum disorder and motor coordination problems were collected primarily by the ECHO study field team. I conducted nine

of the psychiatric interviews and assigned diagnoses. I conducted all of the analyses in this thesis involving the cognitive, psychiatric and other neurodevelopmental data and interpreted the results.

All of the research presented in this thesis was conducted under the guidance of my supervisors, Professor Marianne van den Bree, Professor David Linden and Professor Michael Owen (Cardiff University).

The manuscript based on the analyses from Chapter 4 and 5 of this thesis was written by myself, under the guidance of my supervisors and Dr Khalid Hamandi, Dr Rhys Thomas, Dr Adam Cunningham, Professor Michael Kerr (Cardiff University), Dr Gareth Payne and Dr Siske Struik (both from the University Hospital of Wales).

Publications based on this thesis

Christopher B. Eaton, Rhys H. Thomas, Khalid Hamandi, Michael P. Kerr, Gareth C. Payne, David E.J. Linden, Michael J. Owen, Adam Cunningham, Ullrich Bartsch, Siske S. Struik and Marianne B.M van den Bree. **Epilepsy and seizures in young people with 22q11.2 deletion syndrome: prevalence and links with neurodevelopmental disorders** (Under review).

Summary

Young people with 22q11.2 deletion syndrome (22q11.2DS) are at increased risk for acute symptomatic seizures and epilepsy. Their true prevalence may have been underestimated however, as previous studies used medical record reviews, which may have missed non-convulsive seizures. In this thesis, I aimed to conduct a 'first-hand', systematic assessment of epileptic seizures and epilepsy in young people with 22q11.2DS and their unaffected siblings.

Firstly, using a validated epilepsy screening questionnaire (ESQ), completed by the primary caregiver, I found that whilst 11.1% of deletion carriers were reported as having an epilepsy diagnosis, 48.7% had an afebrile seizure or a paroxysmal event without a diagnosis. 21.1% of deletion carriers were reported with febrile seizures. Deletion carriers screening positive to at least one item of the questionnaire were more likely to have psychopathology, motor problems and a lower performance IQ.

I then conducted a second stage of assessment with a sub-sample of deletion carriers and controls, comprising parental and child interviews, review of medical records and a 24-hour EEG assessment. An epileptologist reviewed these data. In the second stage, all but one of the ESQ-reported epilepsy diagnoses were confirmed. One deletion carrier was newly diagnosed with epilepsy. Only 11.8% reported with an afebrile seizure or paroxysmal event were diagnosed with epileptic seizures ('possible' absence seizures).

These findings reinforce that young people with 22q11.2DS are at increased risk for acute symptomatic seizures (predominantly febrile seizures) and epilepsy. I also provide evidence to suggest that epileptic seizures may not be recognised during routine clinical care in some young deletion carriers, and an epilepsy diagnosis may be overlooked. The high rate of febrile seizures suggests a lower seizure threshold in 22q11.2DS. The associations of positive screens with impaired cognition, psychopathology and motor problems may suggest shared neurobiological risk pathways, although false-positives could be a confounding factor.

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1 Introduction

1.1 Overview of copy number variation

Copy number variants (CNVs) are structural rearrangements of DNA. They can encompass either part of, or all, of an entire chromosome. In CNVs, sequences of base pairs can be deleted, duplicated, inverted or translocated (transferred to a new position). A CNV is formally defined as a chromosomal rearrangement of 1000 base pairs (1 kilobase, kb) or more. CNVs are littered throughout an individual's genome and they often have little to no effect on development. However, some CNVs are pathogenic and disrupt core developmental processes. Such pathogenic CNVs have been linked with risk for medical conditions and neuropsychiatric disorders. Examples includes deletions and duplications of DNA in chromosome 16p11.2, which have been associated with micro and macrocephaly, respectively, as well as cognitive impairment, language delay, seizures, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD) and schizophrenia[1-4].

1.2 Background to 22q11.2 Deletion Syndrome

22q11.2 Deletion Syndrome (22q11.2DS) is the most common microdeletion syndrome in humans. It is caused by a hemizygous deletion of 90 genes (46 of which are protein-coding) in region 11.2 on the long arm (q) of chromosome 22[5]. The deletion is thought to occur in 1 in every 2,000-4,000 live births[6-9]. The vast majority (90-95%) of people with 22q11.2DS have *de novo* (spontaneous) deletions. In the remaining patients the deletion is inherited from an affected parent[10] and these individuals may have poorer cognitive outcomes[11]. The high rate of *de novo* deletions in 22q11.2DS is due to the presence of low copy repeats (LCRs; sections of DNA that share >96% of sequence identity) in the 22q11.2 region, increasing the probability of non-allelic homologous recombination during meiosis[12]. Figure 1-1 provides a visual overview of the variety of typical and atypical deletions that can occur on chromosome 22q11.2. In around 90% of people with 22q11.2DS, a deletion of 3 million base pairs (megabase, Mb) occurs between LCR22A and LCR22D, the two largest breakpoints in the 22q11.2 region[13,14]. Smaller nested 1.5Mb and 2Mb deletions can occur between LCR22A and LRC22B or LCR22C, respectively, and account for around 8% of patients[14], who show similar phenotypic outcomes to patients with a 3Mb deletion[13]. Atypical nested deletions can also occur between LCR22B or LCR22C and LCR22D; these are more frequently inherited and lead to a milder phenotype[15].

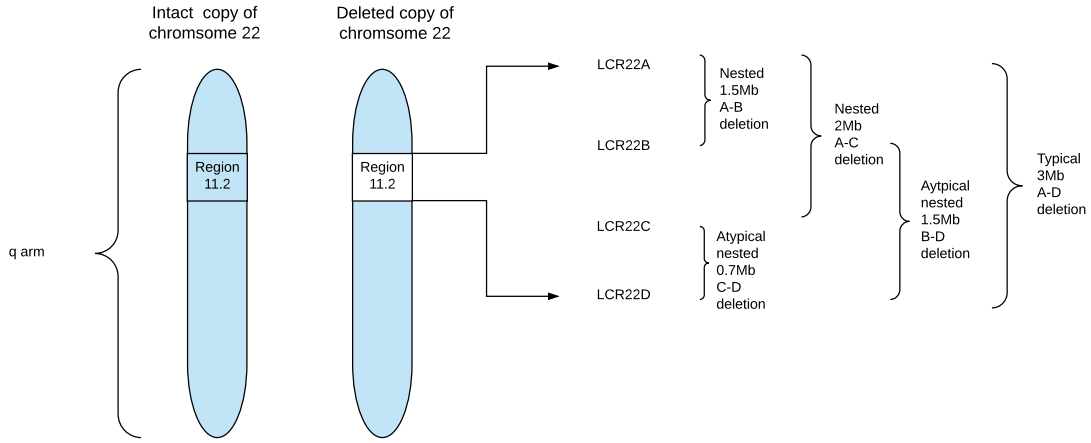

Figure 1-1. Diagram of the 22q11.2 deletion. An intact long arm (q) of chromosome 22q is shown and is compared with a deleted copy. The variety of typical and atypical deletions that can occur between the low copy repeats (LCRs) 22A-22D are also shown.

The phenotype associated with 22q11.2DS is complex and affects multiple organ systems. As children, patients are referred for genetic testing due to the presence of a constellation of medical conditions. These most commonly include congenital heart disease, velopharyngeal insufficiency, cleft palate, immune dysfunction, hypocalcaemia due to hypoparathyroidism, feeding difficulties, genitourinary abnormalities and facial dysmorphism (e.g. hooded eyelids, bulbous nasal tip)[15]. Developmental delay, impaired cognition and psychopathology have also been associated with this syndrome and during adolescence and adulthood it is the emerging psychiatric phenotype that can lead to referral for genetic testing[16]. Phenotypic outcomes of this syndrome are highly variable: some patients succumb to congenital heart disease as new-borns, whilst others present with few clinical symptoms and may only be referred for genetic testing because they have a child with the deletion[17,18].

People with 22q11.2DS were previously diagnosed with a variety of different syndromes depending on the particular pattern of symptoms they presented with. Examples include DiGeorge Syndrome[19], velocardiofacial syndrome[20], conotruncal anomaly face syndrome[21], Opitz G/BBB[22] and Cayler cardiofacial syndrome[23]. The advent of genetic technologies such as fluorescence *in situ* hybridization (FISH) in the early 1990s led to the realisation that many of the people with these syndromes had the 22q11.2 deletion.

In the following sections the term 'idiopathic' will be used to refer to individuals who have a given neuropsychiatric disorder but who do not have 22q11.2DS. I will also frequently refer to a study of neuropsychiatric development from childhood to adulthood in 1,402 people with 22q11.2DS. This was the largest study to date in this area, compiling data from 15 sites spread across the globe. It was

conducted by the International Consortium on Brain and Behaviour in 22q11.2 Deletion Syndrome and I will refer back to it as the '22q IBBC study'.

1.3 Cognition in 22q11.2DS

Studies have consistently observed an average full-scale IQ (FSIQ) of around 70 in people with 22q11.2DS (e.g. [11,24-26]), which is around 30 points lower than their unaffected siblings[27]. In keeping with the phenotypic variability that characterises 22q11.2DS, FSIQ scores differ considerably between patients, for example, ranging from 40-92 in a study by Moss et al. of 33 children, adolescents and young adults with 22q11.2DS[24] and from 50-109 in a study by De Smedt et al. of 103 children with the deletion[25]. Intellectual disability (ID, defined as an FSIQ <70) is elevated in 22q11.2DS, although in the majority of cases the ID is mild (FSIQ=55-70), less commonly moderate (FSIQ=40-54) and rarely reaches severe levels (FSIQ <40)[11,25,27,28]. Factors associated with poorer intellectual functioning in 22q11.2DS include having an inherited deletion[11] and neonatal hypocalcaemia[29]. The 22q11.2 deletion has been observed in ID/ developmental delay (DD) cohorts at a rate of between 0.61%-2.4%[30,31]; indeed, it is the second most common cause of ID/DD after Down syndrome.

People with 22q11.2DS show impairments in numerous cognitive domains. Relative deficits in non-verbal learning (fluid intelligence) as compared to verbal ability have been observed in some studies[11,24,25]. Other studies have found no difference between verbal IQ (VIQ) and performance IQ (PIQ) and some people with 22q11.2DS have higher PIQ than VIQ scores [25,27]. The psychoeducational profile of people with 22q11.2DS is fairly consistent with a non-verbal learning deficit, with patients having difficulties with mathematics but demonstrating relative strengths in reading and spelling tasks, although some patients exhibit difficulties with expressive and receptive language[24,32]. People with 22q11.2DS also have impairments in neurocognitive domains such as spatial working memory, executive function, planning, attention and processing speed. Interestingly, these deficits are relatively independent of one another and are not related to general intellectual disability[27]. Deficits in face memory, social cognition (e.g. emotion identification) and complex cognition (e.g. language and nonverbal reasoning) have also been observed in 22q11.2DS and may be greater than in people with idiopathic DD[33].

There is considerable debate as to whether IQ declines with increasing age in 22q11.2DS. Cross-sectional studies have observed negative correlations between age and IQ[27,34,35]. In addition, some longitudinal assessments of IQ in 22q11.2DS have noted a decline in IQ with increasing age, although the extent of the reported decrement is quite variable, ranging from two to seven FSIQ points[36-40]. VIQ scores in particular have been reported to show decline, and most notably in those who go onto

to develop psychotic disorders[36,40]. By contrast, other longitudinal assessments have found no evidence for cognitive deterioration in 22q11.2DS[41,42]. A recent study from our group showed a decline in processing speed, attention, spatial planning and spatial working memory in young people with 22q11.2DS over a two-and-half year period. This is one of very few studies in 22q11.2DS to have included a control sample and interestingly, the prevalence of deterioration in the group with 22q11.2DS did not differ from that observed in their unaffected siblings, suggesting that cognitive decline is not specific to 22q11.2DS and instead may represent normal developmental fluctuation[43].

1.4 Psychopathology in 22q11.2DS

1.4.1 Schizophrenia

Schizophrenia is a chronic psychiatric disorder. Affected individuals can experience positive psychotic symptoms, in which an individual is unable to differentiate between real and unreal experiences. Examples include delusions and hallucinations. Negative symptoms are also present in schizophrenia, which are characterised by a loss of normal functioning. Examples include reduced emotional expression and loss of motivation and interest. Disorganised symptoms are another key feature of the syndrome, in which the individual exhibits disordered thoughts and behaviour[44]. Schizophrenia has a typical onset in late adolescence or early adulthood[45], but in rare cases can be diagnosed during childhood[46].

22q11.2DS has been robustly associated with risk for developing schizophrenia. The lifetime prevalence of schizophrenia in the general population is around 0.5%[47], whilst 22-30% of people with 22q11.2DS go on to develop schizophrenia during adulthood[26,48,49]. More broadly, between 30-41% of adults with 22q11.2DS meet criteria for any psychotic disorder[26,48] and nearly half of adolescents and young adults with 22q11.2DS report having psychotic experiences[50], compared to 5% of the general population[51]. The prevalence of the 22q11.2 deletion is also elevated in schizophrenia cohorts; occurring in around 0.3% of cases compared to 0% of controls[4,30] (with an associated p-value of 1.0×10^{-30} in the study by Malhotra and Sebat[30]). The rate of the 22q11.2 deletion is even higher (4%) in children who develop schizophrenia before the age of 13 years[52]. Not all studies have observed a link between 22q11.2DS and schizophrenia however; schizophrenia prevalence was not significantly elevated in 34 deletion carriers drawn from a recent population-based study of ~76,000 people in Denmark. The mean age of the 22q11.2DS sample was however relatively young (17.4 years), meaning that schizophrenia may not have emerged yet in many of these individuals[53].

The presentation of schizophrenia in people with 22q11.2DS is broadly similar to that observed in cases of schizophrenia from the general population. Early studies reported that people with 22q11.2DS and schizophrenia had an earlier[54,55] or later age of onset and fewer negative symptoms[48]. Sample sizes in these studies were small however and some[54,55] did not compare to a control group of patients with idiopathic schizophrenia. Later studies with larger sample sizes failed to replicate these findings[49,56]. One study provided evidence for better global functioning in people with 22q11.2DS and schizophrenia[49], but another found no difference[56]. Finally, there is some evidence that people with 22q11.2DS and schizophrenia have less lifetime substance use and poorer impulse control, cooperativeness and greater hostility than idiopathic schizophrenia patients[56].

1.4.2 Attention deficit hyperactivity disorder

ADHD is a neurodevelopmental disorder characterised by symptoms of inattentiveness and/or hyperactivity or impulsivity that result in a clinically significant impairment in functioning across numerous settings, such as within school[44]. Despite the prevailing idea that ADHD is a disorder limited to childhood and adolescence, symptoms are often chronic and persist well into adulthood[57]. ADHD has three subtypes depending on the particular pattern of symptoms that an individual presents with. Inattentive ADHD is characterised by poor attentional control and high distractibility, whereas in hyperactive-impulsive ADHD the patient is restless and has difficulty controlling impulsive behaviour. Individuals presenting with a combination of inattentive and hyperactive-impulsive symptoms are diagnosed with the combined ADHD subtype. ADHD prevalence is estimated at around 7%[58]. Prevalence rates of the disorder are far higher in males than females, for examples, three times as many adolescent males (13%) than females (4%) were diagnosed with ADHD in a study by Merikangas et al.[59].

Studies have repeatedly shown a high rate of ADHD in people with 22q11.2DS[27,34,60]. The 22q IBBC study found that 37% of children with the deletion had ADHD. This was the most common neurodevelopmental disorder diagnosed in this age range. The prevalence declined to 24% in adolescence and 16% in adults with the deletion, although these rates are still elevated relative to the general population (9% in adolescents[59] and 4% in adults[61]).

In some domains, ADHD in 22q11.2DS is similar to ADHD in people without the deletion. The 22q IBBC study observed that, as in the general population, ADHD seem to predominantly occur in males with 22q11.2DS (61% of males compared to 13% of females)[26]. This gender difference was not replicated in a later study[62], although this may have been the result of a lack of statistical power due to a far smaller sample of people with 22q11.2DS and ADHD (n=44 versus n=253 in the 22q IBBC study[26]). Both

cross-sectional[26] and longitudinal studies[63] in 22q11.2DS have revealed that ADHD persists into adolescence and adulthood in 22q11.2DS, as it does in the general population. Around 65% of children with 22q11.2DS continue to have ADHD into adolescence[26,63], a rate similar to that observed in idiopathic ADHD (70%[64]). There is also overlap in predictors for ADHD persistence into adolescence between 22q11.2DS and idiopathic cohorts, for example, the level of childhood hyperactivity and a family history of ADHD[63].

In other respects the presentation of ADHD in 22q11.2DS differs quite substantially from that observed in idiopathic ADHD. Inattentive ADHD is the predominant ADHD subtype in 22q11.2DS[62,65] (60% in the 22q IBBC study[26]), whereas hyperactive-impulsive and combined subtypes are more common in idiopathic ADHD[62,66]. In particular, deletion carriers show more inattentive symptoms within an academic context than children with idiopathic ADHD (likely related to the psychoeducational difficulties seen in 22q11.2DS[24]), as well as fewer hyperactive-impulsive symptoms[62,65]. In addition, relative to cases of idiopathic ADHD, deletion carriers have lower rates of comorbid disruptive behaviours disorders (e.g. conduct disorder and oppositional defiant disorder) and major depression, as well as a higher rate of generalised anxiety disorder[62,65]. There is evidence that these differences in ADHD profile and comorbidities may be due to the 22q11.2 deletion, as they are not explained by the effects of intellectual disability in 22q11.2DS or by the differences in the prevalence of ADHD subtypes between 22q11.2DS-ADHD and idiopathic ADHD[62].

The treatment of ADHD in 22q11.2DS is complicated by the cardiac problems these individuals experience, as official health authorities advise against the use of stimulants with such populations[26]. When methylphenidate has been used with deletion carriers with ADHD however, it has been shown to improve prefrontal cognitive performance and lead to a 40% reduction in symptom severity six months after treatment, whilst being reasonably well-tolerated[67]. Despite these benefits, evidence suggests that young people with 22q11.2DS are under-treated for their ADHD. Niarchou et al. (2015) diagnosed ADHD in 44 children young people with 22q11.2DS (mean age=9.61 years, SD= 2.1 years, range =6-14 years) using the semi-structured Child and Adolescent Psychiatric Assessment; a semi-structured interview that was conducted with the primary caregiver. Interestingly, only of one these children was being treated for ADHD (stimulant medication)[62]. In addition to a caution to use stimulants with this population due to cardiac problems, a lack of treatment may plausibly be attributed to diagnostic overshadowing brought about by the complex cognitive and psychiatric presentation in 22q11.2DS. For example, clinicians may think of inattentive or hyperactive ADHD symptoms in 22q11.2DS as a general feature of the intellectual disability seen in this syndrome, rather than a specific, treatable psychiatric disorder.

1.4.3 Anxiety disorder

'Anxiety disorder' is an umbrella term for a number of psychiatric disorders, all of which are characterised by excessive fear and worry that leads to behavioural disturbances, such as pervasive strategies to try and avoid the feared/anxiety-inducing situation[44]. Anxiety disorders have been estimated to affect 7% of people worldwide[68].

People with 22q11.2DS commonly experience anxiety and many are diagnosed with anxiety disorders. The 22q IBBC study found that 31% of people with 22q11.2DS had an anxiety disorder. Of these, around a third met criteria for two or more anxiety disorders. The prevalence of generalised anxiety disorder (GAD) and obsessive-compulsive disorder (OCD) appears to remain stable from childhood to adulthood (around 10% and 6% of deletion carriers, respectively). Social and specific phobias, however, predominate during childhood (10% and 22%, respectively), but decrease in prevalence as children grow older (social phobia and specific phobia are present in 1-3% and 2-4% of adult deletion carriers over the age of 25). Panic disorder meanwhile is rare during childhood and adolescence (1%) but increases in prevalence as deletion carriers enter adulthood, reaching a peak prevalence of 14% in mature adults (older than 35 years). Other anxiety disorders seen in 22q11.2DS include separation anxiety disorder (6% of children), posttraumatic stress disorder (although this is very rarely diagnosed across all ages groups) and anxiety disorder not otherwise specified (<1% of cases)[26]. Interestingly, rates of GAD and social phobia in 22q11.2DS are higher than those observed in a cohort of individuals with intellectual disability from the general population[26,69]. This finding, along with the high rates of ASD observed in 22q11.2DS (discussed in the next section) suggest social impairments may be a core deficit in 22q11.2DS[70]. There is some evidence to suggest that anxiety disorders have a relatively greater impact on the daily living skills of people with 22q11.2DS than the other psychiatric disorders seen in this syndrome, for example, schizophrenia spectrum disorders[26].

1.4.4 Autism Spectrum Disorder

Autism spectrum disorder (ASD) is an umbrella term for a spectrum of disorders characterised by chronic deficits in social communication, social interaction, as well repetitive or stereotyped interests and behaviours[44]. Other symptoms include hypo or hyper-sensitivity (e.g. to sounds, smells). ASD is thought to affect around 0.6% of the population[71]. Comorbid ID is common; up to 70% of people with ASD also have ID[72]. The severity of symptoms and degree of intellectual ability in ASD is highly variable, with some individuals able to lead independent lives and others requiring daily care. Males are 3 times more likely to meet criteria for ASD than females[73].

Studies have estimated that between 14-50% of people with 22q11.2DS have ASD[27,60,74-76], a rate significantly higher than in their unaffected sibling controls (5%)[27]. These studies have however relied on screening tools and/or parent-report of ASD symptoms. This may lead to inaccurate estimates of ASD prevalence in 22q11.2DS, because there is a notable degree of symptom overlap between 22q11.2DS and ASD. For example, children with 22q11.2DS fixate less on the eyes and more on the mouth during emotion processing tasks[77], as do people with idiopathic ASD[78]. In addition, the high rate of anxiety disorders in 22q11.2DS (particular in social domains[26]) may erroneously inflate the prevalence of ASD seen in this syndrome, particularly when ASD assessments are preliminary (e.g. screening tools) or limited (e.g. parent-report only)[79]. Difficulties with speech as a result of palatal abnormalities and velopharyngeal dysfunction may also result in misdiagnosis of ASD in 22q11.2DS[80]. Evidence largely does not support this however and instead suggests that 22q11.2DS cases with and without ASD have a broadly similar communication profile[81]. In addition, there are features that distinguish 22q11.2DS cases with and without ASD. Children with 22q11.2DS and ASD are reported by their parents as exhibiting less imaginative play and more motor stereotypies and repetitive behaviours.[81] In addition, deletion carriers with ASD present with a higher rate of comorbid psychiatric disorders than those without ASD (94% versus 60%)[75].

In order to accurately represent the rate of ASD in 22q11.2DS then, studies should employ the gold-standard assessment of taking a clinical history from the parent using the Autism Diagnostic Interview-Revised (ADI-R)[82] in conjunction with direction observation of the child using the Autism Diagnostic Observation Schedule (ADOS)[79,83]. Three of the sites that contributed to the sample in the 22q IBBC study used both the ADI-R and ADOS to assess ASD prevalence in 22q11.2DS. The 22q IBBC study may therefore provide the most accurate estimate of ASD prevalence to date. 19% of their sample was diagnosed with ASD, with prevalence peaking during adolescence (27%). However, 72% of the adolescents diagnosed with ASD were obtained from Utrecht, a site which only used the ADI-R, meaning the rate of ASD during adolescence in 22q11.2DS may have been erroneously inflated[26].

Finally, there are some differences between the presentation of ASD in 22q11.2DS and ASD in people without the deletion. People with 22q11.2DS and ASD have better communication and more socioemotional reciprocity than do people with idiopathic ASD[81]. In addition, the male preponderance of ASD seen in the general population is not observed in 22q11.2DS[26].

1.4.5 Disruptive disorders

Disruptive disorders are characterised by an inability to control one's emotions and behaviour, which leads to conflict with authority figures and violation of societal rules. I will discuss two specific

disruptive disorders in this section. Firstly, oppositional defiant disorder (ODD) is defined by vindictive and defiant behaviour, as well as angry/irritable mood. Conduct disorder (CD) is a more severe disruptive disorder and supersedes a diagnosis of ODD. CD is characterised by behaviours that violate social norms and the rights of other people (e.g. vandalism of property)[44]. ODD has an estimated prevalence of 8% in adolescents (13-17 years old) from the general population, whilst CD is thought to be diagnosed in 5%[84].

Between 14-18% of children and adolescents with 22q11.2DS are diagnosed with ODD[26,27], a rate significantly higher in than their unaffected sibling controls (0%, p=0.005)[27] but similar to that observed in children with ID (14%)[69]. ODD occurs less frequently in adults with 22q11.2DS(6%)[26].

The presentation and developmental course of ODD in 22q11.2DS differs from that seen in idiopathic cohorts. The authors of the 22q IBBC study suggest that ODD symptoms in people with 22q11.2DS are largely limited to a family context. Outside of the family, these individuals are described as being introverted[26]. Very few people with 22q11.2DS seem to transition from ODD to CD. The 22q IBBC study found that no children or adolescents with 22q11.2DS were diagnosed with CD and a rate of only 1% in adults. By contrast, in the general population, people with ODD are nearly 13 times more likely to go on to develop CD[85].

1.5 Other neurodevelopmental problems in 22q11.2DS

1.5.1 Sleep problems

Problems with sleeping have been observed in people with 22q11.2DS, chief amongst them obstructive sleep apnoea (OSA), defined as frequent interruptions of breathing whilst sleeping. Rates of diagnosed OSA are around 10% in 22q11.2DS, but when looking more broadly at obstructive sleep symptoms the prevalence increases to 39%[86-88]. OSA in 22q11.2DS is thought to be caused by muscular hypotonia of the upper airway, pharynx and palate[89]. People with 22q11.2DS are at particular risk for OSA after surgery to correct the velopharyngeal insufficiency commonly seen in this syndrome, as such surgery involves obstructing the velopharynx[87]. More research needs to be done in order to better elucidate the prevalence and nature of sleep problems in 22q11.2DS.

1.5.2 Motor problems

Motor problems are increasingly recognised as a salient feature of 22q11.2DS. Whilst these difficulties are associated to some extent with the ID seen in 22q11.2DS[90], children with the deletion perform worse on tasks assessing manual dexterity, balance and visual-motor integration than IQ-matched

controls, suggesting these motor deficits cannot be solely accounted for by ID[91,92]. Other problems with motor function include hypotonia and tremor or tetany as a result of hypocalcaemia[93,94]. A recent study from our group (Cunningham et al.) found the vast majority of children and adolescents with 22q11.2DS (81%) screened positive for developmental coordination disorder (DCD)[90]. DCD is a neurodevelopmental disorder characterised by impairments fine motor skills, gross motor skills, or both. These individuals also have problems with motor learning. Crucially, these deficits are not explained by a neurological disorder (e.g. cerebral palsy) or cognitive delay and they lead to significant impairments in daily life. In Cunningham et al., DCD was found to be associated with a greater number of anxiety, ADHD and ASD symptoms, as well as poorer performance on neurocognitive tasks assessing visual and sustained attention[90]. This suggests shared neurobiological risk pathways for motor and attentional deficits, as well as psychiatric impairment in 22q11.2DS.

1.6 Neuropsychiatric risk pathways in 22q11.2DS

The many neuroimaging studies of patients with 22q11.2DS have collectively observed numerous neuroanatomical abnormalities, however, specific findings are relatively inconsistent from one study to another and children and adolescents with the deletion show a different profile of changes to adult carriers. Findings common to both young people and adults with the deletion include a decrease in total brain volume relative to typically developing controls, polymicrogyria, white matter abnormalities, reduced volume of the cerebellum and enlarged ventricles[95-97]. Interestingly, enlarged ventricles is a well-established neuroimaging finding in patients with idiopathic schizophrenia[98].

In children and adolescents with 22q11.2DS, regional volumetric reductions follow a rostral-caudal gradient (i.e. anterior areas of the brain are relatively intact whereas posterior regions are more severely affected, notably the parietal lobe and the cerebellum)[95]. Given the association of parietal and cerebellar abnormalities with motor functioning, volume loss in these areas may plausibly account for the motor deficits seen in 22q11.2DS. However, in a recent MRI and DTI study from our group (Cunningham et al.), comparing deletion carriers with unaffected sibling controls, volumetric reduction of bilateral superior parietal areas and white matter abnormalities in the left inferior cerebellar peduncle did not significantly associate with motor coordination scores[99]. Some studies have also reported a reduction in the volume of the hippocampus in young people with 22q11.2DS, which showed a negative correlation with FSIQ and a relationship with impaired association and recall[100,101]. When comparing deletion carriers with typically developing controls however, other studies have failed to observe any differences in hippocampal volume[36,102,103]. Young people with 22q11.2DS and ASD also have a larger right amygdala volume[75], a structure involved in rapid emotional

processing[104]. A larger right amygdala volume has been associated with deficits in social communication and interaction in idiopathic ASD cases[105].

During adulthood, the rostral-caudal gradient of structural abnormalities disappears, with adult deletion carriers showing volumetric reductions in frontal and temporal areas, which are particularly pronounced in deletion carriers with schizophrenia[106,107]. Reductions in grey matter volume of the prefrontal cortex from childhood to late adolescence/adulthood are observed in deletion carriers who go on to develop more severe psychotic symptoms. These changes were associated with the low-activity allele of the catechol-*O*-methyltransferase gene (*COMT*^{L}), which encodes an enzyme involved in the clearance of dopamine in the prefrontal cortex. *COMT* is one of the deleted genes in 22q11.2DS and so the remaining copy on the intact chromosome 22 may subsequently have a greater impact on phenotypic expression. Deletion carriers with the *COMT*^{L} variant also showed greater reductions in verbal IQ over time than did those with the high-activity allele (*COMT*^{H}) [108]. Cognitive decline has long been thought to precede the onset of schizophrenia[109], although as noted previously there is evidence to suggest that IQ does not decline over time when comparing the cognitive trajectory of people with 22q11.2DS with their unaffected sibling controls[43].

Whilst over the last 20 years progress has been made in understanding how the genetic disruption in 22q11.2DS leads to the neurocognitive deficits and psychiatric impairment observed in this syndrome, there are many neuropsychiatric risk pathways that need to be better elucidated.

1.7 Epileptic seizures, epilepsy and 22q11.2DS

1.7.1 Overview of epileptic seizures and epilepsy

1.7.1.1 Epileptic seizures

Background

An epileptic seizure is caused by abnormal excessive or synchronous neuronal activity within the brain. They are transient, clinically observable events and symptoms may include motor, sensory or autonomic disturbances, changes in emotional state or impairments in consciousness and memory. When using the term 'seizure' in this thesis, I will be referring to *epileptic* seizures, and not those which are thought to be psychological in origin (e.g. psychogenic non-epileptic seizures)[110].

Classification

The organisation responsible for deciding upon the classification system for epileptic seizures is the International League Against Epilepsy (ILAE). A plethora of clinical symptoms have been associated

with seizures, therefore grouping these into meaningful diagnostic categories has proven a challenging task. This is reflected through the way in which the classification system has often been amended, extended and revised over the past 40 years[111-115]. According to the most recent revision in 2017, seizures can be broadly classified according to how much of the brain the abnormal activity encompasses. Focal seizures are those in which abnormal neuronal activity begins in one hemisphere. By contrast in a generalised seizure this activity begins in both hemispheres. Seizures in which the onset is uncertain are labelled as 'unknown'. These three seizures types are then further delineated according to whether they feature prominent motor features (e.g. generalised motor/non-motor seizure) and in the case of focal seizures whether they result in impaired awareness (e.g. focal aware/impaired awareness seizure). Finally, seizures are then further classified according to the particular pattern of clinical symptoms they elicit, for example, stiffening and jerking of all of the body is termed a 'tonic-clonic' seizure, whereas brief, sudden interruptions of consciousness in which the individual appears vacant are termed 'absence' seizures[115].

Another mode of classification is to delineate between 'acute symptomatic' and 'unprovoked' epileptic seizures. This classification system is important because it highlights the subtle distinction between an individual experiencing epileptic seizures and an individual who is diagnosed with epilepsy. Acute symptomatic seizures are brought about acute conditions. For example, a high fever in young children may induce an epileptic seizure, known more specifically as a febrile seizure/convulsion. Febrile seizures are found in 2-5% of children between six months and five years old[116]. Febrile seizures can be classified as either 'simple' or 'complex'. Simple febrile seizures are the most common, accounting for 65-90% of all febrile seizures[117]. They have a generalised semiology and a duration of less than 15 minutes. They slightly increase the risk of epilepsy later in life (2%) but do not associate with poorer academic or behavioural outcomes and are therefore though to be relatively benign[118]. Complex febrile seizures affect only one part or side of the body, or are abnormally prolonged, lasting 30 minutes or more (also known as 'febrile status epilepticus', FSE). They substantially increase the risk of epilepsy (6-49%), as well as neurological injury and cognitive impairment[119-122]. Other conditions which can provoke an acute symptomatic seizures include severely low blood sodium or calcium levels, or alcohol withdrawal. Crucially, these conditions are temporary and reversible and can induce epileptic seizures in neurotypical individuals who may never receive a diagnosis of epilepsy. By contrast, unprovoked seizures occur spontaneously. Repeated unprovoked seizures indicate an individual has an underlying difference in their brain leading to a chronic predisposition for seizures. It is these individuals who are diagnosed with epilepsy.

1.7.1.2 Epilepsy

Background

Epilepsy is the most common serious neurological disorder. Between 0.5-1% of the population have epilepsy[123,124] and up to 3% of individuals may be diagnosed at some point during their lifetime[125]. Epilepsy affects individuals of all ages. Throughout history epilepsy has been severely misunderstood, having been associated with madness, the devil, witchcraft, contagion and family misfortune[126]. Even today, people with epilepsy experience stigma. In particular, the intermittent, often spontaneous nature of seizures leads to unease and concern from others about when seizures will occur (felt stigma)[110]. Whilst epilepsy is thought to affect up to 50 million people worldwide[127], the majority of affected people do not receive treatment[128]. Issues of stigma and poor treatment are compounded through the lack of prominent figures in the public eye (e.g. celebrities) who choose to disclose their epilepsy[110,126] and who could advocate for better understanding and treatment of this condition. Individuals with epilepsy also commonly experience learning difficulties, memory problems and psychiatric disorders[124,129-131], meaning that they are less likely to perform highly in education and may therefore may be poorly placed to advocate for, or enact, measures that reduce stigma and improve treatment.

Diagnosis

Epilepsy is normally diagnosed when an individual has at least two unprovoked seizures >24 hours apart[132]. The process of diagnosing epilepsy can be complex and there is no one single test for determining whether epilepsy is present, unlike with other chronic diseases, such as the FEV_1 for lung disease. Instead, epilepsy diagnosis relies on multiple sources, chief among them a description of the clinically suspicious event the patient has experienced. This can be self-report but is very often given by a witness, due to the way in which epileptic seizures can impair consciousness and memory. Electroencephalography (EEG) assessments, which involve measuring the pooled electrical activity of groups of cortical neurons through electrodes systematically across the scalp, can be used to identify epileptiform discharges and areas of cortical epileptogenicity (i.e. aberrant brain regions implicated in generating epileptic seizures). There is a great deal of misunderstanding amongst the public and clinicians alike about the role of EEG in diagnosing epilepsy however; it is strictly an adjunct to the clinical description of the suspicious paroxysm. EEG recordings may fail to capture epileptiform discharges in up to 75% of individuals with an epilepsy diagnosis, particularly during routine clinical recordings (i.e. ~30 minutes)[133]. Specificity of EEG for epilepsy is better, although 0.5% of adults and between 2-4% of children with no history of seizures or epilepsy will show epileptiform discharges during EEG recordings[133,134]. Epileptiform discharges are also more prevalent in people who have ASD;

one study found that 60% of ASD cases with no history of epilepsy showed epileptiform discharges during sleep[135]. Similarly, 30.1% of people with ADHD show epileptiform discharges[136]. Epileptiform discharges may therefore simply reflect broad neuronal network dysfunction in some cases, rather than specifically pointing toward the presence/risk of epilepsy. This potential pitfall in interpretation may be particularly salient when reading the EEG trace from children with neurodevelopmental disorders. The process of diagnosing epilepsy is made more difficult through the numerous conditions that can mimic features of epileptic seizures. An example is vasovagal syncope, in which an individual loses consciousness and may present with myoclonic jerks, head turning, automatisms and incontinence, all of which are features of various epileptic seizures[110]. Ultimately, all of these factors make diagnosing epilepsy a complex process; around 25% of individuals are falsely diagnosed with epilepsy, even in specialist centres[137].

Classification

The ILAE has taken the lead in creating a standardised classification system for the epilepsies[114,138]. Aetiology is of paramount importance when classifying the epilepsies, informing prognosis and treatment options[110].The most recent classification system[114] defines the 'genetic epilepsies' (previously known as the 'idiopathic epilepsies'[138]) as those with a known or presumed genetic cause. The 'structural-metabolic epilepsies' (previously known simply as the 'structural epilepsies'[138]) are those in which the individual has a structural brain lesion or metabolic condition that is known to be associated with increased risk for developing epilepsy. Finally, there are the 'epilepsies of unknown cause' (previously known as the 'cryptogenic epilepsies'[138]). Certain epilepsies present with a highly distinct cluster of symptoms, for example a typical age of onset with specific EEG features and seizure types. These are known as electroclinical syndromes. They have a strong genetic basis and are associated with certain developmental stages. Examples include childhood absence epilepsy and juvenile myoclonic epilepsy[114].

1.7.1.3 Comorbidities

People with epilepsy may experience numerous comorbid neurodevelopmental and psychiatric disorders. Epilepsy is found at higher rates in those with ID (14-44%), particularly those with more severe forms of ID[129,139].

In addition to this deficit in global intellectual functioning, people with epilepsy show deficits in specific neurocognitive domains relative to unaffected controls, such as in executive function and verbal memory[140]. Rates of psychiatric disorders are also elevated in people with epilepsy relative to the general population. People with epilepsy are at an increased risk for anxiety disorders such as generalised anxiety disorder (13%), social phobia (6%) and agoraphobia (5%)[124], although diagnoses

of these disorders were based on a structured psychiatric interview designed for trained lay interviewers, rather than a clinical assessment from a psychiatrist . Between 6-8% are diagnosed with ASD when using gold-standard autism assessments such as the ADOS and the ADI-R , although when screening tools are used the prevalence increases to up to 32%. [124,141,142]. In addition, the prevalence of ASD is higher in epilepsy than in other chronic neurologic conditions (e.g. migraine)[124]. ADHD prevalence is estimated at ~30% in epilepsy, based on diagnoses from a child psychiatrist[143]. In addition, ADHD symptoms are more common in frontal lobe epilepsy, CAE and Rolandic epilepsy and often precede seizure onset[144]. Conduct disorders are diagnosed in 13-24% of epilepsy patients, based on data from semi-structured interviews, the data from which was reviewed by clinicians and evaluated according to DSM-IV-TR criteria [145,146]. Around 12% of individuals with epilepsy report suicidal ideation and the prevalence of lifetime suicide attempts is around 21% (as assessed through structured psychiatric interviews)[147]. Psychotic disorders diagnosed according to DSM and ICD criteria also show an increased prevalence in epilepsy (6%), notably in patients with temporal lobe epilepsy (7%)[148].

Epilepsy also shows a robust, complex relationship with sleep. Certain seizures may occur predominantly during sleep (as in benign partial epilepsy of childhood with centro-temporal spikes, BECTS). Sleep deprivation and sleep-wake transitions can act as triggers for epileptiform discharges and epileptic seizures. Sleep problems are roughly twice as common in epilepsy patients as in healthy controls, notably obstructive sleep apnoea, and can have a serious impact on quality of life. In children with epilepsy, sleep disturbance associates with poorer cognition and behaviour during the daytime[149].

Finally, children with epilepsy also often present with comorbid motor problems. A study of 21 children and adolescents with BECTS observed that nearly half (48%) of patients exhibited difficulties in one or more areas of motor functioning, such as manual dexterity, balance and aiming and catching[150].

Comorbidities between epilepsy and impaired cognition, psychopathology, sleep disturbance and motor problems suggest shared neurobiological risk pathways for these conditions. For example, both ASD and epilepsy are thought to be the result of aberrant synaptic plasticity, leading to an imbalance in neuronal excitation-inhibition. However, it may also be the case that epileptic seizures have deleterious effects on neurodevelopment and give rise to, or worsen, cognitive impairments, psychopathology and other developmental problems. For example, abnormalities in synaptic plasticity can arise due to changes in receptors, signalling molecules or neurotrophins and it is known that early-life seizures can alter these molecules[151]. More striking examples are the epileptic encephalopathies,

in which epileptic activity is thought to contribute to the severe cognitive and behavioural impairments associated with these conditions[114]. It is important to highlight here that whilst prolonged seizures/status epilepticus (as seen in many of the epileptic encephalopathies) have been shown to lead to neurological damage, evidence in the general population does not suggest that the repeated occurrence of other, more 'simple' epileptic seizures themselves maintain the epileptogenic process and directly contributes to cognitive deterioration[152]. Instead, the co-occurrence of frequent epileptic seizures and impaired cognition may simply reflect a more severe underlying epileptogenic process. Alternatively, epileptic activity may increase the risk of cognitive impairment by disrupting neurological mechanisms that are independent of the process of epileptogenesis. For example, prolonged epileptic activity during sleep may disrupt slow-wave activity that is associated with a homeostatic reduction of synaptic strength, a process hypothesized to be crucial for the ability to learn[153]. Whilst progress has been made, future longitudinal designs are needed to better tease apart the relationship of epilepsy with numerous neurodevelopmental disorders.

1.7.2 Copy number variation and epilepsy

The genetic aetiologies of the epilepsies are diverse. Mutations in single genes can lead to epilepsy. For example, mutations in *SCN1A* can cause Dravet syndrome, which is an epileptic encephalopathy[154]. By contrast, the genetic aetiology of other epilepsies can be more complex and involve mutations in, or loss/ duplication of, numerous genes. Advances in genetic sequencing technologies over the last 30 years has led to an understanding that CNVs contribute to the genetic aetiology of numerous epilepsies, particularly those with comorbid dysmorphic features, intellectual disability or ASD[154]. Microdeletions of DNA within chromosomes 15p11.2, 15q13, 16p13.11 and 22q11.2 have been strongly associated with risk for genetic generalised epilepsy (GGE) [155,156]. 15q13.3 deletions seem to exclusively predispose to GGE, as these deletions were not observed in a sample of 300 patients with focal epilepsies[157]. By contrast, the 600kb microduplication at 16p11.2 (BP4-BP5) seem to exclusively confer risk for Rolandic epilepsy. Rolandic epilepsy patients are 26 times more likely to show this 16p11.2 microduplication than controls and this duplication was not observed in a sample of 1,738 patients with GGE or TLE[158].

1.7.3 Epileptic seizures and epilepsy in 22q11.2DS

1.7.3.1 Prevalence of seizures and epilepsy in 22q11.2DS

Research studies have suggested that individuals with 22q11.2DS are at an increased risk for both acute symptomatic and unprovoked epileptic seizures, although the reported rates are wide-ranging. Causes for acute symptomatic seizures in 22q11.2DS include hypocalcaemia, with studies indicating between 1%-14.5% of people with the deletion have hypocalcaemia-induced seizures (10-14.5% in children with the deletion)[159-161]. Hypocalcaemia irritates the CNS[162] and enhances neuronal excitability[163]. This disturbance of neuronal excitation-inhibition may mean that hypocalcaemia could plausibly lead to a predisposition for acute symptomatic seizures in response to other triggers, such as a high fever. Indeed, hypocalcaemia is one of the most important risk factors for febrile seizures[164]. However, to date, the prevalence of febrile seizures in 22q11.2DS is estimated at between 2-6% of cases[159-161], which is a rate similar to the general population (2-5%[116]). 17.6% of adults with 22q11.2DS who take psychotropic drugs have been reported to have seizures[161]. The increased rate of acute symptomatic seizures in 22q11.2DS suggests the deletion may lower the 'seizure-threshold' (i.e. the likelihood that an individual will have a seizure)[161]. Estimates of repeated unprovoked seizures (i.e. epilepsy) in 22q11.2DS range from 4.4%-36.8% [96,97,160,161].

1.7.3.2 The epilepsy phenotype in 22q11.2DS

Between 1-7% of individuals with 22q11.2DS are diagnosed with structural/metabolic epilepsy[97,159-161] and these patients present with GTCS, myoclonic seizures, focal clonic seizures, focal seizures with impaired awareness and focal to bilateral tonic-clonic seizures. Neuroanatomical abnormalities present in 22q11.2DS that can confer risk for epileptic seizures include diffuse cerebral atrophy, polymicrogyria, hippocampal malrotation, grey and white matter heterotopia and focal cortical dysplasia. However, other than these abnormalities, there is a dearth of research into biomarkers for epilepsy risk in 22q11.2DS. Whilst focal epilepsy is the most common type in 22q11.2DS (44% of those with unprovoked seizures)[165], the deletion has also been strongly linked with risk for genetic generalised epilepsy (GGE). Between 1-8.3% of people with 22q11.2DS are diagnosed with GGE (27% of deletion carriers with unprovoked seizures) and the deletion is also found in significant excess in GGE cohorts relative to controls[155,160,161,165,166].

A specific GGE electroclinical syndrome that the 22q11.2 deletion may confer risk for is juvenile myoclonic epilepsy (JME), which typically onsets in adolescence and is characterised predominantly by myoclonic jerks, GTCS, and less often absence seizures. Several case studies have reported myoclonic seizures in people with 22q11.2DS[167-170] and a 2016 review of patients with epilepsy and

22q11.2DS found that 15% had GGE with myoclonic features[166]. Absence seizures are not frequently observed in 22q11.2DS. When they do occur, they are generally atypical[168,171], which is in keeping with a JME diagnosis. One possible genetic mechanism for GGE in 22q11.2DS is haploinsufficiency of the genes *DGCR6* and *DGCR6L*, which may affect the expression levels of the $GABA_{B1}$ subunit, the gene for which (*GABBR1*) is located on chromosome 6p21.3[166]. $GABA_{B1}$-deficient mice exhibit spontaneous epileptiform discharges, clonic, tonic-clonic and atypical absence seizures[172].

Little is known about how often individuals with 22q11.2DS have epileptic seizures. Case reports have sometimes described the frequency of seizures on an individual basis (e.g., daily for Patient 3 in Roubertie et al.[171] and 'sporadic' for Case 2 in El Tahir et al.[167]), but studies have not reported on the average or range of seizure frequency in a group of people with 22q11.2DS. The average and range of the length of seizures in groups of young people with 22q11.2DS has also not been described.

Epileptiform discharges in patients with epilepsy and 22q11.2DS, as ascertained through EEG recordings, are varied and can occur in a localised area of the brain (focal), in multiple discrete brain regions (multifocal) or across both hemispheres (generalised). These include spikes, polyspikes, sharp waves and spike/sharp-and-slow-wave discharges, which in some cases are elicited by photic stimuli (e.g. flashing lights). More general EEG abnormalities are also present, namely focal or generalised slowing [96,97,160,161,165,166].

1.7.3.3 Association of seizures and epilepsy with neurodevelopmental problems in 22q11.2DS

Research has also begun to explore the association of seizures and epilepsy with neurodevelopmental problems in 22q11.2DS. Cheung et al. assessed a cohort of adults with 22q11.2DS through medical record review and parental interview. People with neonatal seizures (most hypocalcaemia induced, 7/9 cases) were 28 times more likely to have moderate-to-severe ID (as diagnosed according to DSM-IV criteria based on data obtained from medical record reviews)[29]. Their sample size was small however (N=14), meaning that larger studies are warranted to assess how replicable these findings are. Kim et al. found that children and adolescents with 22q11.2DS and developmental delay were 4 times more likely to have epilepsy than deletion carriers without developmental delay. Psychiatric disorders (as diagnosed by a child psychiatrist) were also more common in deletion carriers with epilepsy (23%) than without epilepsy (9%), although this difference was not significant (p=0.057)[97]. This analysis may have been underpowered however, as only a small number of participants were diagnosed with psychiatric disorders (n=16, 11%), which contrasts with prior findings of high rates of psychopathology in 22q11.2DS (e.g. 54% in Niarchou et al.[27]). There is ultimately a lack of research into the cognitive, psychiatric and other neurodevelopmental profiles that associate with an increased risk for epilepsy in 22q11.2DS.

1.7.3.4 Limitations of research into seizures and epilepsy in 22q11.2DS

All studies investigating the prevalence of seizures and epilepsy in young people with 22q11.2DS, as well as the associations with neuropsychiatric development, have relied primarily on historical medical record review. Differences between medical centres and clinicians in diagnostic methods (e.g. classification system) and completeness of records may have led to inaccurate reports of epilepsy prevalence and seizure type in this syndrome. In addition, such reviews may fail to account for deletion carriers who are experiencing very brief, subtle paroxysms, such as non-motor absences, that their family either do not notice or do not consider to be epileptic seizures and have therefore not been brought to the attention of a clinician. This may be particularly likely given the many serious medical conditions and psychiatric disorders that families of an individual with 22q11.2DS have to manage. If subtle, stereotyped paroxysms seem to lead to no obvious impairment in an individual with 22q11.2DS, their caregivers may prioritise time with clinicians to speak about other conditions that are having a more obvious or serious impact (e.g. congenital heart disease, ASD). In the general population, non-convulsive seizures usually have to occur several times before the affected individual and their family become concerned and speak to a clinician[173].

The majority of studies reporting epileptiform discharges in 22q11.2DS are similarly based on medical record reviews (however, see Andrade et al[96]. for an first-hand EEG assessment)[97,160,161,165,166]. These studies do not specify the duration of the EEG recordings. If the reported findings were based on routine, interictal clinical EEG (i.e. ~30 minutes), then epileptiform discharges may have been missed in some cases, for example, those occurring during sleep.

Finally, whilst studies have begun to address the links of seizures and epilepsy with neurodevelopmental problems in 22q11.2DS, there are many relationships that have not been explored. Examples include specific psychiatric diagnoses (e.g. ADHD) and other salient manifestations of 22q11.2DS, such as sleep disturbance and motor coordination problems.

1.8 Summary and purpose of thesis

22q11.2DS is a complex disorder characterised by a highly variable physical, cognitive and psychiatric phenotype. People with 22q11.2DS may also be at risk for acute symptomatic seizures (e.g. those associated with hypocalcaemia and psychotropic drug use) and epilepsy. The true prevalence of seizures and epilepsy, as well as the range of different seizure types in 22q11.2DS may not be known however, as the majority of studies in this area (including all in children and adolescents) have relied primarily on historical medical record review. These records are not suitable for systematic evaluation, given differences between clinical centres in diagnosis, classification and documentation of seizures

and epilepsy. Crucially, this approach will also fail to detect individuals who are having brief, non-convulsive, epileptic seizures that have not been brought to the attention of a clinician. Seizure length and frequency in 22q11.2DS also needs to be better delineated. Furthermore, whilst seizures and epilepsy have been shown to be associated with poorer intellectual functioning and developmental delay in 22q11.2DS, the relationships with specific psychiatric diagnoses (e.g. ADHD) and other salient features of this syndrome (e.g. sleep disturbance, motor coordination problems) remain unexplored.

In this thesis I aimed to address these gaps in the literature by conducting a systematic assessment of seizures and epilepsy in children and adolescents with 22q11.2DS. The data I used relied on 'first-hand' accounts of seizures and other clinically suspicious events from affected individuals and their primary caregivers, supplemented by 24-hour EEG measurement and review of relevant medical records. I also conducted the same assessments in siblings without the deletion (referred to from now on as 'control siblings'). Finally, I explored the associations of seizures and epilepsy with cognition, psychiatric health and other salient developmental domains (e.g. sleep quality and motor functioning) in the cohort of young people with 22q11.2DS.

More specifically, in this thesis I aimed to use systematic and standardised assessments to:

1. Explore whether epileptic seizures, epilepsy and epileptiform discharges occur in children and adolescents with 22q11.2DS at significantly higher rates than in their control siblings.
2. Better characterise the aetiology, semiology and frequency of epileptic seizures in young people with 22q11.2DS.
3. Examine the associations of epileptic seizures and epilepsy with impaired brain activity, cognition, psychopathology and other salient neurodevelopmental problems in young people with 22q11.2DS.

In order to address these aims, the following key hypotheses were tested:

1. The rate of epileptic seizures, epilepsy and epileptiform discharges would be higher in children and adolescents with 22q11.2DS than in control siblings.
2. Epileptic seizures and epilepsy would be associated with impaired brain activity and cognition, as well as higher rates of psychopathology and other neurodevelopmental problems in young people with 22q11.2DS.

In addition to better elucidating the prevalence, type and frequency of epileptic seizures, epilepsy and epileptiform discharges in young people with 22q11.2DS, this systematic epilepsy assessment can arguably provide important insights beyond this patient group. This is because the vast majority of children with 22q11.2DS (~95%) have a homogenous genetic lesion[15], allowing us to precisely model

one possible biological risk pathway for epileptic seizures and epilepsy and the interaction of these phenomena with other important developmental domains, such as cognition and psychiatric health.

1.9 References

1 McCarthy, S. E. *et al.* Microduplications of 16p11. 2 are associated with schizophrenia. *Nature genetics* **41**, 1223-1227 (2009).

2 Shinawi, M. *et al.* Recurrent reciprocal 16p11. 2 rearrangements associated with global developmental delay, behavioral problems, dysmorphism, epilepsy, and abnormal head size. *Journal of medical genetics*, jmg. 2009.073015 (2009).

3 Sanders, S. J. *et al.* Multiple recurrent de novo CNVs, including duplications of the 7q11. 23 Williams syndrome region, are strongly associated with autism. *Neuron* **70**, 863-885 (2011).

4 Rees, E. *et al.* Analysis of copy number variations at 15 schizophrenia-associated loci. *British Journal of Psychiatry* **204**, 108-114, doi:10.1192/bjp.bp.113.131052 (2014).

5 Guna, A., Butcher, N. J. & Bassett, A. S. Comparative mapping of the 22q11. 2 deletion region and the potential of simple model organisms. *Journal of neurodevelopmental disorders* **7**, 18 (2015).

6 Du Montcel, S. T., Mendizabai, H., Aymé, S., Lévy, A. & Philip, N. Prevalence of 22q11 microdeletion. *Journal of Medical Genetics* **33**, 719 (1996).

7 Oskarsdottir, S., Vujic, M. & Fasth, A. Incidence and prevalence of the 22q11 deletion syndrome: a population-based study in Western Sweden. *Archives of disease in childhood* **89**, 148-151 (2004).

8 Goodship, J., Cross, I., LiLing, J. & Wren, C. A population study of chromosome 22q11 deletions in infancy. *Archives of disease in childhood* **79**, 348-351 (1998).

9 Devriendt, K., Fryns, J.-P., Mortier, G., Van Thienen, M. & Keymolen, K. The annual incidence of DiGeorge/velocardiofacial syndrome. *Journal of Medical Genetics* **35**, 789 (1998).

10 Mcdonald-Mcginn, D. M. *et al.* Phenotype of the 22q11. 2 deletion in individuals identified through an affected relative: cast a wide FISHing net! *Genetics in Medicine* **3**, 23 (2001).

11 Swillen, A. *et al.* Intelligence and psychosocial adjustment in velocardiofacial syndrome: a study of 37 children and adolescents with VCFS. *Journal of Medical Genetics* **34**, 453-458 (1997).

12 Edelmann, L., Pandita, R. K. & Morrow, B. E. Low-copy repeats mediate the common 3-Mb deletion in patients with velo-cardio-facial syndrome. *The American Journal of Human Genetics* **64**, 1076-1086 (1999).

13 Carlson, C. *et al.* Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. *The American Journal of Human Genetics* **61**, 620-629 (1997).

14 Shaikh, T. H. *et al.* Chromosome 22-specific low copy repeats and the 22q11. 2 deletion syndrome: genomic organization and deletion endpoint analysis. *Human Molecular Genetics* **9**, 489-501 (2000).

15 McDonald-McGinn, D. M. *et al.* 22q11. 2 deletion syndrome. *Nature Reviews Disease Primers* **1**, 15071 (2015).

16 Swillen, A. & McDonald-McGinn, D. in *American Journal of Medical Genetics Part C: Seminars in Medical Genetics.* 172-181 (Wiley Online Library).

17 McDonald-McGinn, D. *et al.* in *AMERICAN JOURNAL OF HUMAN GENETICS.* 198-198 (UNIV CHICAGO PRESS 1427 E 60TH ST, CHICAGO, IL 60637-2954 USA).

18 McDonald-McGinn, D. M. & Zackai, E. H. Genetic counseling for the 22q11.2 deletion. *Developmental Disabilities Research Reviews* **14**, 69-74, doi:10.1002/ddrr.10 (2008).

19 DiGeorge, A. Discussion on a new concept of the cellular immunology. *J. Pediatr* **67**, 907-908 (1965).

20 Driscoll, D. *et al.* Prevalence of 22q11 microdeletions in DiGeorge and velocardiofacial syndromes: implications for genetic counselling and prenatal diagnosis. *Journal of Medical Genetics* **30**, 813-817 (1993).

21 Burn, J. *et al.* Conotruncal anomaly face syndrome is associated with a deletion within chromosome 22q11. *Journal of Medical Genetics* **30**, 822-824 (1993).

22 McDonald-McGinn, D. M. *et al.* Autosomal dominant "Opitz" GBBB syndrome due to a 22q11. 2 deletion. *American Journal of Medical Genetics Part A* **59**, 103-113 (1995).

23 Giannotti, A., Digilio, M. C., Marino, B., Mingarelli, R. & Dallapiccola, B. Cayler cardiofacial syndrome and del22qll: Part of the CATCH22 phenotype. *American journal of medical genetics* **53**, 303-304 (1994).

24 Moss, E. M. *et al.* Psychoeducational profile of the 22q11. 2 microdeletion: a complex pattern. *The Journal of pediatrics* **134**, 193-198 (1999).

25 De Smedt, B. *et al.* Intellectual abilities in a large sample of children with velo–cardio–facial syndrome: an update. *Journal of Intellectual Disability Research* **51**, 666-670 (2007).

26 Schneider, M. *et al.* Psychiatric disorders from childhood to adulthood in 22q11. 2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11. 2 Deletion Syndrome. *American Journal of Psychiatry* (2014).

27 Niarchou, M. *et al.* Psychopathology and cognition in children with 22q11. 2 deletion syndrome. *The British Journal of Psychiatry* **204**, 46-54 (2014).

28 Chow, E. W., Watson, M., Young, D. A. & Bassett, A. S. Neurocognitive profile in 22q11 deletion syndrome and schizophrenia. *Schizophrenia research* **87**, 270-278 (2006).

29 Cheung, E. N. *et al.* Neonatal hypocalcemia, neonatal seizures, and intellectual disability in 22q11.2 deletion syndrome. *Genet Med* **16**, 40-44, doi:10.1038/gim.2013.71 (2014).

30 Malhotra, D. & Sebat, J. CNVs: harbingers of a rare variant revolution in psychiatric genetics. *Cell* **148**, 1223-1241 (2012).

31 Rauch, A. *et al.* Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. *American journal of medical genetics Part A* **140**, 2063-2074 (2006).

32 De Smedt, B., Swillen, A., Verschaffel, L. & Ghesquiere, P. Mathematical learning disabilities in children with 22q11. 2 deletion syndrome: a review. *Developmental Disabilities Research Reviews* **15**, 4-10 (2009).

33 Gur, R. E. *et al.* Neurocognitive development in 22q11. 2 deletion syndrome: comparison with youth having developmental delay and medical comorbidities. *Molecular psychiatry* **19**, 1205 (2014).

34 Green, T. *et al.* Psychiatric disorders and intellectual functioning throughout development in velocardiofacial (22q11. 2 deletion) syndrome. *Journal of the American Academy of Child & Adolescent Psychiatry* **48**, 1060-1068 (2009).

35 Niklasson, L. & Gillberg, C. The neuropsychology of 22q11 deletion syndrome. A neuropsychiatric study of 100 individuals. *Research in developmental disabilities* **31**, 185-194 (2010).

36 Gothelf, D., Penniman, L., Gu, E., Eliez, S. & Reiss, A. L. Developmental trajectories of brain structure in adolescents with 22q11. 2 deletion syndrome: a longitudinal study. *Schizophrenia research* **96**, 72-81 (2007).

37 Antshel, K. M. *et al.* Cognitive and psychiatric predictors to psychosis in velocardiofacial syndrome: a 3-year follow-up study. *Journal of the American Academy of Child & Adolescent Psychiatry* **49**, 333-344 (2010).

38 Duijff, S. N. *et al.* Cognitive and behavioral trajectories in 22q11DS from childhood into adolescence: a prospective 6-year follow-up study. *Research in developmental disabilities* **34**, 2937-2945 (2013).

39 Gothelf, D. *et al.* Risk factors and the evolution of psychosis in 22q11. 2 deletion syndrome: a longitudinal 2-site study. *Journal of the American Academy of Child & Adolescent Psychiatry* **52**, 1192-1203. e1193 (2013).

40 Vorstman, J. A. *et al.* Cognitive decline preceding the onset of psychosis in patients with 22q11. 2 deletion syndrome. *JAMA psychiatry* **72**, 377-385 (2015).

41 Hooper, S. R. *et al.* A longitudinal examination of the psychoeducational, neurocognitive, and psychiatric functioning in children with 22q11. 2 deletion syndrome. *Research in developmental disabilities* **34**, 1758-1769 (2013).

42 Schaer, M. *et al.* Deviant trajectories of cortical maturation in 22q11. 2 deletion syndrome (22q11DS): a cross-sectional and longitudinal study. *Schizophrenia Research* **115**, 182-190 (2009).

43 Chawner, S. J. *et al.* Childhood cognitive development in 22q11. 2 deletion syndrome: case–control study. *The British Journal of Psychiatry*, bjp. bp. 116.195651 (2017).

44 Association, A. P. *Diagnostic and statistical manual of mental disorders (DSM-5®)*. (American Psychiatric Pub, 2013).

45 Häfner, H., Maurer, K., Löffler, W. & Riecher-Rössler, A. The influence of age and sex on the onset and early course of schizophrenia. *The British Journal of Psychiatry* **162**, 80-86 (1993).

46 Nicolson, R. *et al.* Lessons from childhood-onset schizophrenia. *Brain Research Reviews* **31**, 147-156, doi:https://doi.org/10.1016/S0165-0173(99)00032-6 (2000).

47 Saha, S., Chant, D., Welham, J. & McGrath, J. A systematic review of the prevalence of schizophrenia. *PLoS medicine* **2**, e141 (2005).

48 Murphy, K. C., Jones, L. A. & Owen, M. J. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Archives of general psychiatry* **56**, 940-945 (1999).

49 Monks, S. *et al.* Further evidence for high rates of schizophrenia in 22q11. 2 deletion syndrome. *Schizophrenia research* **153**, 231-236 (2014).

50 Baker, K. D. & Skuse, D. H. Adolescents and young adults with 22q11 deletion syndrome: psychopathology in an at-risk group. *The British Journal of Psychiatry* **186**, 115-120 (2005).

51 Van Os, J., Linscott, R. J., Myin-Germeys, I., Delespaul, P. & Krabbendam, L. A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness–persistence–impairment model of psychotic disorder. *Psychological medicine* **39**, 179-195 (2009).

52 Ahn, K. *et al.* High rate of disease-related copy number variations in childhood onset schizophrenia. *Mol Psychiatry* **19**, 568-572, doi:10.1038/mp.2013.59 (2014).

53 Olsen, L. *et al.* Prevalence of rearrangements in the 22q11.2 region and population-based risk of neuropsychiatric and developmental disorders in a Danish population: a case-cohort study. *The Lancet Psychiatry*, doi:https://doi.org/10.1016/S2215-0366(18)30168-8.

54 Bassett, A. S. *et al.* 22q11 deletion syndrome in adults with schizophrenia. *American journal of medical genetics* **81**, 328 (1998).

55 Gothelf, D. *et al.* Clinical characteristics of schizophrenia associated with velo-cardio-facial syndrome. *Schizophrenia research* **35**, 105-112 (1999).

56 Bassett, A. S. *et al.* The schizophrenia phenotype in 22q11 deletion syndrome. *American Journal of Psychiatry* **160**, 1580-1586 (2003).

57 Polanczyk, G. & Rohde, L. A. Epidemiology of attention-deficit/hyperactivity disorder across the lifespan. *Current opinion in psychiatry* **20**, 386-392 (2007).

58 Thomas, R., Sanders, S., Doust, J., Beller, E. & Glasziou, P. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. *Pediatrics* **135**, e994-e1001 (2015).

59 Merikangas, K. R. *et al.* Lifetime Prevalence of Mental Disorders in U.S. Adolescents: Results from the National Comorbidity Survey Replication–Adolescent Supplement (NCS-A). *Journal of the American Academy of Child & Adolescent Psychiatry* **49**, 980-989, doi:https://doi.org/10.1016/j.jaac.2010.05.017 (2010).

60 Niklasson, L., Rasmussen, P., Óskarsdóttir, S. & Gillberg, C. Autism, ADHD, mental retardation and behavior problems in 100 individuals with 22q11 deletion syndrome. *Research in developmental disabilities* **30**, 763-773 (2009).

61 Kessler, R. C. *et al.* The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. *American Journal of psychiatry* **163**, 716-723 (2006).

62 Niarchou, M., Martin, J., Thapar, A., Owen, M. J. & van den Bree, M. The clinical presentation of attention deficit-hyperactivity disorder (ADHD) in children with 22q11. 2 deletion syndrome. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* **168**, 730-738 (2015).

63 Antshel, K. M. *et al.* The longitudinal course of attention deficit/hyperactivity disorder in velo-cardio-facial syndrome. *The Journal of pediatrics* **163**, 187-193. e181 (2013).

64 Langley, K. *et al.* Adolescent clinical outcomes for young people with attention-deficit hyperactivity disorder. *The British Journal of Psychiatry* **196**, 235-240 (2010).

65 Antshel, K. M. *et al.* Comparing ADHD in velocardiofacial syndrome to idiopathic ADHD: a preliminary study. *Journal of attention disorders* **11**, 64-73 (2007).

66 Lars, W. *et al.* Prevalence of psychiatric disorders in preschoolers. *Journal of Child Psychology and Psychiatry* **53**, 695-705, doi:doi:10.1111/j.1469-7610.2011.02514.x (2012).

67 Green, T. *et al.* The effect of methylphenidate on prefrontal cognitive functioning, inattention, and hyperactivity in velocardiofacial syndrome. *Journal of child and adolescent psychopharmacology* **21**, 589-595 (2011).

68 Baxter, A., Scott, K., Vos, T. & Whiteford, H. Global prevalence of anxiety disorders: a systematic review and meta-regression. *Psychological medicine* **43**, 897-910 (2013).

69 Dekker, M. C. & Koot, H. M. DSM-IV disorders in children with borderline to moderate intellectual disability. I: Prevalence and impact. *Journal of the American Academy of Child & Adolescent Psychiatry* **42**, 915-922 (2003).

70 Baker, K. & Vorstman, J. A. Is there a core neuropsychiatric phenotype in 22q11. 2 deletion syndrome? *Current opinion in neurology* **25**, 131-137 (2012).

71 Elsabbagh, M. *et al.* Global prevalence of autism and other pervasive developmental disorders. *Autism Research* **5**, 160-179 (2012).

72 Matson, J. L. & Shoemaker, M. Intellectual disability and its relationship to autism spectrum disorders. *Research in developmental disabilities* **30**, 1107-1114 (2009).

73 Loomes, R., Hull, L. & Mandy, W. P. L. What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. *Journal of the American Academy of Child & Adolescent Psychiatry* **56**, 466-474 (2017).

74 Fine, S. E. *et al.* Autism spectrum disorders and symptoms in children with molecularly confirmed 22q11. 2 deletion syndrome. *Journal of autism and developmental disorders* **35**, 461-470 (2005).

75 Antshel, K. M. *et al.* Autistic spectrum disorders in velo-cardio facial syndrome (22q11. 2 deletion). *Journal of autism and developmental disorders* **37**, 1776-1786 (2007).

76 Vorstman, J. A. *et al.* The 22q11. 2 deletion in children: high rate of autistic disorders and early onset of psychotic symptoms. *Journal of the American Academy of Child & Adolescent Psychiatry* **45**, 1104-1113 (2006).

77 Glaser, B. *et al.* Eye gaze during face processing in children and adolescents with 22q11. 2 deletion syndrome. *Journal of the American Academy of Child & Adolescent Psychiatry* **49**, 665-674 (2010).

78 Klin, A., Jones, W., Schultz, R., Volkmar, F. & Cohen, D. Visual fixation patterns during viewing of naturalistic social situations as predictors of social competence in individuals with autism. *Archives of general psychiatry* **59**, 809-816 (2002).

79 Angkustsiri, K. *et al.* Social impairments in chromosome 22q11. 2 deletion syndrome (22q11. 2DS): autism spectrum disorder or a different endophenotype? *Journal of autism and developmental disorders* **44**, 739-746 (2014).

80 Eliez, S. Autism in children with 22q11. 2 deletion syndrome. *Journal of the American Academy of Child & Adolescent Psychiatry* **46**, 433-434 (2007).

81 Kates, W. R. *et al.* Comparing phenotypes in patients with idiopathic autism to patients with velocardiofacial syndrome (22q11 DS) with and without autism. *American Journal of Medical Genetics Part A* **143**, 2642-2650 (2007).

82 Rutter, M., Le Couteur, A. & Lord, C. Autism diagnostic interview-revised. *Los Angeles, CA: Western Psychological Services* **29**, 30 (2003).

83 Lord, C., Rutter, M., DiLavore, P. C. & Risi, S. Autism diagnostic observation schedule-WPS (ADOS-WPS). *Los Angeles, CA: Western Psychological Services* (1999).

84 Kessler, R. C. *et al.* Prevalence, persistence, and sociodemographic correlates of DSM-IV disorders in the National Comorbidity Survey Replication Adolescent Supplement. *Archives of general psychiatry* **69**, 372-380 (2012).

85 Nock, M. K., Kazdin, A. E., Hiripi, E. & Kessler, R. C. Lifetime prevalence, correlates, and persistence of oppositional defiant disorder: results from the National Comorbidity Survey Replication. *Journal of Child Psychology and Psychiatry* **48**, 703-713 (2007).

86 Kennedy, W. P. *et al.* 22q11.2 Deletion syndrome and obstructive sleep apnea. *Int J Pediatr Otorhinolaryngol* **78**, 1360-1364, doi:10.1016/j.ijporl.2014.05.031 (2014).

87 Crockett, D. J., Goudy, S. L., Chinnadurai, S. & Wootten, C. T. Obstructive sleep apnea syndrome in children with 22q11.2 deletion syndrome after operative intervention for velopharyngeal insufficiency. *Front Pediatr* **2**, 84, doi:10.3389/fped.2014.00084 (2014).

88 Perkins, J. A., Sie, K. & Gray, S. Presence of 22q11 deletion in postadenoidectomy velopharyngeal insufficiency. *Archives of Otolaryngology–Head & Neck Surgery* **126**, 645-648 (2000).

89 Heike, C. L. *et al.* Sleep disturbances in 22q11. 2 deletion syndrome: a case with obstructive and central sleep apnea. *The Cleft palate-craniofacial journal* **44**, 340-346 (2007).

90 Cunningham, A. C. *et al.* Developmental coordination disorder, psychopathology and IQ in 22q11. 2 deletion syndrome. *The British Journal of Psychiatry* **212**, 27-33 (2018).

91 Van Aken, K., Caeyenberghs, K., Smits-Engelsman, B. & Swillen, A. The motor profile of primary school-age children with a 22q11. 2 deletion syndrome (22q11. 2DS) and an age-and IQ-matched control group. *Child Neuropsychology* **15**, 532-542 (2009).

92 Roizen, N. J. *et al.* 22q11. 2 deletion syndrome: are motor deficits more than expected for IQ level? *The Journal of pediatrics* **157**, 658-661 (2010).

93 Swillen, A. *et al.* The behavioural phenotype in velo-cardio-facial syndrome (VCFS): from infancy to adolescence. *Genetic Counseling* **10**, 79-88 (1999).

94 Weinzimer, S. A. Endocrine aspects of the 22q11. 2 deletion syndrome. *Genetics in Medicine* **3**, 19 (2001).

95 Drew, L. J. *et al.* The 22q11. 2 microdeletion: fifteen years of insights into the genetic and neural complexity of psychiatric disorders. *International Journal of Developmental Neuroscience* **29**, 259-281 (2011).

96 Andrade, D. M., Krings, T., Chow, E. W., Kiehl, T.-R. & Bassett, A. S. Hippocampal malrotation is associated with chromosome 22q11. 2 microdeletion. *The Canadian Journal of Neurological Sciences* **40**, 652-656 (2013).

97 Kim, E.-H. *et al.* Epilepsy and Other Neuropsychiatric Manifestations in Children and Adolescents with 22q11. 2 Deletion Syndrome. *Journal of Clinical Neurology* **12**, 85-92 (2016).

98 Styner, M. *et al.* Morphometric analysis of lateral ventricles in schizophrenia and healthy controls regarding genetic and disease-specific factors. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 4872-4877 (2005).

99 Cunningham, A. *Coordination and sensorimotor difficulties in children with 22q11. 2 deletion syndrome: relationships with cognition and psychopathology*, Cardiff University, (2017).

100 Debbané, M., Schaer, M., Farhoumand, R., Glaser, B. & Eliez, S. Hippocampal volume reduction in 22q11. 2 deletion syndrome. *Neuropsychologia* **44**, 2360-2365 (2006).

101 DeBoer, T., Wu, Z., Lee, A. & Simon, T. J. Hippocampal volume reduction in children with chromosome 22q11. 2 deletion syndrome is associated with cognitive impairment. *Behavioral and Brain Functions* **3**, 54 (2007).

102 Eliez, S. *et al.* Velocardiofacial syndrome: are structural changes in the temporal and mesial temporal regions related to schizophrenia? *American Journal of Psychiatry* **158**, 447-453 (2001).

103 Kates, W. R. *et al.* Temporal lobe anatomy and psychiatric symptoms in velocardiofacial syndrome (22q11. 2 deletion syndrome). *Journal of the American Academy of Child & Adolescent Psychiatry* **45**, 587-595 (2006).

104 LeDoux, J. E. Brain mechanisms of emotion and emotional learning. *Current opinion in neurobiology* **2**, 191-197 (1992).

105 Munson, J. *et al.* Amygdalar volume and behavioral development in autism. *Archives of general psychiatry* **63**, 686-693 (2006).

106 Gothelf, D., Schaer, M. & Eliez, S. Genes, brain development and psychiatric phenotypes in velo-cardio-facial syndrome. *Developmental disabilities research reviews* **14**, 59-68 (2008).

107 van Amelsvoort, T. *et al.* Brain Anatomy in Adults With Velocardiofacial Syndrome With and WithoutSchizophrenia: Preliminary Results of a Structural Magnetic Resonance Imaging Study. *Archives of general psychiatry* **61**, 1085-1096 (2004).

108 Gothelf, D. *et al.* COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11. 2 deletion syndrome. *Nature neuroscience* **8**, 1500 (2005).

109 Murray, R. M. & Lewis, S. W. Is schizophrenia a neurodevelopmental disorder? *British Medical Journal (Clinical Research Ed.)* **295**, 681 (1987).

110 Smith, P. The bare essentials: epilepsy. *Practical neurology* **8**, 195-202 (2008).

111 Angeles, D. Proposal for revised clinical and electroencephalographic classification of epileptic seizures. *Epilepsia* **22**, 489-501 (1981).

112 Engel Jr, J. International League Against Epilepsy (ILAE). A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. *Epilepsia* **42**, 796-803 (2001).

113 Jerome, E. Report of the ILAE Classification Core Group. *Epilepsia* **47**, 1558-1568, doi:doi:10.1111/j.1528-1167.2006.00215.x (2006).

114 Berg, A. T. *et al.* Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia* **51**, 676-685 (2010).

115 Fisher, R. S. *et al.* Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia* **58**, 522-530 (2017).

116 Stafstrom, C. E. in *Febrile seizures* 1-25 (Elsevier, 2002).

117 Waruiru, C. & Appleton, R. Febrile seizures: an update. *Archives of Disease in childhood* **89**, 751-756 (2004).

118 Verity, C. M., Greenwood, R. & Golding, J. Long-term intellectual and behavioral outcomes of children with febrile convulsions. *New England Journal of Medicine* **338**, 1723-1728 (1998).

119 Lewis, D. V. *et al.* Hippocampal sclerosis after febrile status epilepticus: the FEBSTAT study. *Annals of neurology* **75**, 178-185 (2014).

120 Martinos, M. M. *et al.* Early developmental outcomes in children following convulsive status epilepticus: a longitudinal study. *Epilepsia* **54**, 1012-1019 (2013).

121 Graves, R. C., Oehler, K. & Tingle, L. E. Febrile seizures: risks, evaluation, and prognosis. *American family physician* **85** (2012).

122 Martinos, M. M. *et al.* Recognition memory is impaired in children after prolonged febrile seizures. *Brain* **135**, 3153-3164 (2012).

123 Sanders, J. & Shorvon, S. Epidemiology of the epilepsies. *J Neurol Neurosurg Psychiatry* **61**, 433-443 (1996).

124 Rai, D. *et al.* Epilepsy and psychiatric comorbidity: A nationally representative population-based study. *Epilepsia* **53**, 1095-1103 (2012).

125 Ottman, R. *et al.* Validation of a brief screening instrument for the ascertainment of epilepsy. *Epilepsia* **51**, 191-197 (2010).

126 Thomas, R. H. & Smith, P. E. M. Epilepsy is different. *Journal of the Royal Society of Medicine* **104**, 141-143, doi:10.1258/jrsm.2011.100412 (2011).

127 Leonardi, M. & Ustun, T. B. The global burden of epilepsy. *Epilepsia* **43**, 21-25 (2002).

128 Meyer, A.-C., Dua, T., Ma, J., Saxena, S. & Birbeck, G. Global disparities in the epilepsy treatment gap: a systematic review. *Bulletin of the World Health Organization* **88**, 260-266 (2010).

129 Bowley, C. & Kerr, M. Epilepsy and intellectual disability. *Journal of Intellectual Disability Research* **44**, 529-543 (2000).

130 Blake, R., Wroe, S., Breen, E. & McCarthy, R. A. Accelerated forgetting in patients with epilepsy: evidence for an impairment in memory consolidation. *Brain* **123**, 472-483 (2000).

131 Hoppe, C., Elger, C. E. & Helmstaedter, C. Long-term memory impairment in patients with focal epilepsy. *Epilepsia* **48**, 26-29 (2007).

132 Fisher, R. S. *et al.* ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* **55**, 475-482 (2014).

133 Smith, S. EEG in the diagnosis, classification, and management of patients with epilepsy. *Journal of Neurology, Neurosurgery & Psychiatry* **76**, ii2-ii7 (2005).

134 Gregory, R., Oates, T. & Merry, R. Electroencephalogram epileptiform abnormalities in candidates for aircrew training. *Electroencephalography and clinical Neurophysiology* **86**, 75-77 (1993).

135 Chez, M. G. *et al.* Frequency of epileptiform EEG abnormalities in a sequential screening of autistic patients with no known clinical epilepsy from 1996 to 2005. *Epilepsy & Behavior* **8**, 267-271 (2006).

136 Hughes, J. R., DeLeo, A. J. & Melyn, M. A. The Electroencephalogram in Attention Deficit–Hyperactivity Disorder: Emphasis on Epileptiform Discharges. *Epilepsy & Behavior* **1**, 271-277, doi:https://doi.org/10.1006/ebeh.2000.0073 (2000).

137 Smith, D., Defalla, B. & Chadwick, D. The misdiagnosis of epilepsy and the management of refractory epilepsy in a specialist clinic. *Qjm* **92**, 15-23 (1999).

138 COMMISION, O. C. TERMINOLOGY OF THE INTERNATIONAL LEAGUE AGAINST EPILEPSY. PROPOSAL FOR REVISED CLASSIFICATION OF EPILEPSIES AND EPILEPTIC SYNDROMES. *Epilepsia* **30**, 389 (1989).

139 Arshad, S. *et al.* Epilepsy and intellectual disability: Does epilepsy increase the likelihood of co-morbid psychopathology? *Research in developmental disabilities* **32**, 353-357 (2011).

140 Kernan, C. L. *et al.* Neurocognitive profiles in children with epilepsy. *Epilepsia* **53**, 2156-2163 (2012).

141 Saemundsen, E., Ludvigsson, P., Hilmarsdottir, I. & Rafnsson, V. Autism Spectrum Disorders in Children with Seizures in the First Year of Life—A Population-Based Study. *Epilepsia* **48**, 1724-1730 (2007).

142 Clarke, D. F. *et al.* The prevalence of autistic spectrum disorder in children surveyed in a tertiary care epilepsy clinic. *Epilepsia* **46**, 1970-1977 (2005).

143 Thome-Souza, S. *et al.* Which factors may play a pivotal role on determining the type of psychiatric disorder in children and adolescents with epilepsy? *Epilepsy & Behavior* **5**, 988-994 (2004).

144 Parisi, P., Moavero, R., Verrotti, A. & Curatolo, P. Attention deficit hyperactivity disorder in children with epilepsy. *Brain and Development* **32**, 10-16 (2010).

145 Jones, J. E. *et al.* Psychiatric comorbidity in children with new onset epilepsy. *Developmental Medicine & Child Neurology* **49**, 493-497 (2007).

146 Davies, S., Heyman, I. & Goodman, R. A population survey of mental health problems in children with epilepsy. *Developmental medicine and child neurology* **45**, 292-295 (2003).

147 Jones, J. E. *et al.* Rates and risk factors for suicide, suicidal ideation, and suicide attempts in chronic epilepsy. *Epilepsy & Behavior* **4**, 31-38 (2003).

148 Clancy, M. J., Clarke, M. C., Connor, D. J., Cannon, M. & Cotter, D. R. The prevalence of psychosis in epilepsy; a systematic review and meta-analysis. *BMC psychiatry* **14**, 75 (2014).

149 van Golde, E. G., Gutter, T. & de Weerd, A. W. Sleep disturbances in people with epilepsy; prevalence, impact and treatment. *Sleep medicine reviews* **15**, 357-368 (2011).

150 Kirby, A. *et al.* Benign childhood epilepsy with centrotemporal spikes (BECTS) and developmental co-ordination disorder. *Epilepsy & Behavior* **72**, 122-126 (2017).

151 Brooks-Kayal, A. Epilepsy and autism spectrum disorders: are there common developmental mechanisms? *Brain and Development* **32**, 731-738 (2010).

152 Avanzini, G., Depaulis, A., Tassinari, A. & De Curtis, M. Do seizures and epileptic activity worsen epilepsy and deteriorate cognitive function? *Epilepsia* **54**, 14-21 (2013).

153 Tonelli, A. R., Kosuri, K., Wei, S. & Chick, D. Seizures as the first manifestation of chromosome 22q11.2 deletion syndrome in a 40-year old man: a case report. *J Med Case Rep* **1**, 167, doi:10.1186/1752-1947-1-167 (2007).

154 Thomas, R. H. & Berkovic, S. F. The hidden genetics of epilepsy [mdash] a clinically important new paradigm. *Nature Reviews Neurology* **10**, 283-292 (2014).

155 Lal, D. *et al.* Burden Analysis of Rare Microdeletions Suggests a Strong Impact of Neurodevelopmental Genes in Genetic Generalised Epilepsies. (2015).

156 Mefford, H. C. *et al.* Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet* **6**, e1000962 (2010).

157 Heinzen, E. L. *et al.* Rare deletions at 16p13. 11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *The American Journal of Human Genetics* **86**, 707-718 (2010).

158 Reinthaler, E. M. *et al.* 16p11. 2 600 kb Duplications confer risk for typical and atypical Rolandic epilepsy. *Human molecular genetics*, ddu306 (2014).

159 Ryan, A. *et al.* Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *Journal of Medical Genetics* **34**, 798-804 (1997).

160 Kao, A. *et al.* Increased prevalence of unprovoked seizures in patients with a 22q11. 2 deletion. *American Journal of Medical Genetics Part A* **129**, 29-34 (2004).

161 Wither, R. G. *et al.* 22q11. 2 deletion syndrome lowers seizure threshold in adult patients without epilepsy. *Epilepsia* (2017).

162 Castilla-Guerra, L., Fernández-Moreno, M. d. C., López-Chozas, J. M. & Fernández-Bolaños, R. Electrolytes disturbances and seizures. *Epilepsia* **47**, 1990-1998 (2006).

163 Han, P., Trinidad, B. J. & Shi, J. Hypocalcemia-induced seizure: demystifying the calcium paradox. *ASN neuro* **7**, 1759091415578050, doi:10.1177/1759091415578050 (2015).

164 Delpisheh, A., Veisani, Y., Sayehmiri, K. & Fayyazi, A. Febrile seizures: etiology, prevalence, and geographical variation. *Iranian journal of child neurology* **8**, 30-37 (2014).

165 Mudigoudar, B., Nune, S., Fulton, S., Dayyat, E. & Wheless, J. W. Epilepsy in 22q11. 2 Deletion Syndrome: A Case Series and Literature Review. *Pediatric Neurology* (2017).

166 Strehlow, V. *et al.* Generalized epilepsy and myoclonic seizures in 22q11. 2 deletion syndrome. *Molecular syndromology* **7**, 239-246 (2016).

167 El Tahir, M., Kerr, M. & Jones, R. Two cases of generalized seizures and the Velocardiofacial syndrome–a clinically significant association? *Journal of Intellectual Disability Research* **48**, 695-698 (2004).

168 Bernhard, M., Kantzer, A.-K., Kujat, A. & Merkenschlager, A. 22q11. 2-Mikrodeletion mit atypischen Absencen, Dysmorphie und Skoliose. *Monatsschrift Kinderheilkunde* **155**, S62-S64 (2007).

169 Boot, E. *et al.* Movement disorders and other motor abnormalities in adults with 22q11.2 deletion syndrome. *Am J Med Genet A* **167A**, 639-645, doi:10.1002/ajmg.a.36928 (2015).

170 Lemke, J. R. *et al.* Juvenile myoclonic epilepsy with photosensitivity in a female with Velocardiofacial syndrome (del(22)(q11.2))--causal relationship or coincidence? *Seizure* **18**, 660-663, doi:10.1016/j.seizure.2009.07.008 (2009).

171 Roubertie, A. *et al.* Neurological presentation of three patients with 22q11 deletion (CATCH 22 syndrome). *Brain & Development* **23**, 810-814 (2001).

172 Schuler, V. *et al.* Epilepsy, hyperalgesia, impaired memory, and loss of pre-and postsynaptic GABAB responses in mice lacking GABAB (1). *Neuron* **31**, 47-58 (2001).

173 Pohlmann-Eden, B., Beghi, E., Camfield, C. & Camfield, P. The first seizure and its management in adults and children. *BMJ: British Medical Journal* **332**, 339 (2006).

2 General methodology

This chapter will describe the measures used in the pilot study (Chapter 3) and in the experimental chapters (Chapters 4 and 5). The structure of the systematic assessment of epileptic seizures and epilepsy in young people with 22q11.2DS will also be outlined.

2.1 The ECHO study cohort

In this thesis, findings are reported from a sample of young people with 22q11.2DS and their control siblings, drawn from the Experiences of CHildren with cOpy number variants (ECHO) study.

The ongoing ECHO study describes the physical, cognitive and psychiatric profiles of children and adolescents with 22q11.2DS. People with 22q11.2DS are referred to the ECHO study via UK genetics clinics, charities for children with chromosomal conditions (Unique, The 22Crew and Max Appeal!) and word of mouth. To date, more than 150 young people with 22q11.2DS have been assessed cross-sectionally. Biological siblings without the deletion and who are closest in age to the person with 22q11.2DS are also invited to take part (to date, ~75 control siblings have been assessed). Participants have to be six years of age or older to take part in the study, so that the results of the psychiatric assessments (Section 2.5) are valid. Participants with the 1.5Mb A-B deletion, 2Mb A-C and typical 3Mb A-D deletion are recruited, given that there is little evidence for different phenotypic outcomes based on deletion size in people with 22q11.2DS (see Section 1.2). General information about the study can be found at the following web address:

http://www.cardiff.ac.uk/mrc-centre-neuropsychiatric-genetics-genomics/research/themes/developmental-disorders/echo-study-cnv-research.

The ECHO study also seeks to explore the development of young people with 22q11.2DS over time. Children who have taken part in the first wave of assessment are therefore re-contacted and consented into further waves of assessment. These longitudinal assessments employ the same measures used in the first wave. Children are assessed on average every two-and-a-half years. Children are still being recruited into the first wave of assessment, so different children are at different waves of assessment. So far, 90 people with 22q11.2DS and 44 control siblings have taken part in the second wave of assessment, 54 deletion carriers and 30 siblings have taken part in the third and two deletion carriers have been seen for a fourth wave of assessment.

Within the ECHO study, I conducted a systematic assessment of epileptic seizures and epilepsy. This assessment comprised two stages. Below I describe how these two stages were implemented within

the larger ECHO study. The 'first stage' of this assessment comprised an epilepsy screening questionnaire (described in Section 2.3.1). This questionnaire was a part of the main battery of ECHO study measures (i.e. it was used across the four waves of the ECHO study in tandem with the cognitive and psychiatric measures). The measures constituting the 'second stage' of the systematic assessment of epileptic seizures and epilepsy (see Sections 2.3.2 to 2.3.8) formed an additional assessment battery and were only completed once. For each consenting family, this additional battery was completed at the same time as the main battery at a given ECHO wave, or as close in time as possible to the family's most recent ECHO wave.

2.2 Participants and procedure

The sample that took part in the systematic assessment of epileptic seizures and epilepsy consisted of 108 young people with 22q11.2DS (57.4% male, mean age= 13.6 years, s.d.=3.3 years, range=6.2-20.5 years) and 60 of their unaffected biological control siblings (50% male, mean age= 13.1 years, s.d.= 3.2 years, range=6.3-18.9 years).

The presence of the deletion was confirmed in UK genetics clinics and/or in the laboratory of the MRC Centre for Neuropsychiatric Genetics and Genomics using standard FISH/microarray techniques. These techniques were also used by the MRC laboratory to confirm the absence of any CNV in the control siblings. In 13.9% (15/108) young people with 22q11.2DS, deletion aetiology was not known. In the remaining deletion carriers, 90.3% (84/93), had a de novo deletion, whilst 9.7% (9/93) inherited the deletion from a parent. In 15.8% (17/108) of deletion carriers, deletion size was unknown; the report from the genetic clinic did not specify the deletion size (and a biological sample was unable to be collected from these participants for testing in the MRC laboratory). In the remaining individuals, 90.1% (82/91) had the typical 3Mb A-D deletion. 7.7% (7/91) had a 1.5Mb A-B deletion and 1.1% (1/91) had a 2Mb A-C deletion, with another individual (1.1%) having an atypical B-D deletion. 1.9% (2/108) of deletion carriers had additional 'second-hit' CNVs, one with a duplication at 8q22.2 and another with a duplication at 4q31.3.

Informed written consent was obtained from the primary caregivers of participants, and from participants themselves if they were 16 years of age or older. The National Health Service Wales Research Ethics Committee approved our protocols (REC number 12/WA02/32).

2.3 Systematic assessment of epileptic seizures and epilepsy

Fig 2-1 provides an overview of the structure of the systematic assessment of seizures and epilepsy. Briefly, families who had completed the Epilepsy Screen Questionnaire (ESQ, i.e. they had screened positive or negative) from the main battery of measures in the ECHO study were invited to take part in the second stage of the epilepsy assessment. Within the second stage of assessment, children who had screened positive (see Section 2.3.1 for a definition) or negative on the ESQ were invited to take part in a 24-hour electroencephalography assessment. For these individuals, the primary caregiver was asked about a family history of epileptic seizures and epilepsy. For children screening positive on the ESQ, I also interviewed the primary caregiver and child about the unusual spells reported in this questionnaire and asked for copies of relevant medical records and video-recordings of the child's unusual spells. If during the interview the child was reported as having repeated, stereotyped events, occurring within the last year, I gave a diary to the primary caregiver to record the frequency of the unusual spells over a two-month period.

A limitation of my study design is a possible selection bias when recruiting during the second stage of assessment: families with a child with a milder cognitive and psychiatric phenotype may have been more willing to take part, particularly with respect to the 24-hour EEG assessment. This does not appear to be the case for the total sample of deletion carriers who took part in the second assessment stage; they did not differ in their full-scale IQ (FSIQ) score, rate of 'any psychiatric diagnosis' or the highest level of parental education from deletion carriers who did not take part in the second assessment stage. They were however significantly older (14.62 years versus 12.39 years). Control siblings who took part in the second assessment stage also showed no differences on these measures from those that did not take part, with the exception of their FSIQ score (114.05 versus 105.97, respectively). Please see Section 5.4.1 for a full description of these results, and Section 5.5.1 for a discussion of why these differences occurred and their implications for the generalisability of my findings from the second assessment stage.

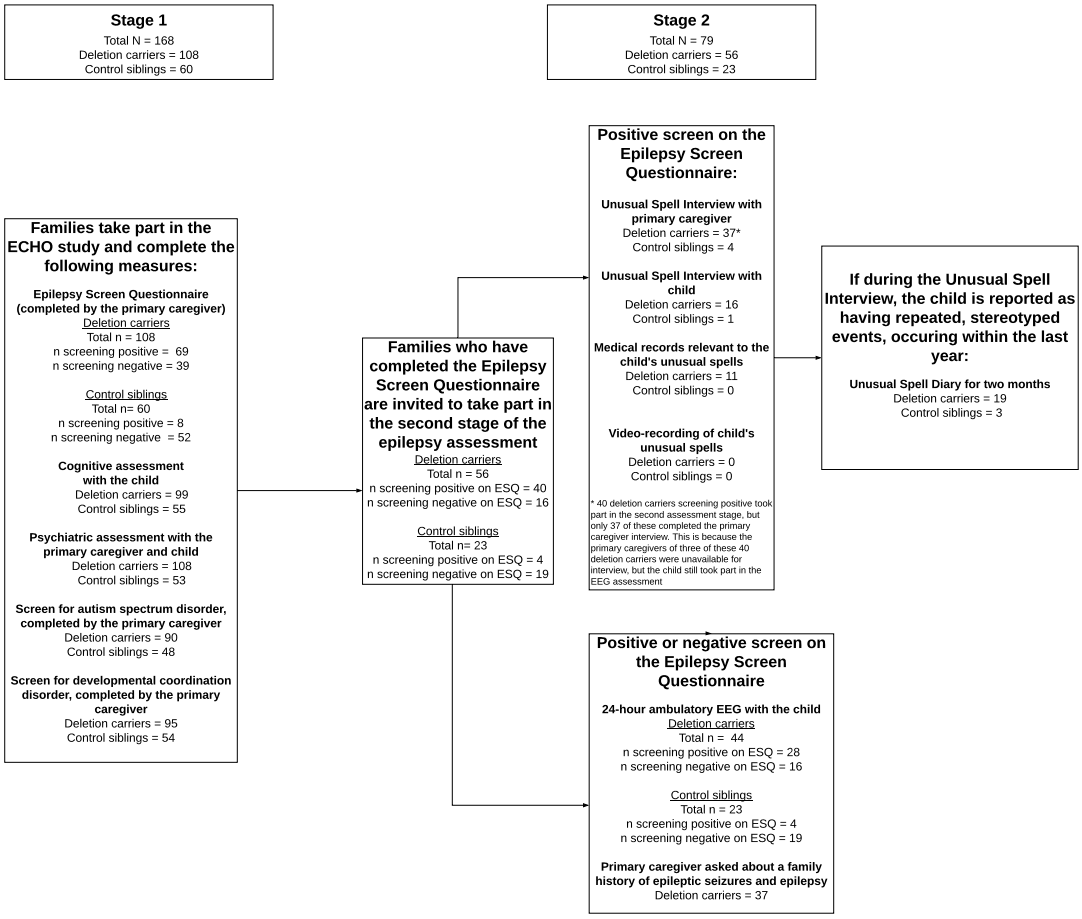

Figure 2-1. Flow chart outlining the systematic assessment of epileptic seizures and epilepsy. A positive screen on the Epilepsy Screen Questionnaire was defined as a 'Yes' or 'Possibly' response to at least one item.

2.3.1 Epilepsy Screen Questionnaire

2.3.1.1 Overview

We used an amended version of the validated 'Epilepsy Screening Questionnaire' (ESQ) developed by Ottman et. al[1] to screen for a lifetime history of an epilepsy diagnosis, seizures and paroxysmal events in the child. Paroxysmal events are behaviours that could be clinical manifestations of seizures, such as uncontrolled jerking or twitching movements or frequent vacant episodes. They may reflect epileptic seizures that have not yet been clinically recognised. The items of the ESQ can be found in Table 2-1. Primary caregivers completed all nine items of this questionnaire. Response options were 'Yes' 'No', 'Possibly' or 'Don't know'. A positive response to a given item was classified as 'Yes' or

'Possibly'. A positive screen was defined as a positive response to at least one item of the questionnaire ('any positive').

The validity of the ESQ was assessed in a study by Ottman et al.[1]. The ESQ was given to 168 participants with a medical-record documented epilepsy diagnosis, 54 participants with an isolated unprovoked seizure and 120 participants with no clinical history of epileptic seizures. When a positive screen was defined as a 'Yes' or 'Possibly' response to at least one item of the questionnaire, the sensitivity of the ESQ was 96% for epilepsy patients and 87% for participants with isolated unprovoked seizures. The false-positive rate amongst the seizure-free participants was 7%. Despite this low false-positive rate, the positive predictive value of the ESQ in the general population (PPV, the percentage of participants who screen positive who genuinely have epilepsy) would only be 23%, given the relatively low prevalence of epilepsy (assumed to be ~2% in this validation study). It is important to note that the prevalence of epilepsy in 22q11.2DS is estimated to be between 4.4-36.8%[2-5]. False-positives can still be expected to occur in 22q11.2DS however, due to the way in which behaviours associated with the numerous cognitive and psychiatric deficits associated in 22q11.2DS[6] could mimic epileptic seizures (e.g. a deletion screening positive based on vacant spells which reflect a learning disability of ADHD, rather than non-motor absence seizures). It is also important to note that this questionnaire was not validated for use in a population with mild-moderate intellectual disability. Although epilepsy does associate with cognitive impairment in the general population[7], the Ottman et al. sample would presumably have had a higher average IQ than the young people with 22q11.2DS in this thesis, given that the Ottman et al. participants would not have had a homogenous genetic lesion conferring risk for mild-moderate intellectual disability (although the mean IQ of the sample was not reported in the validation study)[1]. Despite these potential limitations of using the ESQ with young people with 22q11.2DS, it was important for me employ a measure with high sensitivity (96% for epilepsy in the ESQ), in order to capture non-motor absence or focal non-motor seizures with impaired awareness that may have been mistaken for behaviours associated with cognitive delay and/or psychopathology by families (e.g. daydreaming).

Table 2-1. The items of the Epilepsy Screen Questionnaire.

Item 1. Did your son/daughter ever have a seizure or convulsion caused by a high fever?
Item 2. Other than the seizures associated with high fevers, has your son/daughter ever had a seizure, convulsion, fit, or spell - under any circumstance?
Item 3. Other than the seizures associated with high fevers, has your son/daughter ever had uncontrolled movements of part or all of their body such as twitching, jerking, shaking or going limp?
Item 4. Other than the seizures associated with high fevers, has your son/daughter ever had an unexplained change in their mental state or level of awareness; or an episode of 'spacing out' that they could not control?
Item 5. Does your son/daughter daydream or stare into space more than other children?
Item 6. Have you ever noticed them to have any unusual body movements when exposed to strobe lights, video games, flickering lights, or sun glare?
Item 7. Shortly after waking up, either in the morning or after a nap, have you ever noticed that your son/daughter has had uncontrollable jerking or clumsiness, such as dropping things or things suddenly flying from their hands?
Item 8. Has your son/daughter ever had any other type of repeated unusual spells?
Item 9. Has your son/daughter ever been diagnosed with a seizure disorder or epilepsy?

2.3.1.2 Amendments to the original questionnaire

The ESQ is designed to be self-reported and in the Ottman et al. validation study[1], participants were only asked about afebrile seizures and paroxysmal events if they didn't have an epilepsy diagnosis. I asked the primary caregiver to complete the ESQ, given the cognitive deficits associated with 22q11.2DS[8-11] and the relatively young age of our sample of deletion carriers (13.6 years). I also asked the primary caregiver to complete all nine items of the ESQ, regardless of whether or not their child had an epilepsy diagnosis. This is so we obtained as complete information about seizures and paroxysmal events for each child as possible.

2.3.2 Unusual Spell Interview

I completed all of the interviews described in this thesis.

2.3.2.1 Overview

If the primary caregiver gave a positive response to at least one item of the ESQ, I asked them to complete the Unusual Spell Interview about the child. A short, supplementary section was also completed with the child, if they were 6 years or older at the time when they were having their unusual spells (and were therefore likely to remember these events). The interview can be found in the Appendices. The Unusual Spell Interview was originally developed by Ottman et. al [12,13] and a modified version has been used within several studies conducted as part of the Epilepsy Phenome/Genome Project[14-16]. The interview is either self-report or informant-report. The interview can accurately classify major seizure categories: when comparing interview and neurologist seizure classifications, the positive predictive value (the proportion of patients whose seizure type was correctly classified by the interview) was very high for both focal (0.95) and generalised onset (1.0) seizures. Non-chance agreement between the interview and neurologist diagnoses is also fair-to-excellent for more specific seizure categories, such as focal seizures with and without impaired awareness (k=0.56 and k=0.54, respectively) and generalised tonic-clonic seizures (GTCS, k=0.76)[12]. Reliability of seizure classification from the interview is also good; agreement between lay interviewers is moderate-substantial for generalised onset seizures (k= 0.46-0.77) and substantial- perfect for focal onset seizures (k=0.64-1.00)[13].

2.3.2.2 Amendments to the original interview

In the studies conducted by Ottman et al.[12,13] and the Epilepsy Phenome-Genome project[14-16], the interview was conducted with patients who had reported having an epilepsy diagnosis. By contrast, in this thesis, I interviewed the primary caregiver and child if they had responded positively to at least one item of the ESQ. This was done in order to maximize the sensitivity of the interview with respect to detecting epileptic seizures, for example, a primary caregiver may only have reported that their child "frequently daydreams or stares into space more than other children" (Item 5, Table 2-1), but may not realise that these behaviours could reflect non-motor absence seizures. During the interview, I referred to the events being discussed as 'unusual spells', to reflect the broad nature of my assessment of epileptic seizures and epilepsy. The original interview is divided into sections on "grand mal" seizures (a traditional name for generalised tonic-clonic seizures, GTCS) and "small seizures" (e.g. focal motor seizures). Given that I was using the interview for broader purposes and interviewing some participants who didn't report a history epilepsy or seizures, I combined these two sections into an "unusual spell" section. I also added in new sections for febrile seizures, clinical investigation/medication and hypocalcaemia diagnosis and treatment (given the elevated rate of hypocalcaemia-induced seizures in 22q11.2DS, 1-14.5%[2,4,17]). Finally, I created a new, supplementary

section that was conducted with the child. The purpose of this was to probe for any subjective phenomena occurring with the unusual spell that the primary caregiver may not have been aware of, such as any auras or triggers that the child may have experienced.

2.3.2.3 Structure and content of the interview

The first part of the interview asked the primary caregiver about any febrile seizures the child may have had ('Part 1') and was only completed if the primary caregiver responded positively to Item 1 of the ESQ (Table 2-1). Firstly, the primary caregiver was asked to provide a detailed description of the event, starting with the very first thing that happened, through to how the participant felt, or what happened, after the event. The interviewer could probe for more details about any aspect of the event, such as the typical features of a febrile seizure (e.g. whether the participant vomited or foamed at the mouth, lost consciousness, displayed any jerking/shaking or the body etc, see 'Supplementary: Febrile Seizure Probes'). After the primary caregiver had described the event, the interviewer asked a series of closed questions, probing for features such as the length, lifetime frequency and age of onset and offset of the event, as well as whether the child was prescribed any medication.

The next section asked about any other unusual spells the child might have experienced ('Part 2: Other Unusual Spells'). In 'Section A: Description of Unusual Spells', the interviewer firstly read out the primary caregiver's responses on the ESQ and asked the primary caregiver to identify how many different types of unusual spells the child had experienced in their lifetime. My definition of a 'different type' of unusual spell was if the child felt different during the spell, or if what happened before, during or after the spell was different from the other types. The primary caregiver was then asked to give each unusual spell a name of their choice that summarised its main features, e.g. 'staring spell', 'twitching spell'. Taking each unusual spell in turn, and starting with the most common unusual spell, the interviewer asked the primary caregiver to provide a detailed description of the beginning, middle, end and aftermath of the spell. The interviewer then asked as series of closed questions about the unusual spell, probing for features such as the length, lifetime frequency, onset and offset, as well as the child's level of awareness during the event and whether the child experienced any warnings or auras. Finally, the interviewer took the primary caregiver through a checklist of epileptic seizure symptoms ('Seizure/Unusual Spell Symptoms'), e.g. uncontrollable jerking, shaking, head turn, eye rolling, biting of the cheek or the side of the tongue, repetitive lip smacking etc. If the primary caregiver identified a particular symptom as having occurred with the child's unusual spell, the interviewer probed for more details, such as whether this occurred before, during or after the unusual spell and which parts of the body were involved. The interviewer then asked the primary caregiver about potential triggers for any of the child's unusual spells ('Section B: Seizure Triggers'), e.g. flashing

or blinking lights, being touched, poor sleep. Finally, to probe for particularly prolonged or recurrent seizures, the primary caregiver was asked whether any of the child's unusual spells had lasted for 10 minutes or more, or whether they had experienced several unusual spells one right after the other ('Section C: Screen for Status Epilepticus, Prolonged Seizures or Recurrent Seizures').

In 'Part 3: Further Information', the primary caregiver was firstly asked questions relating to the clinical investigation and treatment of the child's unusual spells ('Section A: Investigation and Medication'). The interviewer then asked about whether the child had been diagnosed with hypocalcaemia and whether a clinician had ever suggested that hypocalcaemia was the cause of the child's unusual spells. Finally, the primary caregiver was asked about whether they had any further details they would like to add ('Section B: Wrap-up').

In the supplementary section conducted directly with the child, the interviewer firstly asked for any further details that the child could provide about their unusual spells, as well as how the child felt, or what happened, before the unusual spell started ('Section A: Description of Auras'). The child was then asked to identify any triggers for their unusual spells ('Section B: Seizure triggers').

2.3.3 Unusual Spell Diary

If in the Unusual Spell Interview the child was reported as having repeated, stereotyped events that had occurred within the last year, primary caregivers were given the 'Unusual Spell Diary' to complete. This measured the frequency of the child's unusual spells over a two-month period. Every time an unusual spell occurred, the primary caregiver was asked to record the date, the time it began, how long it lasted for (in minutes), whether the child was awake or asleep at the time and any additional salient features, for example, what the child was doing before the unusual spell, or whether there were any triggers. The Unusual Spell Diary was based off seizure diaries provided by charities such as the Epilepsy Society:

https://www.epilepsysociety.org.uk/seizure-diaries#.WwV6l4oh1aR

2.3.4 Video-recordings of unusual spells

Primary caregivers were asked to provide a copy of any existing recordings of the child's unusual spells. During the two-month Unusual Spell Diary period, primary caregivers were also asked to record videos of unusual spells on a mobile phone, if they occurred. I asked for a separate video for each different type of unusual spell. Primary caregivers were asked to record as much of the unusual spell as possible, from when it began to when it ended.

2.3.5 Medical records related to unusual spells

Primary caregivers were asked to provide copies of any medical records they had related to the child's unusual spell(s). The various medical records I asked for are displayed Table 2-2.

Table 2-2. The types of medical records primary caregivers were asked for during the second stage of the systematic assessment of seizures and epilepsy.

Medical correspondence or clinic letters
Discharge summaries from the hospital
Results of any scans, such as MRI, CAT or EEG scans
Any records confirming a diagnosis of epilepsy or a seizure disorder
Any medical records related to blood-calcium-level tests, or showing a diagnosis of hypocalcaemia
Any other medical records relevant to the child's unusual spells

2.3.6 Family history of seizures and epilepsy

Primary caregivers were asked whether anyone in the child's family had a history of febrile seizures, other (afebrile) seizures or epilepsy. Further questions included which side of the participant's family and which generation the individual with the history came from (e.g. mother's or father's side of the family, father or grandfather), whether a cause had ever been suggested for their seizures and/or epilepsy and whether the individual had received treatment. The purpose of this was to account for a family history of seizures or epilepsy, which may explain the occurrence of seizures/epilepsy in a participant, instead of, or in tandem with, the 22q11.2 deletion.

2.3.7 24-hour ambulatory EEG studies

2.3.7.1 Summary

Young people with 22q11.2DS and control siblings who had completed the ESQ were asked to part in a 24-hour ambulatory electroencephalography (EEG) assessment, with simultaneous electrocardiography (ECG) and with overnight video-recording. The recording took place in the child's home. I will refer to this from now on as the 'case-control EEG study' (Chapter 5). The equipment to be used in the case-control EEG assessment was piloted in a 24-hour EEG study with children and adolescents from the general population (Chapter 3). I will refer to this from now on as the 'pilot EEG study'.

As described in the 'Contributions' section of this thesis, I conducted both the pilot and case-control EEG studies with a fellow PhD student from Cardiff University (Hayley Moulding) and a postdoctoral research fellow from the University of Bristol (Dr Ullrich Bartsch), both of whom were exploring sleep in young people with 22q11.2DS. When describing the EEG studies in this chapter I refer to myself and these individuals as 'the researchers'.

2.3.7.2 Criteria for selection for the case-control EEG study

Children who had screened positive or negative screen on the ESQ were asked to participate. This was so that I could compare the brain activity of children with and without seizures and/or epilepsy. This would allow me to assess whether epileptiform discharges correlated with epileptic seizures and epilepsy in 22q11.2DS, or were a general feature of the cognitive impairment and psychopathology conferred by the deletion[8,18]. However, as outlined in 1.7.1.2, epileptiform discharges are not wholly specific for epilepsy; they are particularly likely to occur in individuals with neurodevelopmental disorders such as ASD and ADHD, the rates of which are elevated in 22q11.2DS[6,19,20]. Therefore, even if I were to observe a preponderance of epileptiform discharges in deletion carriers with epileptic seizures and/or epilepsy, these waveforms could still be representative of a more non-specific dysfunction in neuronal circuitry.

2.3.7.3 Overview of EEG

EEG is a measure of the electrical activity of the brain. In EEG recordings, electrodes placed on the scalp record the pooled postsynaptic excitatory and inhibitory potentials of groups of pyramidal neurons, oriented perpendicularly to the cortical surface. Electrodes can be placed on the scalp in accordance with numerous standardized systems. The most widely-used is the '10-20' system. This identifies anatomical landmarks on the skulls, such as the nasion, the depressed area between the eyes, and the inion, a protruding section of bone situated at the base of the skull at the back of the

head. Electrodes are then placed between these landmarks at intervals of 10% or 20%. In the 10-10 system, electrodes are more closely spaced at 10% intervals. This standardised, proportional placement system means that the same scalp positions can be identified across numerous participants, regardless of head size. Electrode sites are assigned a letter depending on which part of the brain they record from: 'Fp' for frontopolar, 'F' for frontal, 'T' for temporal, 'O' for occipital, 'C' for central, 'P' for parietal and 'A' for the mastoid bones behind the ears, common sites for reference electrodes. Sites are also denoted with odd or even numbers, or a lowercase 'z', depending on whether they are situated on the left or right hemisphere, or midline, respectively (e.g. 'F4', 'C6', 'Oz'). Diagrams of the 10-20 and 10-10 systems are provided in Figure 2-2. In EEG recordings, electrodes can be individually glued to the head. In clinical EEG recordings, the most common method is to individually glue 21 electrodes to the scalp (referred to from now on as the 'traditional clinical method'). Alternatively, electrodes can be applied simultaneously via structures which hold all electrodes together, such as an electrode cap or net. Figure 2-3 shows the EEG recording setups used in this thesis; namely the traditional clinical method and a 64-channel Hydrocel Geodesic Sensor Net. Before applying an electrode to a given recording site, the site has to be prepared using a cleaning paste which removes dead skin cells and helps to improve the signal impedance (i.e. signal quality, measured in ohms).

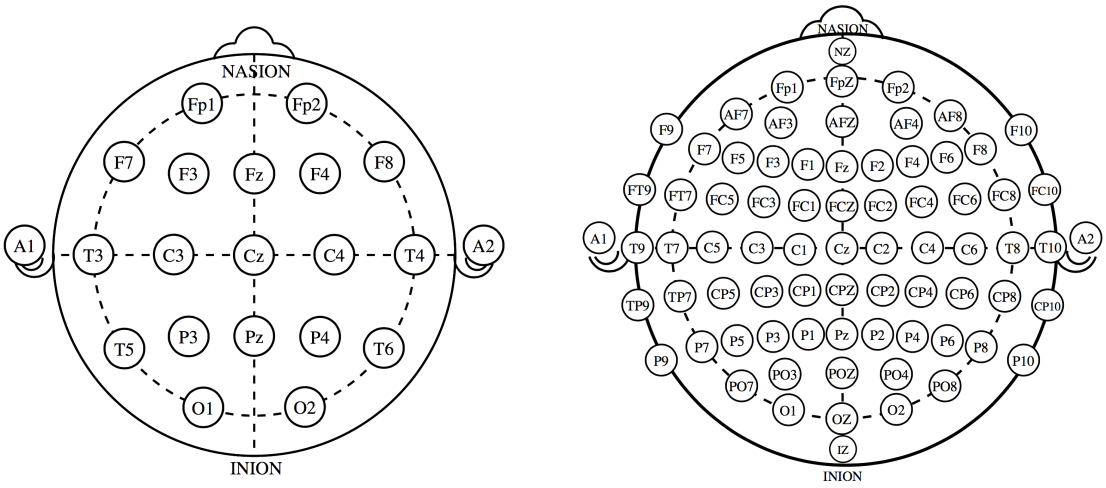

Figure 2-2. The international, standardised systems for electrode placement during EEG recordings. Left: The 10-20 system. Right: The 10-10 system.

Differential amplifiers are then used to amplify the voltage difference between pairs of electrodes (one amplifier per electrode pair), normally by 1,000-100,000 times. Comparing the voltage difference

between pairs of electrodes removes much of the biological and ambient (i.e. external) artifact that is common to both electrodes in a given pair, leaving the signal of interest. Traditionally, EEG was displayed via an analogue method, in which the output signal caused a galvanometer (a coil of wire inside a magnetic field) to move up and down. This galvanometer was attached to a pen and thus drew a signal onto paper moving beneath it. However, modern EEG systems display the trace digitally, allowing for flexible manipulation of the signal. Analogue-to-Digital conversion transforms the waveform into numerical values, at a sampling rate of 256-512Hz. The EEG trace can be viewed in numerous different montages. These are determined by the way in which pairs of electrodes are connected to the differential amplifiers. The most commonly used montages are bipolar, common reference or average reference. In a bipolar montage, the voltage of pairs of adjacent electrodes are compared, either longitudinally (i.e. from the front to the back of the scalp, e.g. Fp1-F7) or transversely (i.e. across the side of the head, e.g. Fp1-Fp2). In a common reference montage, the voltage difference between a given scalp electrode and a common reference is recorded (for example, Fp1-A2). In an average reference montage, the average activity of all scalp electrodes forms the reference electrode.

The EEG signal is then passed through numerous filters, so that activity in frequencies of interest (normally 1-30Hz in clinical EEG) can be viewed clearly. EEG systems currently apply three different filters:), a high-pass filter that attenuates waveforms with a lower frequency than the set value (normally 0.3-1Hz), a low pass filter that attenuates waveforms with a higher frequency than the set value (normally set at 70Hz and a notch filter to remove electrical line noise (set at 50Hz in Europe and 60Hz in North America). Digital EEG systems can apply these filters either during or after the recording. Digital EEG systems also allow manipulation of the size of the waveforms displayed. The size, or sensitivity, of the waveforms is the ratio of signal amplitude (voltage) to the signal deflection (the amount of space the deflection takes up on the recording paper or display). For example, with a sensitivity of 10 μV/mm, a 100μV waveform will present as a 1cm vertical deflection. Finally, the amount of the recording displayed on a particular page can be manipulated, for example, '30mm/s' means that 30mm of page is displayed for each second.

EEG recordings are used for a variety of purposes with suspected and confirmed epilepsy patients. For example, recordings can be used as an adjunct for epilepsy diagnosis, in helping to classify seizure type, in localizing areas likely to generate epileptic seizures ('epileptogenicity') and in assessing response to treatment and risk of seizure recurrence. EEG recordings may pick up on background abnormalities, such as focal or generalised slowing, that are indicative of cerebral dysfunction but are not strongly correlated with epilepsy. EEG recordings may also pick up on epileptiform discharges, waveforms that are strongly correlated with risk for seizures and epilepsy[21]. They are clearly distinguishable from the background activity and are very often of negative polarity at the surface of

the scalp, leading to an upwards deflection on the EEG trace (given that the epileptogenic electrode site is more negative than the common reference electrode in a referential montage, or the neighbouring electrode in a bipolar montage). Epileptiform discharges include spikes, lasting for between 20 to 70 milliseconds, sharp waves (lasting between 70 to 200 msec) and spike/sharp-and-slow-wave complexes. In a referential montage, an epileptiform discharge is localised by looking at the channel with the highest amplitude deflection (i.e. the site of maximal electronegativity). In a bipolar montage, the reviewer looks for 'phase-reversal' when localising the abnormal waveforms; this is where an epileptogenic electrode site (e.g. T5) is shared between two neighbouring channels (e.g. T3-T5 and T5-O1), resulting in a surface-positive downward deflection in one channel (i.e. T3 is more positive than T5) and a surface-negative upward deflection in the other (i.e. T5 is more negative than O1), leading the two deflections to point toward one another.

2.3.7.4 Equipment used in 24-hour ambulatory EEG recordings

The BE PLUS LTM and Galileo Suite Software

The BE PLUS LTM is an EEG amplifier developed by EB Neuro S.p.A (Florence, Italy). It supports both wired and wireless EEG recordings, shown in Figure 2-3. The amplifier supports EEG recording via individual electrodes glued to the head (up to 64), or EEG cap/net. In the pilot and case-control EEG studies, we used a traditional clinical setup and a 64-channel Hydrocel Geodesic Sensor Net (HCGSN) with the amplifier. Four additional channels on the amplifier allow other physiological measures to be implemented. In the pilot and case-control EEG studies, we used these one of these additional channels to record 24-hour electrocardiography (ECG).

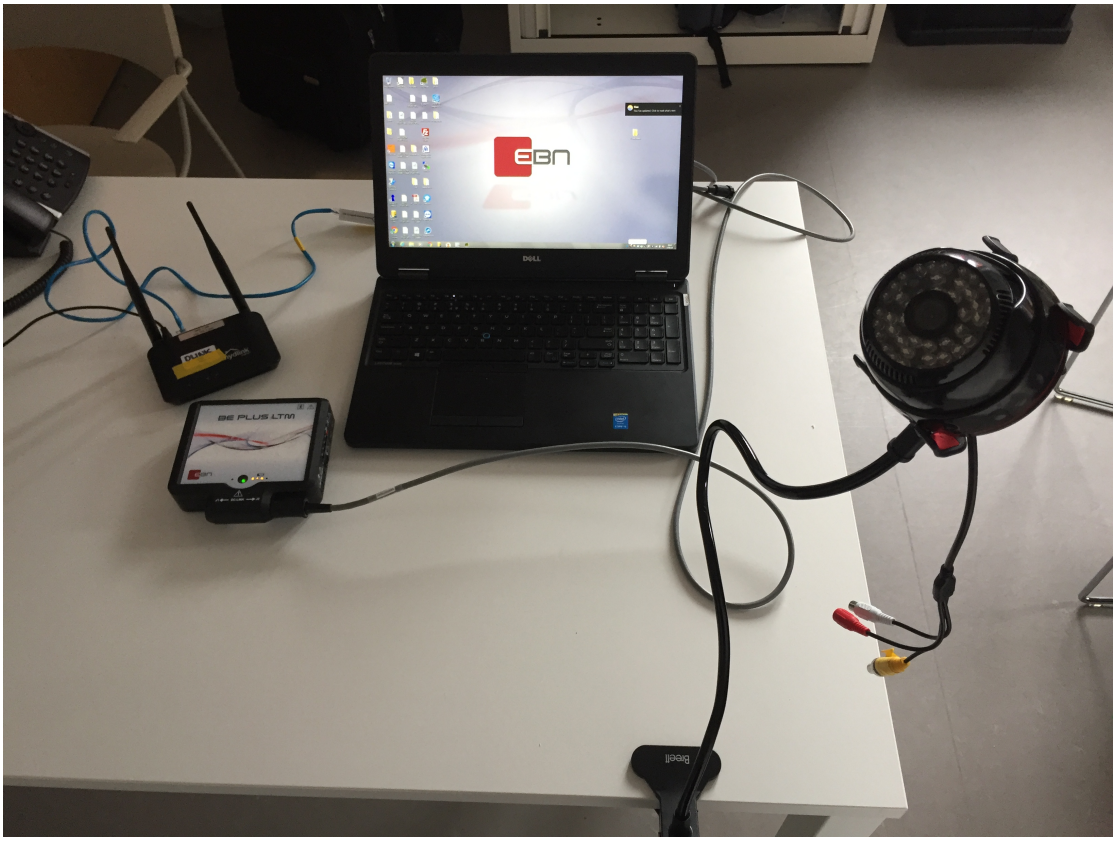

Figure 2-3. Recording setups with the BE PLUS LTM used in my 24-hour EEG studies. Data could be recorded via a wired LAN connection, or wirelessly via an access point using Wi-Fi technology (EB Neuro S.p.A).

The Galileo Suite Software, also developed by EB Neuro S.p.A allows the EEG recording to visualised. Traces can be displayed in various different montages that the user can create (e.g. longitudinal, common reference). High-pass and low-pass filters can be applied, as well as a notch filter, set at 50Hz. The sensitivity of the waveforms can be manipulated, as can the amount of data displayed on each page. The Galileo Suite Software is shown in Figure 2-4.

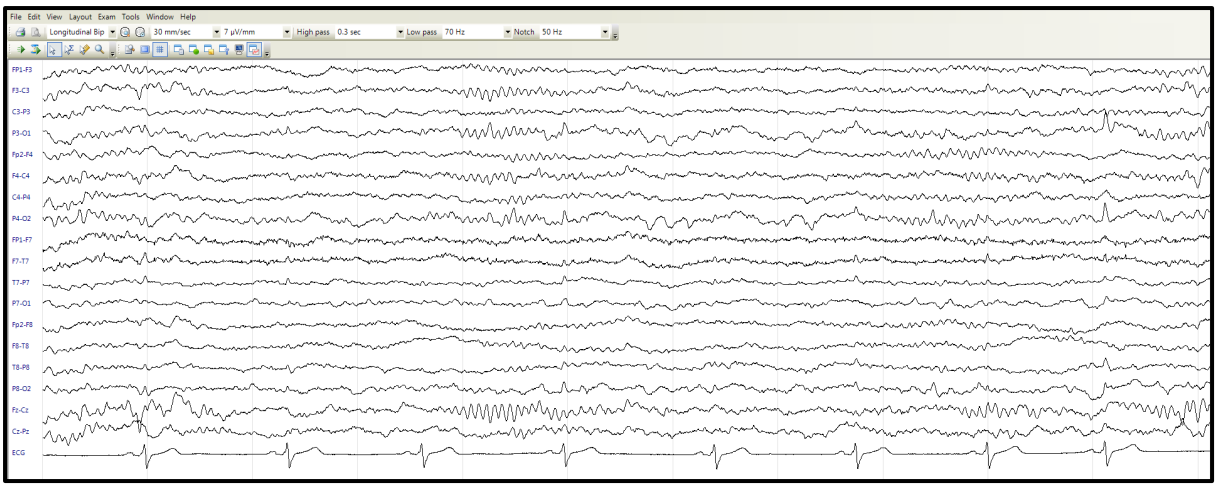

Figure 2-4. The Galileo Suite Software (EB Neuro S.p.A). Options in the top left-hand corner of the screen allow manipulation of the montage, amplitude and filters.

The 64-channel Hydrocel Geodesic Sensor Net

The 64-channel HCGSN (Figure 2-5), developed by Electrical Geodesics Inc. (Eugene, Oregon, U.S.A.), was used to record brain activity in the pilot and case-control EEG studies. This net holds 64 equally spaced electrodes in an elastomer geodesic structure, allowing complete coverage of the entire head. Silver-chloride electrodes are contained within soft pedestals that help to improve comfort. The foot of the pedestal forms a seal with the skin, keeping conductive gel within the chamber, which improves the duration of the recording. The net also contains stabiliser pedestals which do not record, which prevent recording pedestals from overturning. Cut-out sections for the participant's ear are also present, as well as cord locks around the chin that allow the tension of electrodes around the ears and eyes to be adjusted. The electrode wires are held together in a protective wire wrap, which connects the net to an amplifier.

The electrodes on the HCGSN are broadly positioned according the 10-10 electrode placement system (Figure 2-2). Most electrodes on the HCGSN are within 2cm of their 10-10 system equivalent (e.g. the electrode designated as F3 on the net is within 1cm of the F3 position of the 10-10 system). Some electrodes are more imprecisely mapped, for example temporal electrodes are within 2-2.5cm of their 10-10 equivalents. This does not mean that potential epileptiform discharges at temporal sites will be missed however, as the 64-channel HCGSN compensates through the greater head coverage it provides (as compared to the traditional clinical method, using only 21 channels).

The HCGSN has been used to record EEG signals from several paediatric cohorts with neurodevelopmental disorders. It has been used to assess event-related potentials in children with

autism, Down's syndrome and 16p11.2 microdeletion and microduplication syndrome[22,23]. The net has also been used for overnight study of sleep EEG architecture in adolescents with schizophrenia spectrum disorder[24]. Finally, the HCGSN has been used to study the onset and spread of epileptiform discharges in patients with juvenile myoclonic epilepsy[25].

Figure 2-5. The 64-channel Hydrocel Geodesic Sensor Net (Electrical Geodesics, Inc.).

Silver-chloride electrodes, individually glued to the head

The traditional clinical method (i.e. 21 silver-chloride electrodes, individually glued to the head according to the 10-20 electrode placement system) was used during pilot EEG study. In the case-control study, one child had 9 electrodes glued to the head during the overnight portion of the recording (see Section 5.3.3.5 for further details). The silver-chloride electrodes are displayed in Figure 2-6.

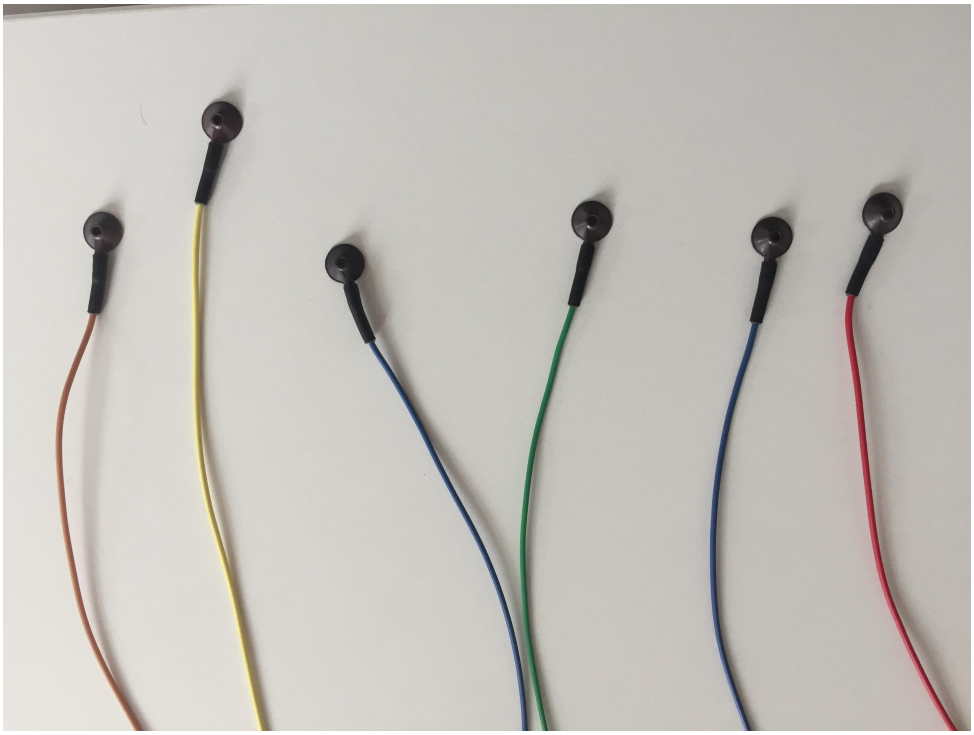

Figure 2-6. The silver-chloride electrodes, used in the EEG studies described in this thesis.

Camera for overnight video-recording

In the case-control EEG study (Chapter 5), children were video-recorded during the overnight sleep period, using an infrared camera. This helped differentiate background and epileptiform abnormalities from artifacts (e.g. from participant movement or external sources), as well as to aid in epileptic seizure diagnosis and classification. The camera was connected to the computer running the Galileo Software Suite, allowing the EEG signal to be synchronised with the video recording.

Electrocardiography

During both the pilot and case-control EEG studies, simultaneous 24-hour electrocardiography was recorded using two silver-chloride electrodes. These were placed under the left and right collarbones. ECG was conducted in order to be able to distinguish epileptiform discharges from heartbeat artifact.

2.3.7.5 Recording procedure

I will firstly provide an overview of elements of the recording procedure common to the various setups used in this study (i.e. 64-channel HCGSN versus traditional clinical method, wired versus wireless recordings) . I then provide further details about each specific setup and describe which study they were used in (pilot versus case-control).

General procedure

All 24-hour EEG recordings were undertaken by trained researchers. Recordings took place in the participant's home. Researchers arrived in the evening, a few hours before the child's bedtime (to allow time for setup). The child was seated and the vertex (the centre of the head) was identified. To do this, the distance between the nasion and inion was measured and a mark (using a china marker pen) was made on the scalp perpendicular to the midpoint. The researchers then applied the electrodes and inserted conductive electrode gel into each one, to improve the signal quality. The ECG electrodes were then applied under the left and right collarbones. The impedances of all electrodes were subsequently checked in the Galileo Suite Software. Electrodes were adjusted (e.g. re-gelled and moved slightly to ensure better contact with the skin) until all electrodes were under 50 kilo-ohms (kohms). During a traditional clinical EEG recording, impedances are to be kept below 5kohms. However, it would have been impractical to attempt to keep the impedances below this level; this would have taken a long time (particularly with the HCSGSN, which does not involve cleaning of the scalp before electrode application). Given their cognitive deficits and psychopathology, individuals with 22q11.2DS may have poorly tolerated an overly-prolonged setup period. A study Ferre et al.[26] found that modern amplifier systems can support high-quality signal acquisition (i.e. minimal attenuation of signals) with a scalp impendances of 40kohms, with further analyses suggesting that this could extend to up to 200kohms.

A short, 'bio-calibration' recording then took place, in which the child was asked to undertake a series of activities, designed to check whether electrodes were positioned and working properly. These activities, and other more general checks, are listed in Table 2-3.

Table 2-3. Checks carried out during the bio-calibration recording, within the pilot and case-control EEG studies.

Check	Purpose
Ask the participant to relax their muscles and to look straight ahead for 30 seconds, keeping their head and eyes still. Ask the participant to try not to swallow during this period	Check for low amplitude, mixed frequency waveforms, identify any channels where there is a lot of artifact. Check that the signal is of sufficient quality
Ask the participant to relax their muscles and to close their eyes for 30 seconds. Ask the participant to try not to swallow during this period	Check for posterior dominant rhythm (alpha activity) across posterior channels. Identify any sources of artifact in these channels
Ask the participant to look up, moving their eyes only and keeping their head still. Ask the participant to return their gaze to a centred position. Use a pen to guide the participant	Check that eye movements are detected by frontal electrodes
Ask the participant to look down, moving their eyes only and keeping their head still. Ask the participant to return their gaze to a centred position. Use a pen to guide the participant	Check that eye movements are detected by frontal electrodes
Ask the participant to look to the left, moving their eyes only and keeping their head still. Ask the participant to return their gaze to a centred position. Use a pen to guide the participant	Check that eye movements are detected by frontal electrodes
Ask the participant to look to the right, moving their eyes only and keeping their head still. Ask the participant to return their gaze to a centred position. Use a pen to guide the participant	Check that eye movements are detected by frontal electrodes
Ask the participant to blink five times	Check that eye movements are detected by frontal electrodes
Ask the participant to grind their teeth as hard as they can	Check that temporal electrodes are working correctly
Researcher monitors the ECG trace	Check that the ECG electrodes are working properly and that the signal is of sufficient quality

After the bio-calibration session had ended, the researchers started the overnight (sleep) recording and left the family home. The researchers then returned to the family home in the morning. Epileptiform discharges are particularly likely to occur during sleep/wake transitions[27]. The researchers therefore returned to the family home to readjust the electrodes around two hours after the participant had woken up, so as not to disturb the recording and miss any epileptiform discharges that could have potentially occurred. Any electrodes showing a high impedance were re-gelled and the researchers conducted another bio-calibration session. After this, the researchers started the daytime recording and left the family home. At the end of the daytime recording (defined as when roughly 24 hours had passed since the start of the study the night before), the participants returned and removed the EEG and ECG electrodes and ended the study. The children and their primary caregiver were told at the beginning of the study however that they could stop taking part at any time they chose and this was also stated in the consent form the parent signed.

It is important to note that in routine clinical EEG recordings, patients with a clinically suspicious paroxysmal event are asked to hyperventilate and are presented with photic flashes at varying frequencies. These are activation procedures which increase the sensitivity of EEG for epileptiform discharges and which may also induce an epileptic seizure[28]. Given that I was not clinically trained and EEG assessments were carried out in the participant's home (with no EEG technicians or neurophysiologists present), it was not ethically appropriate to include these activation procedures in my EEG protocol.

Procedure when using the 64-channel HCGSN

Recording using the 64-channel HCGSN were conducted in both the pilot EEG study (Chapter 3) and the case-control EEG study (Chapter 5). Three different net sizes were used; 51-54cm, 54cm-56cm and 58cm-60cm. Before applying the net, the child's head circumference was measured to determine which net size should be used. The researchers then found and marked the vertex on the scalp. The researchers then placed conductive gel into the recording pedestals of the HCGSN. To do this, researchers tilted each pedestal upwards slightly (making sure to keep part of the pedestal in contact with the skin). They then used a curved syringe to inject the conductive gel into the pedestal, filling it to just below the surface. The pedestal was then placed back onto the scalp, and scrubbed from side-to-side to ensure good contact. The cord locks around the chin were used to adjust the tension of the electrodes around the eyes and ear, until the child was comfortable. Researchers then connected the HCGSN to the BE PLUS LTM amplifier.

Procedure when using the traditional clinical method

Recordings using the traditional clinical method were conducted with two children in the pilot EEG study (Chapter 3). For one child in the case-control EEG study (Chapter 5), 9 electrodes were individually glued to the head for the overnight portion of the 24-hour recording, see Section 5.3.3.5 for further details. Researchers manually identified and marked each electrode site on the scalp, according to the 10-20 electrode placement system. More specifically, sites were identified by measuring the distance between anatomical landmarks of the skull (e.g. the nasion and inion) and then dividing the distance into 10% or 20% intervals. Each site was then cleaned using a specialised paste, which was subsequently removed with an alcohol wipe. Each silver-chloride electrode was then glued to the head. This involved holding the electrode in place and applying collodion around its edge. After the collodion had set, conductive gel was then injected through a small hole in the top of each electrode, using a syringe. At the end of the recording, each electrode was removed by applying a small amount of acetone to a cotton wool ball and gently rubbing this over the electrode site.

Procedure when using wired recordings

Wired recordings were only used in Chapter 3, during the pilot EEG study (the wireless access point was not functioning properly at the time of the pilot study). They were used with both the 64-channel HCGSN and the traditional clinical method. The BE PLUS LTM was initially connected to the computer hosting the Galileo Software Suite via a LAN cable. After the recording was started, the BE PLUS LTM was disconnected from the computer and the data were temporarily stored within the internal memory of the BE PLUS LTM. After the recording had ended, the BE PLUS LTM was reconnected to the computer and the data were downloaded into the Galileo Suite Software.

Procedure when using wireless recordings, with overnight video-monitoring

Wireless recordings were only used in Chapter 5, during the case-control EEG study. They were used with the 64 HCGSN and with the single child who had 9 electrodes individually glued to the head for the overnight recording (see Section 5.3.3.5). In the wireless recordings, the EEG recordings were continually downloaded to the computer via an access point, using Wi-Fi technology. When the child moved out-of-range of the access point, the recording was temporarily stored in the internal memory of the BE PLUS LTM, and transferred to the computer when the child was back in range. Overnight video-monitoring of the child, synchronised to the EEG trace, was conducted in tandem with the wireless recordings. The initial set-up, impedance check and bio-calibration recording needed to be carried in a spacious area, which in the vast majority of cases was in the family dining/living room. As the equipment would then need to be moved to the child's bedroom for overnight video-monitoring, there were a short gap (~20-30minutes) between the end of the bio-calibration recording and the

start of the overnight recording. Similarly, in the morning the equipment was then moved back to the family living/dining room to ensure enough space for readjustment of the net and further bio-calibration checks, so there was another 20-30 minute gap between the overnight recording and the subsequent daytime recording. To ensure that any epileptiform discharges that may have occurred during sleep-wake transitions were captured (periods in which these abnormalities are particularly likely to happen[27]), children did not go to bed until the overnight recording had been started and in the morning the equipment was not moved until around two hours after the child had woken up.

2.3.8 Clinical specialist review

I initially screened the EEG data from the case-control EEG study (Chapter 5) for background abnormalities and/or epileptiform discharges. A consultant epileptologist (Dr Khalid Hamandi, University Hospital of Wales), then reviewed any recordings (in full) that I had highlighted during the screening process. If the epileptologist was unsure about a potential abnormality, the recording was then also reviewed by a consultant neurophysiologist (Dr Gareth Payne, University Hospital of Wales). The epileptologist and neurophysiologist were blind as to whether a recording was from a deletion carrier or control sibling. The clinicians then decided whether a given participant had a background abnormality and/or an epileptiform discharge and in which case they provided a more precise classification of the abnormality (e.g. focal/generalised slowing, focal/generalised spike). To ensure that I was able to accurately identify normal EEG variants, background abnormalities and epileptiform discharges, at the beginning of the review process the epileptologist and I together reviewed the entire EEG trace for five deletion carriers and five sibling controls. It must be acknowledged however that the sensitivity of the EEG review process (for background abnormalities and epileptiform discharges) may have been improved if the epileptologist and neurophysiologist had reviewed all of the EEG recordings in full.

For each child, the epileptologist was presented with background information (e.g. age, gender, IQ), the results from the first stage of the systematic assessment of epileptic seizures and epilepsy assessment (i.e. their responses on the ESQ) and all of the available data from the second stage of assessment (including the results from the EEG review). The epileptologist stated whether each unusual spell could be diagnosed as an epileptic seizure and whether the child could be diagnosed with epilepsy. Diagnostic categories were ‘Yes’, ‘Possible’, ‘No’ and ‘Uncertain’. The epileptologist then made further classifications of seizure semiology and aetiology; this was to be as specific as the available information would allow (for example for seizure semiology: focal seizure – further categorised as focal motor seizure and for seizure aetiology, acute symptomatic seizure – further classified as hypocalcaemia-induced seizure). Diagnosis and classification of epileptic seizures and

epilepsy was made in accordance with the most recent systems put forward by the International League Against Epilepsy (see Section 1.7.1 for a description)[29-31].

2.4 Cognitive assessments

2.4.1 Overview

All cognitive assessments were completed by trained researchers, either in the child's home or at Cardiff University.

2.4.2 Wechsler Abbreviated Scale of Intelligence

Full-scale IQ (FSIQ), performance IQ (PIQ) and verbal IQ (VIQ) scores were derived from the Wechsler Abbreviated Scale of Intelligence (WASI)[32]. The WASI consisted of four subtests, with two probing verbal abilities and two probing non-verbal abilities. The four tasks are described in more detail below:

2.4.2.1 Vocabulary (verbal abilities)

Children were asked to provide a description of each of 42 items. The first four items are pictures, the remainder are words. This subtest was a measure of expressive language.

2.4.2.2 Similarities (verbal abilities)

This subtest measured abstract verbal reasoning and verbal concept formation. It consisted of 26 items, the first four of which were pictures. For the picture items, children were shown three pictures of common objects on the top row of the page and were then asked to choose which picture best matched these from a list of four on the bottom row. For the remaining items, children were presented with a pair of words and asked to explain why the objects/concepts they represented were similar.

2.4.2.3 Block Design (non-verbal abilities)

Children were shown each of 13 geometric patterns and were asked to replicate these using two-colour cubes. A time-limit was imposed for each item. This subtest measured perceptual organisation, spatial visualisation, visual-motor coordination and abstract conceptual abilities.

2.4.2.4 Matrix Reasoning (non-verbal abilities)

Children were shown each of 35 incomplete grid patterns and were asked to complete the grid by choosing one of five possible options. This subtest measured nonverbal fluid reasoning.

2.5 Psychiatric assessments

2.5.1 Child and Adolescent Psychiatric Assessment

The semi-structured Child and Adolescent Psychiatric Assessment (CAPA)[33] interview was conducted with the primary caregiver and with children who were able to understand and answer the questions. All interviews were audiotaped. Data obtained from these interviews were used to diagnose psychiatric disorders according to DSM-IV-TR criteria[34]. Diagnoses were made during consensus meetings, led by a child and adolescent psychiatrist. The CAPA can be used to diagnose a wide range of psychiatric disorders, however, in this thesis I explored only the most common diagnoses seen in young people with 22q11.2DS, namely: attention deficit hyperactivity disorder (ADHD) and anxiety disorder (at least one of the following: generalised anxiety disorder, social phobia, specific phobia, separation anxiety, panic disorder with and without agoraphobia, agoraphobia and obsessive compulsive disorder).

2.5.2 Screening for autism spectrum disorder

Autism spectrum disorder (ASD) was screened for in the child using the Social Communication Questionnaire, also completed by the primary caregiver. The SCQ asks about repetitive and stereotyped behaviours, reciprocal social interaction and communication ability. Total scores range from 0-39: a score of 15 or more is indicative of ASD.[35]

2.6 Assessment of other neurodevelopmental problems

2.6.1.1 Sleep disturbance

The CAPA was also used to screen for sleep disturbance in the child. Sleep disturbance was defined as a score of two or three on at least one item of the sleep section of the CAPA, which asks about insomnia, hypersomnia, nightmares, tiredness, fatigability, night terrors and somnambulism.

2.6.1.2 Screening for development coordination disorder

The fine motor skills, gross motor control and control during movement of the child were assessed using the validated Developmental Coordination Disorder Questionnaire (DCDQ)[36,37], completed by the primary caregiver. Scores range from 15 to 75, with the discrimination thresholds for indicative developmental coordination disorder (DCD) based on age.

2.6.1.3 Physical health problems

Primary caregivers provided information (about the child) on preterm birth (before 37 weeks for a single baby and before 34 weeks for twins), cardiovascular problems and recurrent infections (of the ears and chest/airways) through a health questionnaire and during the CAPA interview.

Sample sizes for the epilepsy, cognitive, psychiatric, sleep, motor and physical health problem assessments differ due to either the child having difficulty completing the WASI or the primary caregiver not completing part of the CAPA or part/all of one or more of the questionnaires.

2.7 Correcting for multiple comparisons

All findings presented within this thesis have not been corrected for multiple comparisons. Much of the work presented in this thesis is novel, exploratory and obtained from a cohort of young people with a rare chromosomal disorder. Therefore, both my supervisors and I agreed that it would be appropriate to present all results before correction, so that salient findings would not be obscured by correction for multiple comparisons. However, this does mean that statistically significant findings presented in this thesis should be viewed with caution and need to be replicated by future studies in order to confirm their validity and reliability.

2.8 References

1 Ottman, R. *et al.* Validation of a brief screening instrument for the ascertainment of epilepsy. *Epilepsia* **51**, 191-197 (2010).

2 Wither, R. G. *et al.* 22q11. 2 deletion syndrome lowers seizure threshold in adult patients without epilepsy. *Epilepsia* (2017).

3 Andrade, D. M., Krings, T., Chow, E. W., Kiehl, T.-R. & Bassett, A. S. Hippocampal malrotation is associated with chromosome 22q11. 2 microdeletion. *The Canadian Journal of Neurological Sciences* **40**, 652-656 (2013).

4 Kao, A. *et al.* Increased prevalence of unprovoked seizures in patients with a 22q11. 2 deletion. *American Journal of Medical Genetics Part A* **129**, 29-34 (2004).

5 Kim, E.-H. *et al.* Epilepsy and Other Neuropsychiatric Manifestations in Children and Adolescents with 22q11. 2 Deletion Syndrome. *Journal of Clinical Neurology* **12**, 85-92 (2016).

6 Schneider, M. *et al.* Psychiatric disorders from childhood to adulthood in 22q11.2 deletion syndrome: Results from the international consortium on brain and behavior in 22q11.2 deletion syndrome. *American Journal of Psychiatry* **171**, 627-639, doi:http://dx.doi.org/10.1176/appi.ajp.2013.13070864 (2014).

7 Bowley, C. & Kerr, M. Epilepsy and intellectual disability. *Journal of Intellectual Disability Research* **44**, 529-543 (2000).

8 Niarchou, M. *et al.* Psychopathology and cognition in children with 22q11. 2 deletion syndrome. *The British Journal of Psychiatry* **204**, 46-54 (2014).

9 Swillen, A. *et al.* Intelligence and psychosocial adjustment in velocardiofacial syndrome: a study of 37 children and adolescents with VCFS. *Journal of Medical Genetics* **34**, 453-458 (1997).

10 De Smedt, B. *et al.* Intellectual abilities in a large sample of children with velo–cardio–facial syndrome: an update. *Journal of Intellectual Disability Research* **51**, 666-670 (2007).

11 Chow, E. W., Watson, M., Young, D. A. & Bassett, A. S. Neurocognitive profile in 22q11 deletion syndrome and schizophrenia. *Schizophrenia research* **87**, 270-278 (2006).

12 Ottman, R., Hauser, W. A. & Stallone, L. Semistructured interview for seizure classification: agreement with physicians' diagnoses. *Epilepsia* **31**, 110-115 (1990).

13 Ottman, R. *et al.* Reliability of seizure classification using a semistructured interview. *Neurology* **43**, 2526-2526 (1993).

14 Phenome, E. & Consortium, E. K. De novo mutations in epileptic encephalopathies. *Nature* **501**, 217-221 (2013).

15 Friedman, D., Fahlstrom, R. & Investigators, E. Racial and ethnic differences in epilepsy classification among probands in the Epilepsy Phenome/Genome Project (EPGP). *Epilepsy research* **107**, 306-310 (2013).

16 Shain, C. *et al.* Polymicrogyria-associated epilepsy: A multicenter phenotypic study from the Epilepsy Phenome/Genome Project. *Epilepsia* **54**, 1368-1375 (2013).

17 Ryan, A. *et al.* Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *Journal of Medical Genetics* **34**, 798-804 (1997).

18 Schneider, M. *et al.* Psychiatric disorders from childhood to adulthood in 22q11. 2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11. 2 Deletion Syndrome. *American Journal of Psychiatry* (2014).

19 Chez, M. G. *et al.* Frequency of epileptiform EEG abnormalities in a sequential screening of autistic patients with no known clinical epilepsy from 1996 to 2005. *Epilepsy & Behavior* **8**, 267-271 (2006).

20 Hughes, J. R., DeLeo, A. J. & Melyn, M. A. The Electroencephalogram in Attention Deficit–Hyperactivity Disorder: Emphasis on Epileptiform Discharges. *Epilepsy & Behavior* **1**, 271-277, doi:https://doi.org/10.1006/ebeh.2000.0073 (2000).

21 Louis, E. K. S., & Frey, L.C. *Electroencephalography (EEG): An Introductory Text and Atlas of Normal and Abnormal Findings in Adults, Children and Infants*. (American Epilepsy Society, 2016).

22 Hudac, C. M. *et al.* Modulation of mu attenuation to social stimuli in children and adults with 16p11. 2 deletions and duplications. *Journal of neurodevelopmental disorders* **7**, 25 (2015).

23 Yoder, P. J., Camarata, S., Camarata, M. & Williams, S. M. Association between differentiated processing of syllables and comprehension of grammatical morphology in children with Down syndrome. *American journal on mental retardation* **111**, 138-152 (2006).

24 Tesler, N. *et al.* Reduced sleep spindle density in early onset schizophrenia: a preliminary finding. *Schizophrenia Research* **166**, 355-357 (2015).

25 Holmes, M. D., Quiring, J. & Tucker, D. M. Evidence that juvenile myoclonic epilepsy is a disorder of frontotemporal corticothalamic networks. *NeuroImage* **49**, 80-93, doi:https://doi.org/10.1016/j.neuroimage.2009.08.004 (2010).

26 Ferree, T. C., Luu, P., Russell, G. S. & Tucker, D. M. Scalp electrode impedance, infection risk, and EEG data quality. *Clinical Neurophysiology* **112**, 536-544 (2001).

27 Matos, G., Andersen, M. L., do Valle, A. C. & Tufik, S. The relationship between sleep and epilepsy: evidence from clinical trials and animal models. *Journal of the neurological sciences* **295**, 1-7 (2010).

28 Noachtar, S. & Rémi, J. The role of EEG in epilepsy: a critical review. *Epilepsy & Behavior* **15**, 22-33 (2009).

29 Fisher, R. S. *et al.* ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* **55**, 475-482 (2014).

30 Fisher, R. S. *et al.* Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia* **58**, 522-530 (2017).

31 Fisher, R. S. *et al.* Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* **46**, 470-472 (2005).

32 Weschler, D. Weschler abbreviated scale of intelligence (WASI). *London: Psychological Corporation* (1999).

33 Angold, A. *et al.* The child and adolescent psychiatric assessment (CAPA). *Psychological medicine* **25**, 739-753 (1995).

34 Association, A. P. & Association, A. P. *Diagnostic and statistical manual-text revision (DSM-IV-TRim, 2000)*. (American Psychiatric Association, 2000).

35 Rutter, M., Bailey, A. & Lord, C. *The social communication questionnaire: Manual*. (Western Psychological Services, 2003).

36 Wilson, B. N. The Developmental Coordination Disorder Questionnaire 2007. *Physical & occupational therapy in pediatrics* **29**, 182-202 (2012).

37 Wilson, B. N., Kaplan, B. J., Crawford, S. G., Campbell, A. & Dewey, D. Reliability and validity of a parent questionnaire on childhood motor skills. *American Journal of Occupational Therapy* **54**, 484-493 (2000).

3 Training in clinical epilepsy assessment and pilot EEG study

3.1 Chapter overview

In this chapter I discuss several methods that I used to prepare for the second stage of the systematic assessment of epileptic seizures and epilepsy in young people with 22q11.2DS. I shadowed several epilepsy clinics and clinical EEG review sessions, which allowed me to ask focused, relevant questions during the semi-structured Unusual Spell Interview and provided me with the ability to identify salient background and epileptiform abnormalities when screening the data from the case-control EEG study. Along with colleagues from Cardiff University and the University of Bristol, I also conducted a pilot 24-hour ambulatory EEG study on a convenience sample of 11 young people from the general population, which took place within the child's home. This pilot EEG study allowed me to familiarise myself with the protocol to be used in the case-control EEG study. The pilot EEG study also highlighted some problems with our recording equipment, which were subsequently rectified during support sessions with the manufacturers.

3.2 Introduction

The process of diagnosing epilepsy and classifying seizures is complex and is conducted by highly experienced epileptologists. When presented with a patient who has had a "blackout", the clinician must bear in mind that there are numerous conditions which may mimic the features of epileptic seizures. For example, features of epileptic seizures such as myoclonic jerks, head turning, automatisms and incontinence can all occur in vasovagal syncope[1]. Delineating seizure semiology is similarly complex, for example, both absence seizures (a generalised seizure) and focal seizures with impaired awareness may involve an interruption of consciousness with automatisms[1]. As discussed in Chapter 2, all measures within the systematic assessment of seizures and epilepsy were reviewed by a consultant epileptologist and a consultant neurophysiologist. However, I conducted all of the semi-structured Unusual Spell Interviews. It was therefore necessary for me to acquire a thorough understanding of the features that distinguish epileptic seizures from non-epileptic events and different seizure types. This would allow to me ask more diagnostically relevant questions in the interview and to compile a clear description of a given unusual spell, for subsequent review by the epileptologist.

Clinical review of EEG for background abnormalities and epileptiform discharges is a similarly complex process and also requires a large amount of experience. As discussed in Chapter 1, misdiagnosis of normal EEG variants as 'epileptiform' is common[2]. I was responsible for screening the EEG data from

the case-control EEG study (i.e. the 24-hour ambulatory EEG assessment with young people with 22q11.2DS and their control siblings). For the majority of children in this study, the epileptologist only reviewed recordings that I thought may contain waveforms of uncertain significance, rather than reviewing the whole EEG trace (see Section 2.3.8 for further details of the EEG review process). It was therefore essential that I acquired as much experience as possible in clinical EEG review and developed a thorough understanding of normal variants, background abnormalities and epileptiform discharges.

Before conducting the case-control EEG study, it was important to pilot the protocol, for several reasons. I therefore conducted a pilot EEG study on a convenience sample of 11 young people from the general population. As described in Section 2.3.7.1, other researchers helped me to conduct this study. In the pilot study, I aimed to familiarise ourselves with the set-up procedure and identify any equipment malfunctions, or areas of the protocol that could be changed to improve the speed of data collection. This would allow me to collect data as efficiently as possible from young people with 22q11.2DS, a cohort whose tolerance for prolonged EEG monitoring is likely to be lower than populations without the deletion, due to the high rates of cognitive impairment and psychiatric disorder seen in this syndrome[3-7]. I aimed to use the 64-channel Hydrocel Geodesic Sensor Net (HCGSN, see Section 2.3.7.4, '*The 64-channel Hydrocel Geodesic Sensor Net*' for further details) as the method for recording during the case-control EEG study, as opposed to the 'traditional clinical method', in which 21 electrodes are individually glued to the head in accordance with the international 10-20 electrode placement system. However, there was a possibility that the HCGSN net would be very poorly tolerated by children with 22q11.2DS. As a backup, I therefore also piloted the traditional clinical method in this chapter. The number of children who were assessed with the 64-channel HGCSN are however greater (n=8) than with the traditional clinical method (n=2) in this pilot study. The rationale for choosing to focus more on piloting the HGCSN, and keeping the traditional clinical method as a backup, is discussed below.

I predicted that the net would be equal, or superior to, the traditional clinical method across several domains. Firstly, I hypothesized that the HCGSN would on average take less time to set up than the traditional clinical method ('set up' defined as the time between the beginning of the study and when the impedances of all electrodes have been checked and adjusted to ensure they were under 50 kohms). This is because in a traditional clinical method, each electrode site must firstly be identified using the 10-20 system head. Each site then needs to be cleaned with skin preparation gel to remove dead skin cells and improve signal quality. The electrodes must then be individually glued to the head, with time needed to allow the glue (collodion) to set. By contrast, when using the HCGSN the researcher only has to measure and mark the vertex (the centre of the participant's head) and all

electrodes can be applied simultaneously. In addition, each electrode site on the scalp does not have to be cleaned before applying the HCGSN.

I chose to conduct the pilot study with a convenience sample of young people from the general population, given the rarity of research participants with 22q11.2DS.

3.3 Methods

3.3.1 Training for the Unusual Spell Interview

To deepen my understanding of the diagnosis, classification and treatment of epilepsy, I shadowed three epilepsy clinics within The Epilepsy Unit at the University Hospital of Wales (UHW, Cardiff, led by Professor Phillip Smith and by Dr. Khalid Hamandi). The process of diagnosing epilepsy can be more complex in people with intellectual disability (ID). For example, many people with ID have stereotypic movement disorders that can mimic the movements seen in an epileptic seizure[8]. Given that 22q11.2DS is associated with mild-moderate intellectual disability (ID)[3-7], I therefore also shadowed two sessions of an epilepsy and learning disability clinic lead by Professor Michael Kerr at Park View Health Centre, Cardiff. In addition, I attended a one-day Paediatric Epilepsy Training (PET1) course at the University of Oxford in September 2016, ran by the British Paediatric Neurologist Association. This provided an introduction to how epileptic seizures and epilepsy are diagnosed, classified and treated. Key features of the course included an understanding of the core characteristics that distinguish epileptic seizures from other 'mimicking' conditions (e.g. syncope, psychogenic seizures), as well how to categorise epilepsy and seizures according to the International League Against Epilepsy (ILAE) classification systems[9,10].

3.3.2 Training in clinical EEG review

From October 2015-April 2018 I shadowed Dr. Gareth Payne, a consultant neurophysiologist, whilst he reviewed clinical EEG recordings within the Clinical Neurophysiology Department at UHW, Cardiff. These were half-day review sessions which I shadowed on a monthly basis. Within these sessions, we reviewed routine clinical (i.e. 30-minutes) and 24-hour ambulatory EEG recordings from children, adolescents and adults, looking for background abnormalities and epileptiform discharges. EEG recordings were conducted using the traditional clinical method. The EEG traces were viewed in standard clinical montages, including bipolar (longitudinal and tranverse) and reference (common and average). I supplemented this experience by attending a two-and-a-half day 'Introduction to EEG course' at Birmingham Children's hospital in November 2015. Key elements of this course included

distinguishing epileptiform discharges from normal waveforms and artifact, as well as an introduction to the various different types of background abnormalities and epileptiform discharges.

3.3.3 Pilot EEG study

3.3.3.1 Participants

Our sample consisted of 11 young people from the general population (36% male, mean age = 12.8 years, s.d. = 4.0 years, range 6.3-17.7 years). Participants had to be at six least years old to take part (in concordance with the inclusion criteria for our systematic assessment of seizures and epilepsy in young people with 22q11.2DS, see Section 2.1). No further inclusion or exclusion criteria were applied, given that we were recruiting a convenience sample. Participants were recruited via word of mouth and through study advertisements placed within the MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University and Cardiff University Brain Imaging Centre. The School of Medicine Ethics Committee, Cardiff University, approved our protocol (SMREC REF 16/12).

3.3.3.2 Procedure

Overview

Please see Section 2.3.7.4 for a description of the equipment used in this study and section 2.3.7.5 for an overview of the general recording procedures, as well as details specific to recordings using the 64-channel HCGSN and the 21-channel traditional clinical method. I conducted a 'wired' recording with all children in this pilot study (see Section 2.3.7.5 for more details). I also did not include the overnight video monitoring. I was unable to conduct wireless recordings and overnight video monitoring as at the time of this study the manufacturers of the equipment (Electrical Geodesics Inc and EB Neuro S.p.A) had not properly configured our wireless access point, or our video-recording preset in the Galileo Suite Software.

Recordings using the 64-channel HCGSN

Nine participants (44% male, mean age = 12.0 years, s.d. = 4.0 years, range 6.3-17.7 years) took part in recordings using the 64-channel HCGSN. Please see Section 2.3.7.5 for an overview of the recording procedure using the HCGSN.

Recordings using the 21-channel traditional clinical method

Two participants (100% female, mean age = 16.2 years, s.d. = 1.2 years, range 15.3-17.1 years) took part in recordings using 21 silver-chloride electrodes, individually glued to the head. Please see Section 2.3.7.5 for an overview of the recording procedure using the traditional clinical method.

3.4 Results

3.4.1 Training for the Unusual Spell Interview and in clinical EEG review

Shadowing the epilepsy clinics and attending the PET1 course improved my understanding of how to distinguish between epileptic seizures and non-epileptic events, how epilepsy is diagnosed, how epilepsy and seizures are classified according to ILAE criteria and how seizures can manifest differently in individuals with intellectual disability. Shadowing the clinical EEG review sessions, coupled with the 'Introduction to EEG course', developed my ability in distinguishing epileptiform discharges from normal waveforms and artifact. In addition, I became able to recognise and distinguish the various types of background abnormalities and epileptiform discharges.

3.4.2 Pilot EEG study

3.4.2.1 Overview

The average recording length across all 11 participants (encompassing both the HCGSN and traditional clinical method) was 572 minutes (s.d. = 290 minutes, range = 0-1079 minutes). One participant (9.1%) took the HCGSN off immediately after trying it on. Three participants (27.3%) completed the study until the end. However, due to equipment problems only a portion of the recording could be downloaded for two of these participants (760 minutes and 540 minutes, respectively). For a further two participants (18.2%), the recording had to be cut short due to logistical reasons (e.g. the child had to go to a sports club). In the remaining five participants (45.5%), the recording was ended as they didn't want to continue, this was generally because the children didn't want to participate in the daytime recording after having worn the equipment overnight. Nine (81.8%) participants reported that the equipment was uncomfortable to sleep in. Interestingly, of those participants who then continued into the daytime recording (n=5), only one (20%) reported that the equipment was uncomfortable to wear during the daytime.

3.4.2.2 Familiarising myself with the data collection protocol and identifying any equipment malfunctions

Piloting the EEG equipment allowed me to familiarise myself with my protocol, therefore helping to ensure that I would collect data more efficiently during the case-control study. For example, this pilot study helped me to place and position the HCGSN on the participant's head more quickly. During the pilot study, I observed several problems with our equipment (it is important to note that the BE PLUS

LTM was a new system at the time of purchase for use in this thesis). Firstly, I had difficulties with getting the Galileo Suite Software to recognise the connection with the BE PLUS LTM. Similarly, I had problems with downloading recorded data from the BE PLUS LTM and on two occasions this resulted in data loss (see Section 3.4.2.1). I also observed that the physical electrodes on the HCGSN had not been properly mapped to their corresponding channels in the Galileo Suite Software by the manufacturers of my equipment (Electrical Geodesics Inc (EGI) and EB Neuro S.p.A (EBN)). For example, the electrode sitting in the 10-10 'F4' position on the net was not mapped to the F4 channel in the Galileo Suite Software. I remedied these issues through five support sessions with both EGI and EBN, each lasting around two hours, in which I was provided with a software patch and a new channel mapping for the HCGSN. During these sessions, I also configured the wireless access point and the video-recording preset in the Galileo Suite Software, as I had been unable to use these during our pilot study (see Section 3.3.3.2, *Overview*). The equipment was then re-tested through several short (e.g. 30-minute) recordings and two prolonged (overnight) recordings with members of the Division of Psychological Medicine and Clinical Neurosciences, Cardiff University. These recordings included use of the wireless access point and the simultaneous video-recording.

3.4.2.3 Comparing the speed of using the HGCSN and the traditional clinical method

We found that on average the HGCSN took 58.4 minutes to set up (s.d. = 20.3 minutes, range = 44-105 minutes). The traditional clinical method took on average 126.5 minutes to set up (s.d. = 2.1 minutes, range = 125-128 minutes).

3.5 Discussion

3.5.1 Training for the Unusual Spell Interview and for clinical EEG review

Diagnosing epilepsy, classifying seizures and reviewing EEGs for background abnormalities and epileptiform discharges are complex processes. Proficiency takes many years of clinical training. Whilst all of the data from my systematic assessment of seizures and epilepsy (Chapters 4 and 5) in young people with 22q11.2DS would be reviewed by consultants specialising in epilepsy and EEG review, I was responsible for conducting all of the Unusual Spell Interviews and providing an initial screen of the recordings from the case-control EEG study. The training in distinguishing epileptic seizures from non-epileptic events, seizure classification and semiology and clinical EEG review was therefore essential in preparation for this systematic assessment. More specifically, the training allowed me to probe for key features that distinguish epileptic seizures from non-epileptic events, as

well as different seizure types, during the Unusual Spell Interview. It also helped to ensure that I would not miss salient background and epileptiform abnormalities whilst screening the EEG data.

3.5.2 Pilot EEG study

The pilot EEG study proved to be very important in helping me to prepare for the case-control EEG study. I familiarised myself with the recording protocol, increasing the speed and efficiency with which I conducted the recordings. This was essential for reducing the burden of the 24-hour recording on young people with 22q11.2DS in the case-control EEG study, a population whose tolerance for prolonged EEG assessments is likely to be lower due to the cognitive and psychiatric impairments these individuals commonly experience[3-7]. I was also able to identify and resolve several issues with my EEG equipment that could have resulted in significant data loss in the case-control EEG study.

Both the HCGSN and the traditional clinical method were reasonably well tolerated by participants in this pilot study. Only one participant ended the study straightaway, this may have been due to the participant's relatively young age (8.2 years). Many participants ended the study after the overnight recording, stating that the equipment was uncomfortable to sleep with. However, the majority of those who continued and completed the daytime recording reported that the equipment was more comfortable to wear. One possible future direction I could have taken from this is to switch the order of the recordings during the case-control EEG study (i.e. run the study from the morning of the first day to the morning of the second day). There are several reasons however why I did not choose to do this. Firstly, the case-control EEG study was conducted in tandem with a polysomnography (PSG) assessment of young people with 22q11.2DS (the project of another PhD student, not included in this thesis). Secondly, epileptiform discharges are particularly likely to occur during sleep-wake transitions[11]. To avoid the risk of a participant ending the study during the daytime recording, resulting in a failure to capture sleep EEG and crucial sleep-wake transitions, I therefore decided to begin with the overnight (sleep) recording in the case-control EEG study.

As I hypothesized, the HCGSN took less than half the time to set up than the traditional clinical method. I therefore chose to use the HCGSN as the primary method for EEG recording in the case-control EEG study, given that a faster set up time could reduce the burden of the assessment for the young people with 22q11.2DS and decrease the likelihood of them ending the recording prematurely. Piloting the 21-channel traditional clinical method did however provide me with an alternative means of recording EEG data if participants with 22q11.2DS did not want to wear the HCGSN, or could not wear it for medical reasons (see Section 5.3.3.5 for an example of an individual who could not wear the net due to wearing a continuous positive airway pressure mask for sleep apnoea).

In conclusion, the training and pilot research discussed in this chapter allowed me to improve the quality of data collection during the systematic assessment of seizures and epilepsy in young people with 22q11.2DS (Chapters 4 and 5). Shadowing epilepsy clinics and clinical EEG review sessions allowed me to ask focused, relevant questions during the semi-structured Unusual Spell Interview and to identify salient background and epileptiform discharges when screening EEG data. The pilot 24-hour EEG study allowed me to become more efficient in carrying out my protocol, and to identify and remedy salient issues with my recording equipment.

3.6 References

1 Smith, P. The bare essentials: epilepsy. *Practical neurology* **8**, 195-202 (2008).

2 Benbadis, S. The differential diagnosis of epilepsy: a critical review. *Epilepsy & Behavior* **15**, 15-21 (2009).

3 Schneider, M. *et al.* Psychiatric disorders from childhood to adulthood in 22q11. 2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11. 2 Deletion Syndrome. *American Journal of Psychiatry* (2014).

4 Swillen, A. *et al.* Intelligence and psychosocial adjustment in velocardiofacial syndrome: a study of 37 children and adolescents with VCFS. *Journal of Medical Genetics* **34**, 453-458 (1997).

5 De Smedt, B. *et al.* Intellectual abilities in a large sample of children with velo–cardio–facial syndrome: an update. *Journal of Intellectual Disability Research* **51**, 666-670 (2007).

6 Chow, E. W., Watson, M., Young, D. A. & Bassett, A. S. Neurocognitive profile in 22q11 deletion syndrome and schizophrenia. *Schizophrenia research* **87**, 270-278 (2006).

7 Niarchou, M. *et al.* Psychopathology and cognition in children with 22q11. 2 deletion syndrome. *The British Journal of Psychiatry* **204**, 46-54 (2014).

8 Bowley, C. & Kerr, M. Epilepsy and intellectual disability. *Journal of Intellectual Disability Research* **44**, 529-543 (2000).

9 Fisher, R. S. *et al.* ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* **55**, 475-482 (2014).

10 Berg, A. T. *et al.* Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia* **51**, 676-685 (2010).

11 Matos, G., Andersen, M. L., do Valle, A. C. & Tufik, S. The relationship between sleep and epilepsy: evidence from clinical trials and animal models. *Journal of the neurological sciences* **295**, 1-7 (2010).

4 Screening for epilepsy, seizures and paroxysmal events in 22q11.2 deletion syndrome: prevalence and links with neurodevelopmental problems

4.1 Chapter overview

A growing body of research suggests that people with 22q11.2 deletion syndrome (22q11.2DS) are at increased risk of epilepsy. The majority of these studies however have relied on historical medical record reviews, rather than first-hand assessments. Differences between clinicians and medical centres in the quality of clinical documentation, as well as diagnostic methods used, may mean these medical records are not suitable for systematically evaluating epilepsy prevalence in 22q11.2DS. In addition, these reviews may fail to account for deletion carriers with non-motor absence seizures and focal non-motor seizures with impaired awareness) that have not been clinically detected. Therefore, the true prevalence of epileptic seizures and epilepsy in 22q11.2DS may not be known. Research has also begun to examine the associations of epilepsy with cognitive and psychiatric development in 22q11.2DS, but associations with specific psychiatric diagnoses (e.g. ADHD) and other salient manifestations of the deletion (e.g. sleep disturbance and motor coordination problems) have not been explored to date. This chapter compares the rates of an epilepsy diagnosis, seizures and paroxysmal events (behaviours which could be clinical manifestations of seizures) between young people with 22q11.2DS and their control siblings without the deletion. I used a validated epilepsy screening questionnaire for this purpose, completed by the primary caregiver. I also explored the relationship between responses on this questionnaire with cognition, psychiatric disorder, sleep disturbance and motor coordination problems in the young people with 22q11.2DS. I found that deletion carriers were around 12 times more likely to be reported as having at least one item from the questionnaire ('any positive') than control siblings. 11.1% of young people with 22q11.2DS were reported as having an epilepsy diagnosis and when these cases were excluded 21.1% were reported with febrile seizures (24.3% when cases with epilepsy were included), a rate far higher than in previous studies in 22q11.2DS and in the general population. Notably, after excluding deletion carriers with a reported epilepsy diagnosis or a febrile seizure, nearly half were reported with an afebrile "seizure, convulsion, fit or spell" or a paroxysmal event. Young people with 22q11.2DS with 'any positive' had significantly lower performance IQ and higher rates of ADHD, indicative autism spectrum disorder and indicative developmental coordination disorder. These findings reinforce that the 22q11.2 deletion

confers significant risk for epileptic seizures and epilepsy, and suggest that epilepsy may be underdiagnosed in some young people with 22q11.2DS. Given the limitations of the screening questionnaire however, a second stage of assessment of the reported events is warranted to establish if they are true epileptic seizures. Our findings also provide further evidence for a reduced seizure threshold in 22q11.2DS and emphasise the significant risk of febrile seizures conferred by this syndrome. The relationships of epilepsy, seizures and paroxysmal events with impaired cognition, psychopathology and developmental coordination disorder I observed may suggest common neurobiological risk pathways in 22q11.2DS (e.g. aberrant synaptic plasticity), or additionally/alternatively, deleterious effects of epileptic seizures on cognitive, psychiatric and motor development. However, the broad nature of the paroxysmal event items in the ESQ may mean that some deletion carriers may are false-positives (i.e. having non-epileptic events, rather than true epileptic seizures). A second stage of assessment is warranted to distinguish true-positive from false-positives and assess the validity of the relationships of 'any positive' with poorer neurodevelopmental outcomes.

4.2 Introduction

As described in the introduction to this thesis (Section 1.7.3.1), a growing body of research has suggested that people with 22q11.2 deletion syndrome (22q11.2DS) are at an increased risk for both acute symptomatic seizures and repeated unprovoked seizures (i.e. epilepsy). During childhood, the most prominent risk factor for acute symptomatic seizures is hypocalcaemia, which is observed in up to 65% of deletion carriers[1]. Subsequently, between 10-14.5% of children with 22q11.2DS experience hypocalcaemia-induced seizures[2,3]. During adulthood, psychotropic drug-use emerges as a risk factor, with 17.6% of deletion carriers exposed to these drugs having seizures (compared to only 1.6% of the general population)[4,5]. The high rate of acute symptomatic seizures in 22q11.2DS, relative to the general population, has led some authors to suggest that the 22q11.2 deletion may lower the seizure threshold, that is, increase the likelihood of having a seizure[4]. Prevalence estimates for repeated unprovoked seizures (i.e. epilepsy) in 22q11.2DS range from 4.4% to 36.8% [2,4,6-8].

To date however, studies exploring the prevalence of epileptic seizures and epilepsy in people with 22q11.2DS have mainly used historical review of medical records, rather than first-hand assessments. Studies that rely solely on medical record review are arguably poorly suited to accurately estimate the prevalence of these phenomena in 22q11.2DS. This is because the extent and quality of clinical documentation may differ between clinicians and medical centres, as well as the diagnostic and classifications systems used. Factors such as these may explain why estimates of the prevalence of epileptic seizures and epilepsy in 22q11.2DS are wide-ranging. In addition, medical records are

arguably limited in that they will fail to account for deletion carriers who are experiencing non-motor absences and focal non-motor seizures with impaired awareness, that their family either do not notice or do not consider to be epileptic seizures and have therefore not been brought to the attention of a clinician. This may be particularly likely given the many serious medical conditions and psychiatric disorders that families of an individual with 22q11.2DS have to manage. If subtle, stereotyped paroxysms seem to lead to no obvious impairment in an individual with 22q11.2DS, their caregivers may prioritise time with clinicians to speak about other conditions that are having a more obvious or serious impact (e.g. congenital heart disease, autism spectrum disorder, ASD). Non-convulsive seizures usually have to occur several times before the affected individual and their family become concerned and seek clinical help[9].

Only one study has conducted a systematic, first-hand investigation into epileptic seizures and epilepsy in 22q11.2DS, involving examination by an epileptologist, magnetic resonance imaging (MRI) and electroencephalography (EEG) assessment. They found that 36.8% of deletion carriers had epilepsy, although this was only in a small sample (n=19) of adults[7].

Two studies have attempted to characterise the association of epileptic seizures and epilepsy with cognitive and psychiatric development in 22q11.2DS, although these have also primarily relied on historical medical record review. The most robust finding to date has been an association with global intellectual development. For example, Cheung et al. observed that neonatal epileptic seizures (mostly hypocalcaemia-induced) predicted a more severe level of intellectual disability (ID) later in life[10]. Kim et al. found that developmental delay was significantly more common in child and adolescent deletion carriers with epilepsy than in those without epilepsy[6]. This same study also observed a trend towards a greater proportion of patients with epilepsy having any psychiatric disorder, which approached significance (p=0.057). In contrast with prior research however, only a small number of participants with 22q11.2DS were diagnosed with psychiatric disorders (n=16/145, 11%, compared to 43/80, or 54.5%, of the 22q11.2DS sample in the study by Niarchou et al.[11]). This analysis may have therefore been underpowered and their sample of young people with 22q11.2DS may not have accurately represented the population. In addition, the associations of epilepsy with specific psychiatric diagnoses (e.g. ADHD) were not reported[6].

Whilst findings to date of the associations of epileptic seizures and epilepsy with neurodevelopmental trajectories in 22q11.2DS are informative, there are many relationships with other salient manifestations of the syndrome that have not been explored, such as with sleep disturbance[12] and motor coordination problems[13].

In summary then, studies exploring epileptic seizures and epilepsy in 22q11.2DS have shown an elevated prevalence, but the reliance on historical medical record review may explain the wide-ranging estimates. These reviews may have also failed to detect deletion carriers with non-motor absence seizures and focal non-motor seizures with impaired awareness, that have not been brought to the attention of a clinician. The true prevalence of epileptic seizures and epilepsy in 22q11.2DS may therefore not be known. They are associated with poorer global cognitive functioning in 22q11.2DS, but the relationships with specific psychiatric diagnoses (e.g. ADHD) and other important deficits associated with the deletion (e.g. sleep disturbance, motor coordination problems) have not been examined. In this chapter, I sought to address some of these limitations from the existing literature. My two primary aims were:

Firstly, I sought to establish the rates of an epilepsy diagnosis, seizures and paroxysmal events (see Section 4.3.2 for a definition) in young people with 22q11.2DS and whether these differed from their unaffected control siblings. These rates were ascertained through a validated screening questionnaire[14], completed by the primary caregiver. Based on prior research in 22q11.2DS showing an elevated prevalence of acute symptomatic seizures and epilepsy[2-4,6-8,15,16], I hypothesised that the rates of reported epilepsy diagnoses, seizures and paroxysmal events would be greater in deletion carriers than in controls.

Secondly, I also investigated whether rates of an epilepsy diagnosis, seizures and paroxysmal events were predicted by intellectual functioning (IQ and ID), psychopathology (diagnosis of ADHD, anxiety disorder and indicative ASD) motor coordination problems and sleep disturbance in young people with 22q11.2DS. Based on prior research in 22q11.2DS showing a relationship between epilepsy and global intellectual functioning[6,10], as well as research from the general population showing an interaction of epilepsy with psychiatric health, motor coordination problems and sleep disturbance[17-22], I predicted these associations would also be observed in our cohort of deletion carriers.

4.3 Methods

4.3.1 Participants

Young people with 22q11.2DS and their control siblings had to be six years of age or older to take part in the study. I recruited 108 young people with 22q11.2DS (57.4% male, mean age= 13.6 years, s.d.= 3.3 years) and 60 of their unaffected biological siblings (50% male, mean age= 13.1 years, s.d.= 3.2 years) closest in age. The recruitment procedure for the ECHO study, from which my sample was drawn, is described in Chapter 2 (Section 2.1).

4.3.2 Screening for a lifetime history of epilepsy, seizures and paroxysmal events

I used the validated 'Epilepsy Screening Questionnaire' (ESQ), developed by Ottman et al.[14] to screen for a lifetime history of an epilepsy diagnosis (Item 9, Table 4-2), seizures (Items 1 and 2) and paroxysmal events (Items 3-8; behaviours that may be clinical manifestations of epileptic seizures, which in some young people may reflect unrecognised epileptic seizures, such as uncontrolled jerking or twitching movements or frequent daydreaming/vacant episodes). A positive response to each item was classified as 'Yes' or 'Possibly' and a negative response as 'No'. The response 'Don't know' was treated as a missing value. I created one dichotomous summary variables from the ESQ: 'any positive'; a positive response to at least one item. I then created three mutually exclusive constituent variables to parse responses on the ESQ:

- 'Epilepsy diagnosis': a positive response to Item 9 (Table 4-2)
- 'Febrile seizure': a positive response to Item 1 (in those who didn't have epilepsy)
- 'Afebrile seizure, convulsion, fit or spell, or paroxysmal events': a positive response to Item 2 and/or Items 3, 4, 5, 6, 7 or 8 (in those who didn't have epilepsy or a febrile seizure).

The broad nature of the 'afebrile seizure or paroxysmal event' items may have increased the likelihood of false-positives (e.g. deletion carriers reported with behaviours associated with impaired cognition, psychopathology, rather than true epileptic seizures). Indeed, in the Ottman et al. (2010) validation study, the paroxysmal event items (3-8) did not distinguish individuals with epilepsy or an isolated unprovoked seizure from those who were seizure-free on medical record review, although they did slightly increase the sensitivity of the ESQ for epilepsy (from 91% to 94%)[14]. I therefore separated these items from the more specifically-worded items related to an epilepsy diagnosis and febrile seizures (Items 1 and 9).

The amendments I made to the original version of the ESQ are described in Chapter 2 (Section 2.3.1.2).

4.3.3 IQ and ID

I derived full-scale IQ (FSIQ), performance IQ (PIQ) and verbal IQ (VIQ) scores from the Wechsler Abbreviated Scale of Intelligence (WASI)[23], conducted with the child. A detailed description of each

constituent sub-test of the WASI is provided in Section 2.4.2. Intellectual disability (ID) was defined as a FSIQ <70.

4.3.4 Psychiatric disorder

The semi-structured Child and Adolescent Psychiatric Assessment interview (CAPA)[24] was conducted with the primary caregiver (and with the child when they were able to understand and answer the questions), to diagnose ADHD and any anxiety disorder (see Section 2.5.1 for the list of anxiety disorders considered), according to DSM-IV-TR criteria[25]. I screened for ASD symptoms using the Social Communication Questionnaire (SCQ), also completed by the primary caregiver. Total scores range from 0-39: a score of 15 or more is indicative of ASD[26].

4.3.5 Developmental coordination disorder and sleep disturbance

I assessed fine motor skills, gross motor control and control during movement using the validated Developmental Coordination Disorder Questionnaire (DCDQ)[27,28], completed by the primary caregiver. Scores range from 15 to 75, with the discrimination thresholds for indicative developmental coordination disorder (DCD) based on age. Sleep disturbance was defined as a score of two on at least one item of the sleep section of the CAPA, which asks about insomnia, hypersomnia, nightmares, tiredness, fatigability, night terrors and somnambulism.

4.3.6 Physical health problems

Primary caregivers provided information on preterm birth (before 37 weeks for a single baby and before 34 weeks for twins), cardiovascular problems and recurrent infections (of the ears and chest/airways) through a health questionnaire and the CAPA.

Sample sizes for the ESQ, IQ, psychiatric, sleep, motor and physical health problem datasets differ due to either the child having difficulty completing the WASI or the primary caregiver not completing part of the CAPA or part/all of one or more of the questionnaires.

4.3.7 Statistical analyses

Statistical analyses were conducted in R version 3.3.3 (https://www.R-project.org/).

4.3.7.1 Prevalence of epilepsy, seizures and paroxysmal events in young people with 22q11.2DS and control siblings

As mention in Section 2.1, the ECHO study has collected data from children with 22q11.2DS and their control siblings across four different waves of assessment (on average 2.5 years apart). This includes the ESQ. For the below analyses, I wanted to assess the lifetime prevalence of the ESQ-variables under consideration (the 'any positive' summary variable the three constituent variables and the individual items). For all ESQ-variables in this section therefore, I looked at whether a given deletion carrier/control sibling met criteria at any wave of assessment. For example, I looked at whether a deletion carrier/control sibling had met criteria for 'any positive' in at least one of the four waves of assessment.

I used a logistic regression to measure the association between deletion status (22q11.2DS/ control sibling) and the 'any positive' (yes/no) summary variable, whilst factoring in age and gender. Predictors were entered hierarchically, age first, then gender, then deletion status. For all of the following analyses, age and gender were not included as predictors. As a sensitivity analysis, I used a logistic regression to explore whether comorbid health problems (preterm birth and cardiovascular problems) contributed to our findings. Predictors were entered hierarchically, preterm birth, then cardiovascular problems, then deletion status. I assessed the relationship between deletion status and the three constituent variables ('epilepsy', 'febrile seizure' and 'afebrile seizure and/or paroxysmal events') and the response to each individual item of the ESQ (positive/negative) using χ^2 tests (with Fisher's Exact Tests where appropriate). I conducted a logistic regression exploring whether deletion status predicted recurrent infections (yes/no). Given the strong association of 22q11.2DS with immune dysfunction[1], I also used a logistic regression to explore whether recurrent infections predicted febrile seizures (Table 4-2, Item 1, positive/negative) in young people with 22q11.2DS. For this analysis, the overall rate of febrile seizures was used (24.3%, see Table 4-2), i.e. I combined across deletion carriers with and without an epilepsy diagnosis.

4.3.7.2 Association of the ESQ responses with neurodevelopmental problems in 22q11.2DS

In this section, I aimed to carry out cross-sectional analyses of the relationships between the ESQ variables and cognition, psychopathology, sleep disturbance and motor problems. However, the ESQ wasn't introduced into the ECHO study until around three years after it had begun, so not all participants completed the ESQ during the first wave of assessment. To increase the sample size and power of all of the analyses in this section therefore, I used the ESQ data, as well as the data pertaining

to cognition, psychopathology, sleep disturbance, and motor problems, from the wave of assessment at which each participant completed the ESQ for the first time.

I compared FSIQ, VIQ and PIQ scores between young people with 22q11.2DS and control siblings using t-tests. I explored the relationships between deletion status and ID, psychiatric disorders, sleep problems, indicative ASD and indicative DCD using χ^2 tests.

In young people with 22q11.2DS, I examined the associations between the 'any positive' summary variable and FSIQ, PIQ and VIQ scores, ID, psychiatric disorders, indicative ASD, indicative DCD and sleep disturbance, using logistic regressions. Predictors were entered hierarchically, age first, then gender and finally the relevant neurodevelopmental variable. For all of the following analyses, age and gender were not included as predictors: Given that I observed a very high rate of febrile seizures (Item 1, Table 4-2) in deletion carriers in this study (24.3%, see Table 4-2), I also aimed to explore their association with neurodevelopmental trajectories. I therefore repeated these logistic regressions using the response to the febrile seizure item (positive/negative) as the dependent variable. For this analysis, the overall rate of febrile seizures was used (24.3%, see Table 4-2), i.e. I combined across deletion carriers with and without an epilepsy diagnosis.

4.4 Results

4.4.1 Prevalence of epilepsy, seizures and paroxysmal events in 22q11.2DS and control siblings

Descriptive statistics about my sample of young people with 22q11.2DS and their control siblings are shown in Table 4-1. Different levels of primary caregiver education and family income were relatively evenly represented in this study.

Table 4-1. Descriptive statistics about my sample of young people with 22q11.2DS and control siblings.

Family Ethnic Background (%)			
European	92 (85.2)		
Mixed	11 (10.2)		
Non-European	2 (1.9)		
Unknown	3 (2.8)		
Highest Parental Qualification[a]			
	My Sample	**Equivalent categories from Lawson et al. (2013)[b]**	
Low (O-Levels/GCSEs)	25 (23.1)	**High School or below**	45 (15.9)
Middle (A-Levels/highers/vocational training)	35 (32.4)	**Some College**	78 (27.6)
High (university degree and/or other higher postgraduate qualification)	35 (32.4)	**College and above**	160 (56.5)
Unknown	13 (12)	-	
Family Income			
	My Sample	**Equivalent categories from Lawson et al. (2013)[b]**	
≤ £19,999	24 (22.2)	**≤ $25,000**	13 (4.6)
£20,000 - £39,999	26 (24.1)	**$25,001-$50,000**	57 (20.1)
£40,000 - £59,999	23 (21.3)	**$50,001-$75,000**	68 (24)
≥£60,000	25 (23.1)	**> $75,000**	145 (51.2)
Unknown	10 (9.3)	-	
Age			
	Mean	**SD**	**Range**
22q11.2DS	13.6	3.3	6.2-20.5
Control siblings	12.7	2.9	6.3-18.9
Gender			
	Male (%)	**Female (%)**	
22q11.2DS	62 (57.4)	46 (42.6)	
Control siblings	30 (50)	30 (50)	

[a] of the parent completing the questionnaire

[b] This was an MRI study of 283 typically developing children and adolescents (mean age 11.47 years) in the United States of America[29]

22q11.2DS, 22q11.2 deletion syndrome

Table 4-2 shows the rates of positive responses on each item of the ESQ**.** 63.9% (69/108) of young people with 22q11.2DS screened positive on the 'any positive' summary variable compared to 13.3% (8/60) of control siblings (*b*=2.49, z=5.67, OR=12, p<0.001). Age and gender did not influence this relationship. 11.1% (12/108) of young people with 22q11.2DS were reported as having an epilepsy diagnosis, compared to none of the controls (p=0.004). When excluding those with epilepsy, 21.1% of deletion carriers were reported as having a febrile seizure (20/95, the primary caregiver of one deletion carrier missed out the question relating to febrile seizures, please see Table 4-2, controls 0%, $\chi^2(1)=14.3$, p<0.001). After excluding those with epilepsy or a febrile seizure, 48.7% (37/76) of the remaining deletion carriers were reported as having an afebrile seizure or paroxysmal event compared to 13.3% (8/60) of control siblings ($\chi^2(1)=18.9$, OR=6.08, p<0.001).

Rates of four of the paroxysmal event items were elevated in deletion carriers compared to control siblings: Item 3, Item 4, Item 5 and Item 7 (Table 4-2). A greater percentage of deletion carriers (9.3%) than control siblings (0%) had a positive response for Item 8, although this difference was not significant (p=0.051).

Table 4-2. Rates of positive responses on each item of the Epilepsy Screen Questionnaire, in young people with 22q11.2DS and their unaffected control siblings.

Item	Positive response		χ^2	OR	p-value
	22q11.2DS (%)	Sibling controls (%)			
1. Did your son/daughter ever have a seizure or convulsion caused by a high fever?[a]	26 (24.3)	0 (0)	17	_	<0.001
2. Other than the seizures associated with high fevers, has your son/daughter ever had a seizure, convulsion, fit, or spell - under any circumstances?[b]	26 (24.1)	0 (0)	16.8	_	<0.001
3. Other than the seizures associated with high fevers, has your son/daughter ever had uncontrolled movements of part or all of their body such as twitching, jerking, shaking or going limp?[c]	21 (19.8)	2 (3.3)	8.72	7.1	0.003
4. Other than the seizures associated with high fevers, has your son/daughter ever had an unexplained change in their mental state or level of awareness; or an episode of 'spacing out' that they could not control?[d]	19 (17.8)	1(1.7)	9.44	12.6	0.002
5. Does your son/daughter daydream or stare into space more than other children?[d]	43(40.2)	5(8.3)	19	7.31	<0.001
6. Have you ever noticed them to have any unusual body movements when exposed to strobe lights, video games, flickering lights, or sun glare?[b]	3(2.8)	0(0)	Fisher's Exact Test	_	0.553
7. Shortly after waking up, either in the morning or after a nap, have you ever noticed that your son/daughter has had uncontrollable jerking or clumsiness, such as dropping things or things suddenly flying from their hands?[b]	10(9.3)	0(0)	Fisher's Exact Test	_	0.014
8. Has your son/daughter ever had any other type of repeated unusual spells?[b]	7(6.5)	0(0)	Fisher's Exact Test	_	0.051
9. Has your son/daughter ever been diagnosed with a seizure disorder or epilepsy?	12(11.1)	0(0)	Fisher's Exact Test	_	0.004

[a] Data available for 107 probands and 59 control siblings

[b] Data available for 59 control siblings

[c] Data available for 106 probands

[d] Data available for 107 probands

22q11.2DS, 22q11.2 deletion syndrome

13.6% (14/103) of young people with 22q11.2DS and 4.4% (2/46) of control siblings were born prematurely. 64.8% (68/105) of deletion carriers and 1.7% (1/59) of control siblings had cardiovascular problems. These comorbidities did not associate with the 'any positive' summary variable.

Recurrent infections were seen more often in deletion carriers (b=2.89, z=6.02, p<0.001) with 68.9% (73/106) of young people with 22q11.2DS having recurrent infections compared to 10.9% (6/55) of their control siblings. Deletion carriers with recurrent infections were not more likely to report febrile seizures than those without infections however (b=0.78, z=1.41, p=0.157).

4.4.2 Association of the ESQ responses with neurodevelopmental problems in 22q11.2DS

4.4.2.1 IQ and ID

The mean FSIQ, PIQ and VIQ of young people with 22q11.2DS was significantly lower than control siblings (Table 4-3). One deletion carrier had a higher-than-average FSIQ (117). 12.1% (12/99) of deletion carriers had an average FSIQ (86-115) and 40.4% (40/99) had an FSIQ in the borderline range (71-85). 41.4% (41/99) had mild ID (55-70) and 5.1% (5/99) had moderate ID (IQ<55). By contrast, 94.5% (52/55) of control siblings had an average, or higher-than-average, FSIQ and only three (5.5%) had a borderline FSIQ. Positive screens on the 'any positive' summary variable were more common in deletion carriers with a lower PIQ but were not related to FSIQ, VIQ or ID (Table 4-4). Age and gender did not influence these relationships. Febrile seizure prevalence was not predicted by FSIQ, PIQ, VIQ or ID in young people with 22q11.2DS (p>0.05 in all cases).

4.4.2.2 Psychiatric disorder, sleep disturbance and motor coordination problems

I found higher rates of psychiatric disorders, sleep disturbance, indicative ASD and indicative DCD in young people with 22q11.2DS when compared to their control siblings (Table 4-3). Young people with 22q11.2DS and ADHD were around three times more likely to report 'any positive' and those with indicative ASD and indicative DCD were around four times more likely (Table 4-4). Age and gender did not influence these relationships. Febrile seizures were not associated with any psychiatric, motor or sleep comorbidity (p>0.05 in all cases).

Table 4-3. IQ scores and rates of neurodevelopmental problems in young people with 22q11.2 deletion syndrome and control siblings.

	22q11.2 DS			Control siblings					
Measure	**n**	**Mean**	**s.d.**	**n**	**Mean**	**s.d.**	**t**	**95% CI**	**p-value**
FSIQ	99	72.05	12.29	55	108.58	15.3	15.19	31.76, 41.31	<0.001
PIQ	99	75.24	12.83	55	109.29	16.62	13.17	28.91, 39.18	<0.001
VIQ	100	73.13	12.67	55	106.45	14.77	14.12	28.64, 38.01	<0.001
Neurodevelopmental problem	**Total**	**Yes (%)**	**No (%)**	**Total**	**Yes (%)**	**No (%)**	**χ^2**	**OR**	**p-value**
ID	99	42 (42.4)	57 (57.6)	55	0(0)	55 (100)	32.08	-	<0.001
Any psychiatric disorder	108	53 (49.1)	55 (50.9)	53	2 (3.8)	51 (96.2)	32.44	24.19	<0.001
ADHD	106	30 (28.3)	76 (71.7)	47	1 (2.1)	46(97.9)	13.81	17.96	<0.001
Any anxiety disorder	108	31 (28.7)	77 (71.3)	49	2 (4.1)	47 (95.9)	12.3	9.36	<0.001
Any sleep problem	107	63 (58.9)	44 (41.1)	50	10 (20)	40 (80)	20.7	5.66	<0.001
Indicative ASD	90	37 (41.1)	53 (58.9)	48	0 (0)	48 (100)	26.96	-	<0.001
Indicative DCD	95	79 (83.2)	16 (16.8)	54	3 (5.6)	51 (94.4)	83.78	79.88	<0.001

22q11.2DS, 22q11.2 deletion syndrome, FSIQ, full-scale IQ, PIQ, performance IQ, VIQ, verbal IQ, ID, intellectual disability, ADHD, attention deficit hyperactivity disorder, ASD, autism spectrum disorder, DCD, developmental coordination disorder.

Table 4-4. IQ scores and rates of neurodevelopmental problems for young people with 22q11.2DS on the 'any positive' ESQ-summary variable.

Measure	n	Any positive		*b*	z	OR	p-value
		No (s.d.)	Yes (s.d.)				
FSIQ	99	73.94 (13.31)	70.35 (11.15)	-0.02	-1.39	0.98	0.163
PIQ	99	78.49 (14.05)	72.31 (10.94)	-0.04	-2.36	0.96	0.018
VIQ	100	73.98 (13.25)	72.38 (12.20)	-0.01	-0.59	1	0.556
Neurodevelopmental problem	**n affected/total n**	**Any positive**		***b***	**z**	**OR**	**p-value**
		No (%)	**Yes (%)**				
ID	42 / 99	17 (36.2)	25 (48.1)	0.44	1.02	1.55	0.921
Any psychiatric disorder	53 / 108	33 (56.9)	20 (40)	0.76	1.87	2.13	0.062
ADHD	30 / 106	10 (20)	20 (35.7)	1.19	2.32	3.28	0.021
Any anxiety disorder	31 / 108	11 (22.0)	20 (34.5)	0.6	1.36	1.83	0.175
Any sleep problem	63 / 107	31 (62)	32 (56.1)	-0.24	-0.59	0.79	0.558
Indicative ASD	37 / 90	11 (25.6)	26 (55.3)	1.35	2.86	3.86	0.004
Indicative DCD	79 / 95	33 (73.3)	46 (92.0)	1.52	2.31	4.56	0.021

22q11.2DS, 22q11.2 deletion syndrome, FSIQ, full-scale IQ, PIQ, performance IQ, VIQ, verbal IQ, ID, intellectual disability, ADHD, attention deficit hyperactivity disorder, ASD, autism spectrum disorder, DCD, developmental coordination disorder

Comorbidity of positive screens on the 'any positive' summary variable, indicative DCD, ADHD and indicative ASD was common in young people with 22q11.2DS (Figure 4-1). 58.2% (46/79) of deletion carriers with indicative DCD screened positive on the 'any positive' summary variable, as did 70.3% (26/37) of those with indicative ASD and 66.7% (20/30) of those with ADHD. 10.6% (10/94) of deletion carriers had 'any positive', indicative DCD, ADHD and indicative ASD.

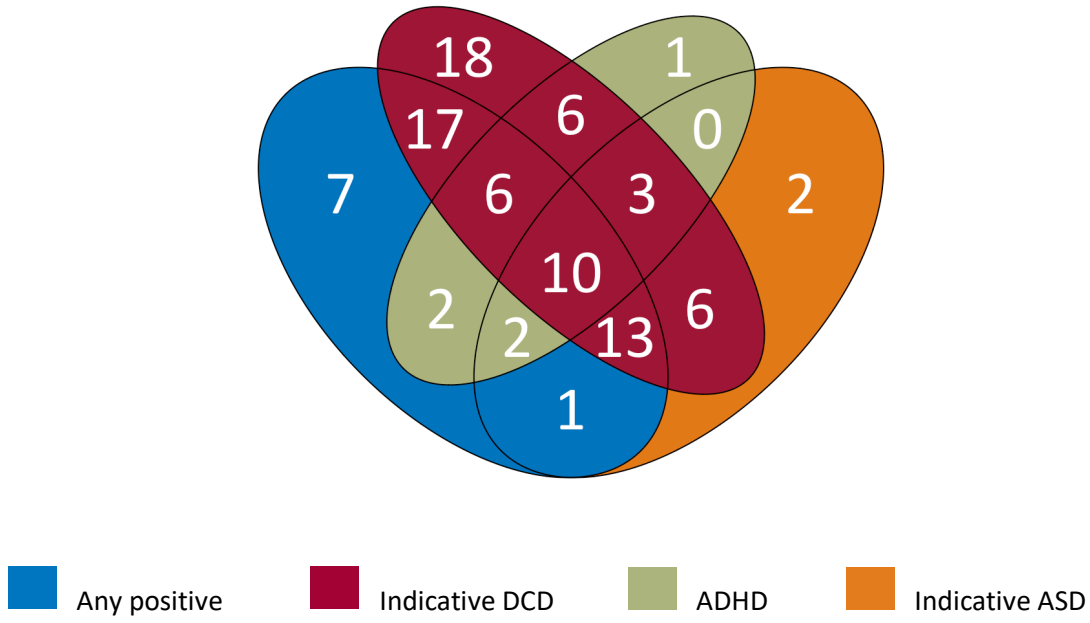

Figure 4-1. The overlap of 'any positive' on the Epilepsy Screen Questionnaire, indicative DCD, ADHD and indicative ASD in 22q11.2DS. DCD, developmental coordination disorder, ADHD, attention-deficit hyperactivity disorder, ASD, autism spectrum disorder.

4.5 Discussion

I found that 11.1% of young people with 22q11.2DS were reported as having an epilepsy diagnosis. When these cases were excluded, 21.1% of deletion carriers were reported having a febrile seizure. After excluding cases with a report of an epilepsy diagnosis or a febrile seizure, nearly half of deletion carriers were reported with an afebrile seizure or a paroxysmal event. Deletion carriers who were reported as having at least one item from the ESQ ('any positive') also had a higher prevalence of neurodevelopmental comorbidities, with high rates of ADHD, indicative ASD, indicative DCD and lower PIQ scores. None of these associations were significant for febrile seizures.

4.5.1 Prevalence of epilepsy, seizures and paroxysmal events in 22q11.2DS and in control siblings

The rate of (reported) epilepsy diagnoses that I observed in young people with 22q11.2DS (11.1%) was in line with previous estimates, as obtained from medical records[2-4,6,8,15] As mentioned in the introduction, one possible limitation of these studies is that the reliance on medical record review may mean that deletion carriers who have not been seen clinically for epileptic seizures may be missed. This may be particularly likely for patients with non-motor absence seizures and focal non-motor seizures with impaired awareness. My preliminary questionnaire findings provide some support to this idea, given that when deletion carriers with an epilepsy diagnosis or a febrile seizure were excluded, 48.7% were reported as having an afebrile "seizure, convulsion, fit or spell" or a paroxysmal event. These reported events may represent hypocalcaemia-induced seizures (10-14.5% of children with 22q11.2DS in previous studies[2,3]) or perhaps an isolated unprovoked seizure, thus explaining why these individuals were not diagnosed with epilepsy. A more serious interpretation is that in some cases these events could represent repeated unprovoked seizures not detected during routine clinical care, in which case epilepsy would be significantly under-diagnosed in 22q11.2DS. This interpretation should be treated with caution however; the broad nature of the paroxysmal event questions (e.g. "Does you son/daughter daydream into space more than other children?") may be indexing the learning difficulties and neurodevelopmental disorders commonly seen in young people with 22q11.2DS[30]. Indeed, individuals with cognitive impairments are particularly likely to be misdiagnosed with epilepsy[31]. In the validation study conducted by Ottman et al.[14] (described in section 2.3.1.1), the paroxysmal event items (3-8, Table 2) increased the sensitivity of the ESQ for epilepsy patients by 3% (from 91% to 94%), however, individuals with and without epilepsy or an isolated unprovoked seizure were equally likely to respond positively to these items, suggesting they are not specific for epilepsy or epileptic seizures. Despite the low false-positive rate in the validation study (7%, amongst those with no history of seizures in their medical records), it was estimated that if the ESQ were to be used to screen for epilepsy in the general population, the positive predictive value (PPV, the proportion of screen-positive people who genuinely have epilepsy) would only be 23%, due to the relatively low prevalence of epilepsy in the general population (lifetime prevalence 0.5-3%)[14]. Despite these limitations, the afebrile seizures and paroxysmal events described in the ESQ are clearly more prevalent in deletion carriers than in control siblings. In addition, rate of epilepsy in this 22q11.2DS is higher (4.4-36.8%)[2,4,6-8,15]. A second stage of assessment of the events described in the ESQ is ultimately needed to establish if they are true epileptic seizures and to better estimate the prevalence of epileptic seizures and epilepsy in 22q11.2DS. This will also establish the specificity and

sensitivity of the ESQ to identify epileptic seizures and epilepsy in a cohort with comorbid cognitive and psychiatric difficulties.

Febrile seizures

The rate of reported febrile seizures in young people with 22q11.2DS was 21.1% when cases with epilepsy were excluded (increasing slightly to 24.3% when cases with epilepsy were included). This is considerably higher than in previous studies in this patient group (2-6%[2-4]) and in the general population (2-5%[32]). This supports the hypothesis of a reduced seizure threshold in 22q11.2DS, for many triggers including hypocalcaemia and psychotropic drug use[3,4]. Interestingly, in this study reported febrile seizures were independent of other indicators of aberrant neurodevelopment – such as ADHD, indicative ASD, DCD and a lower PIQ – which provides preliminary evidence suggesting an independent risk pathway for febrile seizures in 22q11.2DS. One such pathway could be the recurrent infections in this syndrome, due to thymic hypoplasia and impaired T-cell production[1]. I did observe a trend toward a greater percentage of deletion carriers with febrile seizures having recurrent infections as compared to not having recurrent infections (80.8% versus 19.2%), however this was not significant (p=.157), likely due to the fact that the rate of infections was very high in the young people with 22q11.2DS (68.9%). Perhaps therefore recurrent infections increase the risk of febrile seizures only in tandem with other factors in 22q11.2DS, one of which could be a reduced seizure threshold in some young people with this syndrome. These analyses may however be limited by the fact that I did not probe for a lifetime history of recurrent infections, only recurrent infections in the present at a given wave of assessment (e.g. "Does your child have recurrent infections of the chest/airways?"). Therefore, information about infections from the first six years of life (which were no longer ongoing when the caregiver filled out the questionnaire) may have been missing, and the rate of infections I observed could have been inaccurate.

A reduced seizure threshold in 22q11.2DS could be brought about by the structural brain abnormalities associated with 22q11.2DS (e.g. polymicrogyria, focal cortical dysplasia, reduced number and length of dendrites[16,33]) and the impact these may have on the balance of neuronal excitatory-inhibitory signalling. As outlined in section 1.7.3.1, the elevated rate of hypocalcaemia in 22q11.2DS[1] may additionally contribute to this reduced seizure threshold by enhancing neuronal excitability[34]. Future studies should explore the relationships between febrile seizures, infections and potential markers of a reduced seizure threshold, such as a history of seizures in response to psychotropic drug use, hypocalcaemia, or structural brain abnormalities.

Whilst simple febrile seizures (i.e. generalised semiology, lasting less than 15 minutes) are considered relatively benign (for example, they do not associate with poorer academic or behavioural outcomes

in the general population[35]), there is evidence that febrile status epilepticus (FSE, lasting ≥ 30 minutes) leads to acute white matter injury and hippocampal sclerosis (particularly in children with pre-existing neurological vulnerabilities [36]) as well as general cognitive impairment[37] and memory problems[38]. In 22q11.2DS impairments in a variety of neurocognitive domains (including working memory) have been observed[11], as well as white matter abnormalities[39]. In addition, a high rate of hippocampal malrotation (64%) has been observed in 22q11.2DS[7], which is thought to predispose to prolonged febrile seizures[40]. It is important therefore to better characterise febrile seizures in 22q11.2DS in order to understand their relationship with these outcomes, although in this study I did not see associations of reported febrile seizures with any of our neurodevelopmental measures.

4.5.2 Association of the ESQ responses with neurodevelopmental problems in 22q11.2DS

The relationships I observed between the 'any positive' summary variable and ADHD, indicative ASD, indicative DCD and a lower PIQ in our sample of young people with 22q11.2DS have several implications:

1. One the one hand, these relationships are in line with the associations of epilepsy with impaired cognition, psychopathology and motor problems, observed in the general population[17,20,21,41] and therefore may suggest shared neurobiological risk pathways for these disorders. One pathway could be aberrant synaptic plasticity. In the general population, abnormal synaptic plasticity has been implicated in the development of epileptic seizures, as well as behaviours associated with ASD via an imbalance in neuronal excitation-inhibition[42]. In a mouse model of ASD, aberrant synaptic plasticity was also implicated in problems with motor coordination and learning via impaired long-term depression response and synaptic pruning in the cerebellum[43]. Synaptic plasticity in the prefrontal cortex is important in adolescent development of executive function, a behavioural impairment in ADHD[44]. Impaired hippocampal synaptic plasticity shows links with memory deficits[45,46]. Aberrant synaptic plasticity is also implicated in the emergence of the sensory, cognitive, motor and psychotic features that characterise schizophrenia[47], for which 22q11.2DS confers risk (22%)[30,48,49]. Mouse models of 22q11.2DS have demonstrated aberrant synaptic plasticity. For example, hemizygosity for the microRNA biogenesis gene *Dgcr8* leads to enhanced short and long-term synaptic plasticity within hippocampal CA3-CA1 synapses, coinciding with spatial memory deficits, as well as enhanced short-term depression in the prefrontal cortex. Mice that are haploinsufficient for the mitochondrial function gene *Mrpl40* also show abnormal short-term potentiation within the hippocampus and co-occurring working memory deficits. The aberrant synaptic plasticity in these mouse models is mediated by dysregulation of

presynaptic calcium levels and neurotransmitter release (e.g. enhanced glutamate activity)[45,46,50].

Evidence suggests epileptic seizures may also result in neurobiological changes that can lead to impaired cognition and ASD-related behaviours[42] and that seizures may alter the functional organisation of motor control in the brain[51]. In addition, paroxysmal epileptic activity has been shown to associate with poorer performance on tests assessing attentional processes[52]. Early-life seizures may therefore have deleterious effects on a vulnerable neural network in 22q11.2DS for example, within hippocampal and prefrontal regions[7,46,50], leading to the exacerbation of impaired cognition, ADHD symptoms, ASD-related behaviours and motor coordination problems. It is important to highlight that these deleterious effects on cognition, psychiatric health and motor function may be independent of the process of epileptogenesis that initially gave rise to epileptic seizures[53], please see section 1.7.2.3 for a discussion of this issue in the general population. Future longitudinal studies may be able to tease apart the relationship between epileptic seizures and impaired cognition, psychopathology and motor coordination problems in 22q11.2DS observed here.

2. As discussed in Section 4.5.1, the broad nature of the paroxysmal event items of the ESQ may mean that in some cases positive responses represent behaviours associated with ID, psychopathology and other neurodevelopmental problems seen in 22q11.2DS[1,30], rather than true epileptic seizures. If this is the case, then it is no surprise I observed significant associations between 'any positive' on the ESQ and neurodevelopmental problems in deletion carriers. These associations, and conclusions about shared neurobiological risk pathways in 22q11.2DS, therefore need to be interpreted with caution. A second stage of assessment is warranted to delineate these ESQ-reported events (distinguish true-positives from false-positives) and better assess the validity of the relationships with neurodevelopmental problems, observed in this chapter.

Of further interest from my analysis of the ESQ responses with neurodevelopmental problems in 22q11.2DS is that I did not observe any significant relationships of the 'any positive' summary variable with anxiety disorder and sleep disturbance, in contrast to findings seen in the general population[17,22]. My assessment of sleep disturbance was preliminary, relying on parental report, whereas a systematic polysomnography assessment may provide a more accurate estimation of sleep problems in 22q11.2DS and allow us to better assess their relationship with epileptic seizures and epilepsy.

In conclusion, the findings from this chapter clearly reinforce that not only are children with 22q11.2DS at risk of acute symptomatic seizures and epilepsy, but that epilepsy could be underdiagnosed in this population. Risk for epileptic seizures in 22q11.2DS may be symptomatic of underlying network dysfunction, possibly involving aberrant synaptic plasticity, which also gives rise to impairment in fluid intelligence, ADHD, ASD symptoms and motor coordination problems. These associations are however based on a preliminary epilepsy screening questionnaire and true-positives need to distinguished from false-positives in order to assess the validity of these relationships. I demonstrated further evidence for a reduced threshold for acute symptomatic seizures in 22q11.2DS through the high rate of febrile seizures (24.3%) reported in my cohort of deletion carriers. Highlighting the prevalence of epileptic seizures and epilepsy and their relationship with neurodevelopment in 22q11.2DS could allow more focused monitoring of and intervention for epileptic seizures by clinicians. This could improve the outcomes of young people with this syndrome. Ultimately then, it is essential that the events reported in the ESQ undergo a second, detailed stage of assessment (e.g. involving interview, video-EEG recording and review of medical records) to establish whether they are true epileptic seizures. A second stage assessment of epileptic seizures and epilepsy in a sub-group of my sample of young people with 22q11.2DS and control siblings will be discussed in the next chapter.

4.6 References

1 McDonald-McGinn, D. M. *et al.* 22q11. 2 deletion syndrome. *Nature reviews. Disease primers* **1**, 15071 (2015).

2 Kao, A. *et al.* Increased prevalence of unprovoked seizures in patients with a 22q11. 2 deletion. *American Journal of Medical Genetics Part A* **129**, 29-34 (2004).

3 Ryan, A. *et al.* Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *Journal of Medical Genetics* **34**, 798-804 (1997).

4 Wither, R. G. *et al.* 22q11. 2 deletion syndrome lowers seizure threshold in adult patients without epilepsy. *Epilepsia* (2017).

5 Lee, K. C., Finley, P. R. & Alldredge, B. K. Risk of seizures associated with psychotropic medications: emphasis on new drugs and new findings. *Expert opinion on drug safety* **2**, 233-247 (2003).

6 Kim, E.-H. *et al.* Epilepsy and Other Neuropsychiatric Manifestations in Children and Adolescents with 22q11. 2 Deletion Syndrome. *Journal of Clinical Neurology* **12**, 85-92 (2016).

7 Andrade, D. M., Krings, T., Chow, E. W., Kiehl, T.-R. & Bassett, A. S. Hippocampal malrotation is associated with chromosome 22q11. 2 microdeletion. *The Canadian Journal of Neurological Sciences* **40**, 652-656 (2013).

8 Olsen, L. *et al.* Prevalence of rearrangements in the 22q11.2 region and population-based risk of neuropsychiatric and developmental disorders in a Danish population: a case-cohort study. *The Lancet Psychiatry*, doi:https://doi.org/10.1016/S2215-0366(18)30168-8.

9 Pohlmann-Eden, B., Beghi, E., Camfield, C. & Camfield, P. The first seizure and its management in adults and children. *BMJ: British Medical Journal* **332**, 339 (2006).

10 Cheung, E. N. *et al.* Neonatal hypocalcemia, neonatal seizures, and intellectual disability in 22q11.2 deletion syndrome. *Genet Med* **16**, 40-44, doi:10.1038/gim.2013.71 (2014).

11 Niarchou, M. *et al.* Psychopathology and cognition in children with 22q11. 2 deletion syndrome. *The British Journal of Psychiatry* **204**, 46-54 (2014).

12 Crockett, D. J., Goudy, S. L., Chinnadurai, S. & Wootten, C. T. Obstructive sleep apnea syndrome in children with 22q11.2 deletion syndrome after operative intervention for velopharyngeal insufficiency. *Front Pediatr* **2**, 84, doi:10.3389/fped.2014.00084 (2014).

13 Cunningham, A. C. *et al.* Developmental coordination disorder, psychopathology and IQ in 22q11. 2 deletion syndrome. *The British Journal of Psychiatry* **212**, 27-33 (2018).

14 Ottman, R. *et al.* Validation of a brief screening instrument for the ascertainment of epilepsy. *Epilepsia* **51**, 191-197 (2010).

15 Strehlow, V. *et al.* Generalized Epilepsy and Myoclonic Seizures in 22q11. 2 Deletion Syndrome. *Molecular Syndromology* **7**, 239-246 (2016).

16 Mudigoudar, B., Nune, S., Fulton, S., Dayyat, E. & Wheless, J. W. Epilepsy in 22q11. 2 Deletion Syndrome: A Case Series and Literature Review. *Pediatric Neurology* (2017).

17 Rai, D. *et al.* Epilepsy and psychiatric comorbidity: A nationally representative population-based study. *Epilepsia* **53**, 1095-1103 (2012).

18 Saemundsen, E., Ludvigsson, P., Hilmarsdottir, I. & Rafnsson, V. Autism Spectrum Disorders in Children with Seizures in the First Year of Life—A Population-Based Study. *Epilepsia* **48**, 1724-1730 (2007).

19 Clarke, D. F. *et al.* The prevalence of autistic spectrum disorder in children surveyed in a tertiary care epilepsy clinic. *Epilepsia* **46**, 1970-1977 (2005).

20 Parisi, P., Moavero, R., Verrotti, A. & Curatolo, P. Attention deficit hyperactivity disorder in children with epilepsy. *Brain and Development* **32**, 10-16 (2010).

21 Kirby, A. *et al.* Benign childhood epilepsy with centrotemporal spikes (BECTS) and developmental co-ordination disorder. *Epilepsy & Behavior* **72**, 122-126 (2017).

22 van Golde, E. G., Gutter, T. & de Weerd, A. W. Sleep disturbances in people with epilepsy; prevalence, impact and treatment. *Sleep medicine reviews* **15**, 357-368 (2011).

23 Weschler, D. Weschler abbreviated scale of intelligence (WASI). *London: Psychological Corporation* (1999).

24 Angold, A. *et al.* The child and adolescent psychiatric assessment (CAPA). *Psychological medicine* **25**, 739-753 (1995).

25 Association, A. P. & Association, A. P. *Diagnostic and statistical manual-text revision (DSM-IV-TRim, 2000)*. (American Psychiatric Association, 2000).

26 Rutter, M., Bailey, A. & Lord, C. *The social communication questionnaire: Manual*. (Western Psychological Services, 2003).

27 Wilson, B. N. The Developmental Coordination Disorder Questionnaire 2007. *Physical & occupational therapy in pediatrics* **29**, 182-202 (2012).

28 Wilson, B. N., Kaplan, B. J., Crawford, S. G., Campbell, A. & Dewey, D. Reliability and validity of a parent questionnaire on childhood motor skills. *American Journal of Occupational Therapy* **54**, 484-493 (2000).

29 Lawson, G. M., Duda, J. T., Avants, B. B., Wu, J. & Farah, M. J. Associations between children's socioeconomic status and prefrontal cortical thickness. *Developmental Science* **16**, 641-652, doi:doi:10.1111/desc.12096 (2013).

30 Schneider, M. *et al.* Psychiatric disorders from childhood to adulthood in 22q11. 2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11. 2 Deletion Syndrome. *American Journal of Psychiatry* (2014).

31 Britton, J. *et al. Electroencephalography (EEG): An introductory text and atlas of normal and abnormal findings in adults, children, and infants*. (American Epilepsy Society, Chicago, 2016).

32 Stafstrom, C. E. in *Febrile seizures* 1-25 (Elsevier, 2002).

33 Karayiorgou, M., Simon, T. J. & Gogos, J. A. 22q11. 2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. *Nature Reviews Neuroscience* **11**, 402 (2010).

34 Han, P., Trinidad, B. J. & Shi, J. Hypocalcemia-induced seizure: demystifying the calcium paradox. *ASN neuro* **7**, 1759091415578050, doi:10.1177/1759091415578050 (2015).

35 Verity, C. M., Greenwood, R. & Golding, J. Long-term intellectual and behavioral outcomes of children with febrile convulsions. *New England Journal of Medicine* **338**, 1723-1728 (1998).

36 Lewis, D. V. *et al.* Hippocampal sclerosis after febrile status epilepticus: the FEBSTAT study. *Annals of neurology* **75**, 178-185 (2014).

37 Martinos, M. M. *et al.* Early developmental outcomes in children following convulsive status epilepticus: a longitudinal study. *Epilepsia* **54**, 1012-1019 (2013).

38 Martinos, M. M. *et al.* Recognition memory is impaired in children after prolonged febrile seizures. *Brain* **135**, 3153-3164 (2012).

39 Kikinis, Z. *et al.* Abnormalities in brain white matter in adolescents with 22q11. 2 deletion syndrome and psychotic symptoms. *Brain imaging and behavior* **11**, 1353-1364 (2017).

40 Lewis, D. V. *et al.* Himal Is A Malformation That Predisposes To Prolonged Febrile Seizures. *Epilepsia* **47**, 16 (2006).

41 Bowley, C. & Kerr, M. Epilepsy and intellectual disability. *Journal of Intellectual Disability Research* **44**, 529-543 (2000).

42 Brooks-Kayal, A. Epilepsy and autism spectrum disorders: are there common developmental mechanisms? *Brain and Development* **32**, 731-738 (2010).

43 Piochon, C. *et al.* Cerebellar Plasticity and Motor Learning Deficits in a Copy Number Variation Mouse Model of Autism. *Nature communications* **5**, 5586-5586, doi:10.1038/ncomms6586 (2014).

44 Selemon, L. D. A role for synaptic plasticity in the adolescent development of executive function. *Translational psychiatry* **3**, e238 (2013).

45 Devaraju, P. *et al.* Haploinsufficiency of the 22q11. 2 microdeletion gene Mrpl40 disrupts short-term synaptic plasticity and working memory through dysregulation of mitochondrial calcium. *Molecular psychiatry* (2016).

46 Earls, L. R. *et al.* Age-dependent microRNA control of synaptic plasticity in 22q11 deletion syndrome and schizophrenia. *Journal of Neuroscience* **32**, 14132-14144 (2012).

47 Forsyth, J. K. & Lewis, D. A. Mapping the Consequences of Impaired Synaptic Plasticity in Schizophrenia through Development: An Integrative Model for Diverse Clinical Features. *Trends in cognitive sciences* **21**, 760-778 (2017).

48 Monks, S. *et al.* Further evidence for high rates of schizophrenia in 22q11. 2 deletion syndrome. *Schizophrenia research* **153**, 231-236 (2014).

49 Murphy, K. C., Jones, L. A. & Owen, M. J. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Archives of general psychiatry* **56**, 940-945 (1999).

50 Fénelon, K. *et al.* Deficiency of Dgcr8, a gene disrupted by the 22q11. 2 microdeletion, results in altered short-term plasticity in the prefrontal cortex. *Proceedings of the National Academy of Sciences* **108**, 4447-4452 (2011).

51 Woodward, K. E. *et al.* Recent seizure activity alters motor organization in frontal lobe epilepsy as revealed by task-based fMRI. *Epilepsy Res* **108**, 1286-1298, doi:10.1016/j.eplepsyres.2014.06.015 (2014).

52 Aldenkamp, A. & Arends, J. The relative influence of epileptic EEG discharges, short nonconvulsive seizures, and type of epilepsy on cognitive function. *Epilepsia* **45**, 54-63 (2004).

53 Avanzini, G., Depaulis, A., Tassinari, A. & De Curtis, M. Do seizures and epileptic activity worsen epilepsy and deteriorate cognitive function? *Epilepsia* **54**, 14-21 (2013).

5 Seizures, epilepsy and epileptiform discharges in 22q11.2DS: results from the second stage of assessment

5.1 Chapter overview

In Chapter 4, I observed that whilst 11.1% of young people with 22q11.2DS were reported as having an epilepsy diagnosis on the Epilepsy Screening Questionnaire (ESQ), 48.7% were reported with an afebrile seizure, convulsion, fit or spell in the absence of an epilepsy diagnosis or a febrile seizure. One implication of these findings was that epilepsy may be underdiagnosed in some of these deletion carriers. In addition, I observed that 21.1% of deletion carriers were reported as having febrile seizures in the absence of an epilepsy diagnosis, a rate far higher than previous estimates in 22q11.2DS (2-6%). In order to further investigate these events, I conducted a second stage of assessment in a sub-sample of deletion carriers and controls, which involved interviewing the primary caregiver and child; a two-month unusual spell diary completed by the primary caregiver; collecting relevant medical records; and conducting a 24-hour ambulatory electroencephalography (EEG) assessment in the family home (referred to as the 'case-control EEG study' in previous chapters). All of this data was reviewed by a consultant epileptologist, who identified epileptiform discharges and diagnosed and classified epileptic seizures and epilepsy according to the most recent International League Against Epilepsy (ILAE) guidelines. I also sought to better delineate salient characteristics of epileptic seizures in 22q11.2DS that have not been addressed to date in previous studies of deletion carriers, and which associate with poorer developmental outcomes in the general population. These included the phenotype of febrile seizures (simple/complex) and the length and frequency of unprovoked seizures. I aimed to assess whether a family history of epileptic seizures and/or epilepsy confers significant risk for epileptic seizures in young people with 22q11.2DS. In Chapter 4 I observed that the 'any positive' ESQ summary variable associated with a lower performance IQ (PIQ) and higher rates of attention deficit hyperactivity disorder (ADHD), indicative autism spectrum disorder (ASD) and indicative developmental coordination disorder (DCD). In this chapter, I explored whether these relationships remained significant when considering deletion carriers who were diagnosed with epileptic seizures and epilepsy during the second stage of assessment. I also took an alternative approach to address the question of the validity of the relationships observed in Chapter 4 and explored whether screen-positive deletion carriers who were subsequently diagnosed with non-epileptic events had a significantly lower PIQ and higher rates of ADHD, indicative ASD and DCD. I found that 5/6 deletion carriers with an ESQ-reported epilepsy diagnosis (and who were available for interview) were confirmed as having epilepsy by the epileptologist. Interestingly, two deletion carriers who had not

been reported with an epilepsy diagnosis in the ESQ were diagnosed with epilepsy by the epileptologist in this second assessment stage. In one these individuals however, there was evidence from the medical records that they had been previously given a clinical diagnosis of epilepsy. By contrast, only 11.8% (2/17) of individuals who were reported with an afebrile seizure or a paroxysmal event (in the absence of epilepsy or a febrile seizure) were diagnosed with 'possible' epileptic seizures by the epileptologist. These were repeated 'possible' non-motor absence seizures in both cases, resulting in an 'uncertain' epilepsy diagnosis in both cases. These findings suggest that epileptic seizures may not be recognised in some young people with 22q11.2DS during routine clinical care, and an epilepsy diagnosis may be overlooked, although not in as many as the findings from Chapter 4 initially suggested. The vast majority of those with ESQ-reported febrile seizures in the absence of an epilepsy diagnosis (and who were available for interview) had these confirmed by the epileptologist, supporting the findings from Chapter 4 and reinforcing that the 22q11.2 deletion confers significant risk for febrile seizures during childhood, as well as a reduced seizure threshold. These febrile seizures did not present with 'complex' features or meet criteria for febrile status epilepticus and are therefore less likely to lead to neurological injury and cognitive impairment. They were recurrent however, which in the general population increases the risk of future afebrile seizures. Febrile seizures in young people with 22q11.2DS should therefore arguably be closely monitored by clinicians. The majority of unprovoked seizures in this study were of 'primary genetic' origin (e.g. thought to be a direct consequence of the deletion, rather than a secondary consequence of a structural brain abnormality) and were generalised, providing further evidence for a strong association between 22q11.2DS and genetic generalised epilepsy (GGE). Unprovoked seizures did not meet criteria for status epilepticus but 30% of participants reported that, at their most frequent, seizures were occurring daily, which in the general population confers significant risk for a more severe epilepsy phenotype (e.g. drug-resistant epilepsy). 9.1% of deletion carriers showed brief spike-and-slow-wave discharges of atypical morphology on EEG, occurring during sleep and/or shortly after awakening. These were not significantly elevated relative to the control siblings however (0%, p=0.290), or more frequent in deletion carriers with epileptic seizures (15.4%) relative to those without (6.5%, p=0.570). Future longitudinal studies of epileptiform discharges and epilepsy are warranted to assess the extent to which these waveforms index epilepsy risk in 22q11.2DS. I did not observe a significant association between a positive family history and epileptic seizures in young people with 22q11.2DS. However, I only obtained information about family history in a small number of deletion carriers (n=37), meaning that this finding is perhaps due to a lack of power and that future studies should replicate these analyses in larger samples. I did not observe significant associations of epileptic seizures and epilepsy with PIQ, ADHD, indicative ASD and indicative DCD in young people

with 22q11.2DS, although there was a trend toward a greater proportion of deletion carriers diagnosed with epilepsy having ADHD and indicative ASD. I also did not find significant relationships between non-epileptic events and these poorer neurodevelopmental outcomes, but observed a trend toward a greater proportion of deletion carrier diagnosed with non-epileptic events having DCD. These analyses were very likely underpowered however, due to the relatively smaller number of deletion carriers with epileptic seizures, epilepsy and non-epileptic events. Larger studies of deletion carriers diagnosed with epileptic seizures and epilepsy are required to better explore their relationship with neurodevelopmental trajectories.

5.2 Introduction

As discussed in the introduction to this thesis (Chapter 1), people with 22q11.2DS are at an increased risk for acute symptomatic seizures and repeated unprovoked seizures (i.e. epilepsy), although reported rates are wide-ranging. For example, studies have reported that between 1-14.5% of deletion carriers have hypocalcaemia-induced seizures, whilst between 4.4-36.8% have epilepsy[1-7]. Differences in the extent of clinical documentation and diagnostic criteria between clinicians and medical centres may help to explain the wide-ranging prevalence estimates for epileptic seizures and epilepsy in 22q11.2DS. In addition, these reviews may have failed to account for non-motor absence seizures and focal non-motor seizures with impaired awareness, of which primary caregivers and clinicians may be unaware. Indeed, non-convulsive seizures usually have to occur several times before the affected individual and their family visit a clinician[8]. This may mean that prior studies may have underreported the true prevalence of epileptic seizures and epilepsy in young people with 22q11.2DS.

The vast majority of studies exploring epileptiform and background abnormalities through electroencephalography (EEG) recordings in 22q11.2DS have also similarly relied on historical medical record review[2-4,6,9] (however, see the study by Andrade et. al[1] for a first-hand assessment). These studies have identified epileptiform discharges which can be focal, multifocal or generalised and include spikes, polyspikes, sharp waves and spike/sharp-and-slow-wave discharges. Background abnormalities such as focal and generalised slowing has also been observed. However, medical record reviews of EEG findings in young people with 22q11.2DS very often do not specify the duration of the recordings (e.g.[2-4,6,9]). If findings were based on routine, interictal clinical recordings (i.e. ~30 minutes long), epileptiform discharges may have been missed, for example, those only occurring during sleep in a given patient.

In the previous chapter (Chapter 4), I aimed to go beyond the limitations of a medical record review approach and used the validated Epilepsy Screen Questionnaire (ESQ)[10], completed by primary

caregivers, to assess the prevalence of epilepsy, seizures and seizure-like symptoms in young people with 22q11.2DS (see Section 2.3.1.1 for a definition of 'seizure-like symptoms'). I observed that whilst 11.1% of deletion carriers were reported as having an epilepsy diagnosis, nearly half had an afebrile seizure or a paroxysmal event in the absence of an epilepsy diagnosis (or a febrile seizure). One interpretation of these findings is that epilepsy may be underdiagnosed in some young people with 22q11.2DS. However, as discussed in Section 4.5.1, this questionnaire approach has limitations. For example, the broad paroxysmal event items may be indexing behavioural features associated with the cognitive deficits and psychopathology observed in 22q11.2DS[11,12], leading to false-positives. In addition, whilst in the validation study (conducted by Ottman et al[10]) the sensitivity of the ESQ was high for patients with medical-record confirmed epilepsy diagnosis (96%), and the false-positive rate low (7%) for individuals who were seizure-free on medical record, the positive predictive value of the ESQ (i.e. the percentage of screen-positive individuals who genuinely have epilepsy) was estimated at only 23% in the general population. As discussed in Section 4.5.1, this low PPV was due to the rarity of epilepsy in the general population (lifetime prevalence of 0.5-3%, assumed to be 2% when calculating the PPV in the validation study[10]). The prevalence of epilepsy in 22q11.2DS is thought to be higher however (4.4-36.8%[1-5,7]). My first aim of this study was to conduct a second stage of assessment in a sub-sample of deletion carriers and control siblings screening positive on the ESQ, to determine whether their reported events represented epileptic seizures. This second stage comprised interviewing the primary caregiver and child about the ESQ-reported events, review of relevant medical records, a two-month unusual spell diary (completed by the primary caregiver) and a 24-hour ambulatory EEG assessment in the family home (referred to as the case-control EEG study in previous chapters). All of this data was then reviewed by an epileptologist, who made diagnoses of epilepsy and epileptic seizures, and identified epileptiform discharges. The case-control EEG study also included deletion carriers and control siblings who had screened negative on the ESQ. This was done to better delineate whether epileptiform discharges are associated with epilepsy risk in 22q11.2DS, as opposed to being a more general feature of the neurodevelopmental disorders conferred by the deletion (e.g. ADHD and ASD, disorders in which the rate of epileptiform discharges is elevated[13,14]), although see Section 2.3.7.2 for a discussion of the potential pitfalls of interpreting epileptiform discharges in populations with neurodevelopmental disorders. My hypotheses were as follows: 1. Given that the rate of epilepsy we observed from the ESQ (11.1%) was in line with previous estimates in 22q11.2DS, it would be likely that the majority of deletion carriers with an ESQ-reported epilepsy diagnosis would have their diagnosis confirmed by the epileptologist. 2. Given the broad nature of the paroxysmal event questions however, not all those reported as having an afebrile seizure or paroxysmal event would be diagnosed with epileptic seizures by the epileptologist. 3. The rates of

background abnormalities and epileptiform discharges would be significantly higher in deletion carriers relative to control siblings, given prior findings of these phenomena in people with 22q11.2DS[1-4,6,9]. 4. I predicted that epileptiform discharges would be significantly more prevalent in deletion carriers with epileptic seizures, relative to those without.

I also sought to explore salient characteristics of epileptic seizures in young people with 22q11.2DS. One of these was the distribution of various seizure aetiologies. Previous research has indicated that people with 22q11.2DS are at an increased risk for both acute symptomatic seizures and unprovoked seizures. The most common acute symptomatic seizure experienced by young people with this syndrome are hypocalcaemia-induced (10-15% of young people with 22q11.2DS, 43-67.7% of young deletion carriers with epileptic seizures)[3,5]. Amongst deletion carriers unprovoked seizures, focal seizures of varying aetiologies (e.g. focal cortical dysplasia, polymicrogyria) are the most common (44%)[9]. People with 22q11.2DS also show an elevated rate of unprovoked seizures with a 'primary genetic' origin (i.e. seizures are thought to be a direct consequence of the deletion, rather than a secondary consequence of structural brain abnormalities). More specifically, 22q11.2DS shows a strong association with genetic generalised epilepsy. It affects between 1-8.3% of deletion carriers (27% of deletion carriers with unprovoked seizures) and the deletion occurs in significantly more people with GGE (0.2%, 3/1,366) than without (0/5,234, p=8.85E-03 [2-4,6,9,15]. Further characteristics of unprovoked seizures that I aimed to better delineate were seizure onset, length and frequency. Whilst previous studies of groups of individuals with 22q11.2DS have described the age of onset of unprovoked seizures in young people with 22q11.2DS (8 months- 5.99 years)[2,3], they have not described the average length or frequency of seizures. Increased seizure length and frequency associate with poorer developmental outcomes in the general population. For example, particularly prolonged seizures (those over 30 minutes) may lead to neuronal injury, death and alteration of neuronal networks[16]. Similarly, seizure frequency significantly predicts poorer health-related quality of life, including general health, social functioning and mental health[17]. In addition, having weekly epileptic seizures significantly increases the risk of developing drug-resistant epilepsy[18]. In summary, I hypothesised that 1. Whilst the most common unprovoked seizure semiology in deletions carriers would be focal, a notable proportion would have generalised seizures with a 'primary genetic' aetiology. 2. I also predicted that the age of onset of unprovoked seizures would be between 8-60 months and that this study would better elucidate the length and frequency of unprovoked seizures in young people with 22q11.2DS.

In Chapter 4, I highlighted the need to better characterise the febrile seizure phenotype in 22q11.2DS. In the general population, 'simple' febrile seizures account for 65%-90% of all febrile seizures[19]. They have a generalised semiology and a duration of less than 15 minutes. Whilst they slightly increase the

risk of epilepsy later in life (2%), they do not associate with poorer academic or behavioural outcomes. Simple febrile seizures are therefore thought to be relatively benign[20]. By contrast, complex febrile seizures affect only one part of side of the body ('complex') or are abnormally prolonged (when a febrile seizure lasts 30 minutes or more it also known as 'febrile status epilepticus', FSE). Complex febrile seizures associate with substantially increased risk of epilepsy (6-49%), as well as neurological injury and cognitive impairment[21-24]. Delineating the phenotype of febrile seizures in 22q11.2DS could therefore provide important insights into risk for poor developmental outcomes in this syndrome. I therefore sought to collect data about febrile seizure semiology, seizure length, the lifetime number of febrile seizures and their age of onset. It is important to note that in Chapter 4 I did not observe an association of ESQ-reported febrile seizures with impaired cognition, psychopathology, sleep disturbance and motor coordination problems (see Section 4.4.2). I therefore hypothesized that febrile seizures in 22q11.2DS would not present with 'complex' features and would not meet criteria for FSE.

In the general population, a family history of epilepsy is a known risk factor for genetic epilepsy, generalised seizures and abnormal EEG findings[25]. A question of interest in whether a family history of epileptic seizures and epilepsy (referred to from now on as a 'positive family history') adds to the risk conferred by the de novo deletion for these epileptiform phenomena. This may help to explain why some deletion carriers go on to have epileptic seizures, whereas others do not. I therefore sought to address this question in my sub-sample of young people with 22q11.2DS. Kao et al. found a non-significant trend toward an association between a positive family history and a higher rate of unprovoked seizures ($p=0.089$)[3]. However, the reliance on medical record review may have meant that both the rate of unprovoked seizures and the rate of a positive family history may not have been accurate (see Section 1.7.3.4). Given this, and findings of an association between a family history of epilepsy and seizure risk in the general population, I hypothesized that a positive family history would show a significant association with epileptic seizures in young people with 22q11.2DS.

In Chapter 4, I observed that young people with 22q11.2DS who were reported as having 'any positive' in the ESQ (a positive response to at least one item of the questionnaire) were significantly more likely to have ADHD, indicative ASD and indicative DCD, as well as a significantly lower PIQ. Given the possibility that the broad nature of the paroxysmal event items (3-8, Table 4-2) may have lead to false-positives (see Section 4.5.2 for a discussion), the relationships observed in Chapter 4 may not be valid. We may not have been exploring the association of true epileptic seizures with PIQ, ADHD, indicative ASD and indicative DCD in all deletion carriers. I therefore aimed to assess whether these associations were still significant in deletion carriers who in the second stage of assessment were diagnosed with epileptic seizures and epilepsy (with 'negative' cases including those who were either screened

negative on the ESQ or who screened positive but were diagnosed with non-epileptic events during the second assessment stage). I also took an alternative approach in assessing the validity of the relationships I observed in Chapter 4. I looked only at screen-positive deletion carriers and compared whether false-positives (i.e. diagnosed with non-epileptic events during the second assessment stage) had any differences in their PIQ and rate of ADHD, indicative ASD and DCD than the true positives (i.e. diagnosed with epileptic seizures during the second assessment stage). If false-positives were to have a lower PIQ and higher rates of ADHD, indicative ASD and DCD than true positives, this could suggest that these individuals may have been driving the significant relationships of 'any positive' with poorer neurodevelopmental outcomes, which would cast doubt on the validity of these relationships. Given the robust associations of epilepsy with impaired cognition, psychopathology and motor problems observed in the general population[26-29], I hypothesized that individuals with epileptic seizures and epilepsy would have lower performance IQ and higher rates of ADHD, indicative ASD and indicative DCD. In screen-positive deletion carriers who took part in the second stage of assessment, I hypothesized that those diagnosed with non-epileptic events would have a greater PIQ and lower rates of ADHD, indicative ASD and indicative DCD than the true-positives.

5.3 Methods

5.3.1 Participants

This study comprised a sub-sample of the deletion carriers and controls described in Chapter 4. More specifically, I invited 56 deletion carriers (53.6% male, mean age = 14.6 years, s.d. = 3.2 years, range = 7.9-20.4 years) and 23 of their unaffected biological control siblings (47.8% male, mean age = 13.0 years, s.d. = 3.5 years, range = 7.0-18.9 years) to take part in the second stage of assessment. Sections 2.2 and 2.3 describe the recruitment procedure for the entire sample of deletion carriers and controls, as well as how participants were selected for the second stage of assessment.

5.3.2 Epilepsy Screen Questionnaire

The structure and content of the ESQ is described in detail in Section 2.3.1. This section also contains a description of the changes we made to the original version of the ESQ. The main findings based on data from the ESQ were presented in Chapter 4, in this chapter I recap the percentages of my three constituent ESQ- variables : a lifetime history of an epilepsy diagnosis, a febrile seizure in the absence of epilepsy and an afebrile seizure or paroxysmal event (in the absence of epilepsy or a febrile seizure).

5.3.3 Measure used in the second stage of assessment

5.3.3.1 Unusual Spell Interview

The Unusual Spell Interview, originally developed by Ottman et al.[30,31], was conducted with the primary caregiver if they had given a positive response to at least one item of the ESQ . A short, supplementary section was also conducted with the child, where appropriate (see Section 2.3.2.1). I used the Unusual Spell Interview to acquire a more detailed description of the events described in the ESQ. In addition, I used this measure to acquire estimates of seizure onset (in months), length (in seconds), lifetime number (for febrile seizures specifically) and frequency (for unprovoked seizures specifically). 'Frequency' here refers to how often the event occurred when it was at its most frequent- the primary caregiver was asked to select one of four categories, see Section 5.3.6.3). I also asked about a lifetime history of hypocalcaemia in the Unusual Spell Interview. Please see Section 2.3.2 for a more detailed description of the structure and content of the Unusual Spell Interview, the validity and reliability of the original version of the interview and a discussion of the changes I made to the original version.

5.3.3.2 Review of medical records related to unusual spells

Primary caregivers were asked to provide copies of any medical records related to the child's unusual spells. A complete list of the types of medical record I asked the primary caregiver to provide is presented in Table 2-2, within Chapter 2 (see Section 2.3.5).

5.3.3.3 Unusual Spell Diary

In order to obtain a more prospective measure of the frequency of the child's unusual spells, I asked the primary caregiver to record how often they occurred over a two-month period. The Unusual Spell Diary was only given to the primary caregiver if the child was having repeated, stereotyped events occurring within the last year (this was assessed from the data acquired in the Unusual Spell Interview). A detailed description of the information I asked the primary caregiver to provide within the Unusual Spell Diary is presented in Section 2.3.3.

5.3.3.4 Video-recordings of unusual spells

Primary caregivers were asked to provide a copy of any existing recordings of the participant's unusual spells. During the two-month Unusual Spell Diary period, primary caregivers were also asked to record videos of unusual spells on a mobile phone, if they occurred. Further details about this measure are described in Section 2.3.4.

5.3.3.5 24-hour case-control EEG study

Criteria for selection for the case-control EEG study

I conducted a 24-hour ambulatory EEG assessment with young people with 22q11.2DS and their control siblings, in the family home (referred to in previous chapters as the 'case-control EEG study'. As outlined in Section 2.3.7.2, I invited any child for whom I had ESQ data to take part in the EEG assessment, regardless of whether they had screened positive or negative. This was to assess whether epileptiform discharges indexed epilepsy risk in this syndrome, or were a general feature of the cognitive impairment and psychopathology conferred by the deletion[11,12].

Recording procedure

We used wireless recordings, with overnight video-monitoring, for all children in the case-control EEG study (see Section 2.3.7.5 *'Procedure when using wireless recordings, with overnight video-monitoring'* for a description). We used 64-channel Hydrocel Geodesic Sensor Net (HCGSN, Electrical Geodesics Inc.) as our primary method of recording EEG from deletion carriers and control siblings. Please see section 2.3.7.5, '*Procedure when using the 64-channel HCGSN*' for a description of the set-up procedure when using this method. One deletion carrier wore a continuous positive airway pressure (CPAP) mask for sleep apnoea and was therefore unable to wear the HCGSN during the overnight (sleep) recording. For this child, we used the 'traditional clinical method' for the overnight recording. (see Section 2.3.7.5, '*Procedure when using the traditional clinical method'* for a description of the set-up procedure when using this method). This normally involves gluing 21 electrodes to the head, however due the CPAP mask we were only able to use 9 electrodes (F3, F4, CZ, C3, C4, O1, O2, Ground and Reference). This child then subsequently wore the HCGSN for the daytime recording.

5.3.4 Assessment of cognition, psychopathology and indicative DCD

The measures used to assess cognition, psychopathology and indicative DCD have been described in detail in Chapter 2 (see Sections 2.4, 2.5 and 2.6).

5.3.5 Clinical specialist review

A consultant epileptologist reviewed all of the data from the first and second stages of the systematic assessment of epileptic seizures and epilepsy. This review process is described in detail in Section 2.3.8.

5.3.6 Statistical analyses

Statistical analyses were conducted in R version 3.3.3 (https://www.R-project.org/).

For all below analyses, when I describe a participant being 'diagnosed' with an epileptic seizure or epilepsy, I refer to the fact that they were given a 'Yes' or 'Possible' diagnosis by the epileptologist.

5.3.6.1 Testing for bias in the sub-sample of deletion carriers and control siblings

I compared deletion carriers who did take part in the second stage of assessment with those that didn't, across a number of dimensions, in order to test for bias in the sub-sample described in this chapter. I used χ^2 tests (with Fisher's Exact Test when appropriate) to compare gender and the rate of 'any psychiatric diagnosis' (i.e. ADHD and/or anxiety disorder from the Child and Adolescent Psychiatric Assessment[32]). I used independent t-tests to compare mean age and FSIQ. These analyses were repeated to compare siblings who did or didn't take part in the second stage of assessment.

5.3.6.2 Agreement between ESQ-reports and epileptologist diagnoses

For young people with 22q11.2DS, I carried out the following analyses: For the epilepsy diagnosis, febrile seizure and afebrile seizure or paroxysmal event ESQ- constituent variables, I calculated the percentage of individuals who had screened positive and had been assessed with the Unusual Spell Interview. For each of these variables, I then calculated the percentage of those followed up with Unusual Spell Interview who had their ESQ-report confirmed by the epileptologist. For the epilepsy diagnosis variable ,'confirmed' referred to the child being diagnosed with epilepsy, for the febrile seizure variable (in the absence of epilepsy), this referred to the child being diagnosed with a febrile seizure and for the afebrile seizure or paroxysmal event variable (in the absence of epilepsy or a febrile seizure), this referred to the child being diagnosed with an afebrile epileptic seizure. I also calculated the percentage of individuals who had screened negative who were subsequently assessed with EEG, and of those assessed, the percentage showing epileptiform discharges. The same analyses as described above were conducted for the control siblings.

5.3.6.3 Characteristics of epileptic seizures

Analyses of the characteristics of epileptic seizures were conducted on the data from young people with 22q11.2DS only. I used the number of deletion carriers diagnosed with epileptic seizures as the denominator when calculating the percentage for various seizure aetiologies.

Firstly, the percentage of deletion carriers with an acute symptomatic seizure and an unprovoked seizure were calculated (these were not mutually exclusive categories, i.e. both an acute symptomatic

seizure and an unprovoked seizure could occur in the same child). All deletion carriers with acute symptomatic seizures in my sample had febrile seizures. I therefore calculated the percentage of deletion carriers who had febrile seizures and no other acute symptomatic seizure and the percentage who had febrile seizures with other acute symptomatic seizures (these were mutually exclusive categories). With regard to unprovoked seizures, I calculated the percentage of the deletion carriers whose unprovoked seizure was labelled as 'primary genetic' and the percentage labelled as 'structural' by the epileptologist (mutually exclusive categories).

For febrile seizures and unprovoked seizures, I calculated the median age of seizure onset, in months. For deletion carriers with several different types of unprovoked seizure, the earliest age when seizures appeared was used (none of the deletion carriers had different types of febrile seizures). For febrile and unprovoked seizures, I also calculated the median seizure length, in seconds. I used the time of the longest seizure for deletion carriers with seizures of differing lengths. I calculated the median number of febrile seizures across all deletion carriers. For unprovoked seizures, I calculated the percentage of deletion carriers who endorsed each of the four frequency categories (in response to the question '*During the period in [name of child's] life when these events were occurring most frequently, how often did they occur?*') these were: 'less than once a month', 'one to four times a month', 'more than four times a month but less than once a day' and 'once a day or more'.

5.3.6.4 24-hour case-control EEG study

I explored the association between deletion status (22q11.2DS/ control sibling) and epileptiform discharges (present/absent) using a Fisher's Exact Test. A Fisher's Exact Test was also used to explore the association between epileptic seizures (yes/no) and epileptiform discharges (yes/no) in young people with 22q11.2DS.

5.3.6.5 Family history of epileptic seizures and epilepsy in young people with 22q11.2DS

I explored the association between a family history of epileptic seizures and/or epilepsy (present/absent) and whether a deletion carrier was diagnosed with an epileptic seizure (yes/no) using a Fisher's Exact Test.

5.3.7 Association of epileptic seizures, epileptiform discharges and epilepsy with neurodevelopmental problems in 22q11.2DS

In young people with 22q11.2DS, I compared the rate of ADHD, indicative ASD and indicative DCD in people who were and were not diagnosed with any epileptic seizure (by the epileptologist) using χ^2 tests (with Fisher's Exact Test when appropriate). A Mann-Whitney U test was used to explore the

difference in PIQ between these two groups. As a sensitivity analysis, I repeated these tests to compare rates of ADHD, indicative ASD, indicative DCD and PIQ score between deletion carriers who were and were not diagnosed with epilepsy (by the epileptologist). It is important to note that in these analyses, 'negative' cases for epileptic seizures and epilepsy included deletion carriers who screened negative on the ESQ, and those who screened positive but were subsequently diagnosed with non-epileptic events (false-positives). As a further sensitivity analysis, in screen-positive deletion carriers who took part in the second stage of assessment, I repeated these analyses to compare individuals who were and were not diagnosed with non-epileptic events. If deletion carriers with non-epileptic events had a significantly lower performance IQ and higher rates of ADHD, indicative ASD and indicative DCD, this could suggest these individuals were driving the significant association between 'any positive' and these neurodevelopmental outcomes that I observed in Chapter 4. This would subsequently suggest these associations may not be valid.

As mentioned in Section 2.1, the ECHO study has obtained cognitive, psychiatric and other neurodevelopmental data about young people with 22q11.2DS across four different waves of assessment, with an average gap of 2.5 years between waves. Therefore, for each deletion carrier, I used the ADHD, indicative ASD and indicative DCD diagnoses, as well as the PIQ score, from the ECHO wave closest in time to when the second stage of the epilepsy assessment (i.e. Unusual Spell Interview and/or EEG study) was completed. For all of the analyses in this section, I excluded the three deletion carriers who had screened positive on the ESQ, taken part in the 24-hour EEG study but whose primary caregiver was not available for interview (see Section 5.4 below). This is because I had no information about the presentation and semiology of the events they screened positive for, which is crucial for determining whether an event is or is not an epileptic seizure[33].

5.4 Results

In total, 56 young people with 22q11.2DS and 23 control siblings took part in the second stage of assessment. Table 5-1 provides a breakdown of the number of deletion carriers and controls who provided data for each of the measures used within the second stage. The Unusual Spell Interview was unable to be completed with the primary caregivers of three deletion carriers who had reported 'any positive excluding epilepsy' on the ESQ (more specifically, they had only reported seizure-like symptoms) and who took part in the EEG study. In one case the primary caregiver could not remember why they had reported seizure-like symptoms on the ESQ and the other two primary caregivers did not want to take part in the interview. As described in Table 5-1, 19 young people with 22q11.2DS and three control siblings met criteria for repeated, stereotyped events occurring within the last year, based on the data provided in the Unusual Spell Interview, and were subsequently given the Unusual

Spell Diary. However, it is important to note that after the epileptologist had diagnosed each child, only two of the 19 deletion carriers (and no control siblings) had been given the diary for epileptic seizures. The primary caregiver for one of these deletion carriers did not complete the diary and the other deletion carrier did not experience any epileptic seizures during the two-month diary period. As shown in Table 5-1, no video-recordings of unusual spells were obtained.

37 deletion carriers had information available about a lifetime history of hypocalcaemia (i.e. those who took part in the Unusual Spell Interview). The rate of hypocalcaemia in this group was 35% (13/37).

Table 5-1. The number of deletion carriers and control siblings who provided data for each of the measures used within the second stage of assessment.

Measure	22q11.2DS (n=56)	Control siblings (n=23)
Unusual Spell Interview with carer	37	4
Unusual Spell Interview with child	16	1
Unusual Spell Diary	19[a]	3[b]
Medical record	11	0
Video-recordings of unusual spells	0	0
24-hour EEG assessment	44[c]	23[d]
Family history of seizures and epilepsy[e]	37	37

22q11.2DS, 22q11.2 deletion syndrome, EEG, electroencephalography

[a]Of the 19 deletion carriers who met criteria for the Unusual Spell Diary, only two were diagnosed with epileptic seizures by the epileptologist

[b]None of the control siblings who met criteria for the Unusual Spell Diary were diagnosed with epileptic seizures by the epileptologist

[c]28 deletion carriers who took part in the EEG assessment screened positive on the Epilepsy Screening Questionnaire and 16 screened negative

[d]Four control siblings who took part in the EEG assessment screened positive on the Epilepsy Screening Questionnaire and 19 screened negative

[e] In total 37 families were asked about a family history of epileptic seizures and epilepsy (deletion carriers and controls belonged to the same families)

5.4.1 Testing for bias in the sub-sample of deletion carriers and control siblings

Descriptive statistics about the sub-sample of deletion carriers and control siblings who took part in the second stage of assessment, and those that didn't, are presented in Table 5-2. Deletion carriers who took part in the second stage did not differ in terms of gender distribution, mean FSIQ score or the rate of 'any psychiatric diagnosis', from those that didn't take part ($p>0.05$ in all cases). I did however observe that deletion carriers taking part in the second stage were significantly older (14.62 years versus 12.39 years, $p=<0.001$). Control siblings who did or didn't take part in the second stage did not significantly differ in mean age, gender distribution or the rate of 'any psychiatric diagnosis'

(p>0.05 in all cases). Control siblings who took part in the second stage did however have a significantly higher mean FSIQ score (114.05 versus 105.97, p=0.041).

Table 5-2. Descriptive statistics for deletion carriers and control siblings who did or did not take part in the second stage of assessment.

Descriptive statistic	22q11.2DS (n=108)		Control siblings (n=60)	
	Second stage yes (n=56)	**Second stage no (n=52)**	**Second stage yes (n=23)**	**Second stage no (n=37)**
Age: mean (s.d.)	14.62 (3.24)	12.39 (3.02)	13 (3.5)	13.14 (2.99)
Gender: n (%)				
Male	30 (53.6)	32 (61.5)	11 (47.8)	19 (51.4)
Female	26 (46.4)	20 (38.5)	12 (52.2)	18 (48.6)
FSIQ: mean (s.d.)	72.41 (12.94)	70.91 (11.17)	114.05 (13.67)	105.97 (14.30)
Any psychiatric diagnosis: n (%)	24 (42.9)	26 (50)	1 (5.3)	2 (5.8)
22q11.2DS, 22q11.2 deletion syndrome, FSIQ, full-scale IQ				

5.4.2 Agreement between ESQ-reports and epileptologist diagnoses

Table 5-3 presents data from the second stage of assessment for each of the three ESQ-constituent variables used in this chapter: epilepsy diagnosis, febrile seizure and afebrile seizure or paroxysmal event. For each variable, the percentage screening positive is shown, followed by the percentage of those screening positive who were interviewed. Finally, of those interviewed, the percentage whose ESQ-report was 'confirmed' by the epileptologist is shown (see Section 5.3.6.2 for an explanation of what 'confirmed' means for each of the three variables). This data is displayed for young people with 22q11.2DS and control siblings.

In young people with 22q11.2DS, 83.3% (5/6) deletion carriers reported as having an epilepsy diagnosis, who were subsequently interviewed, had their diagnosis confirmed by the epileptologist. The remaining individual did not meet criteria for an epilepsy diagnosis as the epileptologist determined they had only one neonatal unprovoked generalised tonic-clonic seizure (GTCS). Two individuals who were reported with a history of afebrile seizures (Item 2 from the ESQ, see Table 2-1 in Chapter 2), but not an epilepsy diagnosis, were subsequently diagnosed as having a history of epilepsy by the epileptologist during the second stage of assessment. The epilepsy diagnosis for one of these individuals was based on a history of repeated, unprovoked GTCS, reported in the Unusual Spell Interview. Medical records were available for this individual, which suggested that this child had in fact been clinically diagnosed with epilepsy prior to taking part in this study (despite their primary caregiver not reporting this diagnosis in the ESQ or subsequently in the Unusual Spell interview) – 'Epilepsy' was listed as a diagnosis in a child development clinic letter (see Table 5-4). The other individual was diagnosed with epilepsy (by the epileptologist in this study) based on a history of repeated 'staring spells' reported in the Unusual Spell Interview, which the epileptologist labelled as non-motor absence seizures. Medical records were unavailable for this individual, although during the Unusual Spell Interview the primary caregiver reported that they had spoken to a paediatrician about these staring spells, who had described them as 'possible absence seizures'. The primary caregiver reported that clinical MRI and EEG assessments were going to be conducted with the child, but they were never completed as the staring spells stopped. Interestingly, this child showed epileptiform discharges during the 24-hour EEG assessment (see Section 5.4.4 for a description of these abnormalities).

Around ninety-three percent (13/14) of deletion carriers reported as having febrile seizures (without epilepsy) in the ESQ, who were available for interview, had their report confirmed by the epileptologist. In the one remaining participant, fever-related delirium/drowsiness was diagnosed. One deletion carrier who was not reported with a febrile seizure in the ESQ subsequently described a fever-related seizure in the Unusual Spell Interview and was diagnosed with a febrile seizure by the epileptologist in this study.

Around twelve percent (2/17) of deletion carriers who were reported with an afebrile seizure or a paroxysmal event (in the absence of an epilepsy diagnosis or febrile seizure), who were available for interview, were diagnosed with a history of 'possible' absence seizures by the epileptologist. These two cases had only reported a history of 'daydreaming/staring into space more than other children' in the ESQ (Item 5, Table 2-1). Neither of these children had been seen a clinician for these spells. They were subsequently labelled as 'uncertain' with respect to an epilepsy diagnosis. One of these children showed epileptiform discharges during the 24-hour EEG assessment (see Section 5.4.4). The remaining cases (15/17, 88.2%) were diagnosed with non-epileptic events (e.g. behavioural events such as daydreaming). Interestingly, 80% (12/15) of the deletion carriers diagnosed with non-epileptic events had only been reported with paroxysmal events in the ESQ (Items 3-8, Table 2-1).

None of the control siblings reported an epilepsy diagnosis or febrile seizures on the ESQ. All control siblings reported as having an afebrile seizure or a paroxysmal event, and who were available for interview, were diagnosed with non-epileptic events.

Table 5-3. The percentage of ESQ-reported epilepsy, febrile seizures and afebrile seizures or paroxysmal events that were confirmed by the epileptologist after review of the data from the second stage of assessment.

22q11.2DS			
ESQ variable	**n screening positive/total n (%)**	**Interview completed?**	**Diagnosis confirmed by epileptologist? (%)**
ESQ: epilepsy diagnosis	12/108 (11.1)	6/12 (50)	5/6 (83.3)
ESQ: febrile seizure[a]	20/95 (21.1)[b]	14/20 (70)	13/14 (92.9)
ESQ: afebrile seizure or paroxysmal event[c]	37/76 (48.7)	17/37 (54.1)	2/17 (11.8)
Control siblings			
ESQ variable	**n screening positive/total n (%)**	**Interview completed?**	**Diagnosis confirmed by epileptologist? (%)**
ESQ: afebrile seizure or paroxysmal event	8/60 (13.3)	4/8 (50)	0/4 (0)

[a] Excluding cases with an ESQ-reported epilepsy diagnosis

[b] One caregiver did not complete the febrile seizure item in the ESQ

[c] Excluding cases with an ESQ-reported epilepsy diagnosis or a febrile seizure

EEG, electroencephalography, ESQ , Epilepsy Screen Questionnaire.

5.4.3 Characteristics of epileptic seizures

In total, 22 deletion carriers were diagnosed with epileptic seizures and seven of these individuals were diagnosed with epilepsy. Diagnoses were made using information from the Unusual Spell Interview only in 14 deletion carriers, with the remaining 8 deletion carriers additionally providing relevant medical records to support the diagnosis. Table 5-4 provides a breakdown of the available information for each of the 22 deletion carriers diagnosed with epileptic seizures.

Table 5-4. Overview of the 22 deletion carriers diagnosed with epileptic seizures in the second assessment stage.

	ESQ response (item number)	**Diagnosis from second stage of assessment (semiology)**	**Available data for diagnosis**	**Age of onset of seizure**	**Epileptiform discharges observed during EEG recording**	**Structural or functional brain abnormalities reported to the study team (through medical records/ during the USI)**	**Additional information**
Participant							
1	Febrile seizure (1), afebrile seizure, convulsion, fit or spell (2), paroxysmal events (3)	Epilepsy (GTCS), febrile seizures	USI	12 months (febrile seizures), 29 months (epilepsy)	Did not take part in EEG study	No	Review of medical records revealed this deletion carrier had been given a clinical diagnosis of epilepsy, despite not reporting this in the ESQ or the USI

2	Afebrile seizure, convulsion, fit or spell (2), epilepsy diagnosis (9)	Epilepsy (non-motor absences), remote symptomatic seizures secondary to perinatal subdural haematoma (GTCS)	USI	5 months (epilepsy and remote symtopmatic seizures)	Did not take part in EEG study	Yes, perinatal subdural haematoma observed during CT scan (USI)	-
3	Febrile seizure (1), afebrile seizure, convulsion, fit or spell (2), paroxysmal events (3, 4, 5 & 7), epilepsy diagnosis (9)	Epilepsy (GTCS), febrile seizures	USI, medical records	19 months (febrile seizures), 60 months (epilepsy)	Did not take part in EEG study	No	Unprovoked GTCS occurred upon awakening
4	Febrile seizure (1), afebrile seizure, convulsion, fit or spell (2), paroxysmal events (3, 4 & 5)	Epilepsy (non-motor absences), febrile seizures, acute symptomatic seizures after cardiac surgery (focal motor)	USI, EEG recording	Newborn (acute symptomatic seizures), 60 months (epilepsy), 72 months (febrile seizures)	Yes, brief generalised spike-and-slow wave discharges shortly after awakening	No	A paediatrician had previously described the 'staring spell's (which we diagnosed as non-motor absences) as 'possible absence seizures'

5	Afebrile seizure, convulsion, fit or spell (2), paroxysmal events (3, 4 & 5) epilepsy diagnosis (9)	Epilepsy (focal non-motor seizures with impaired awareness, evolving to bilateral tonic-clonic)	USI, EEG recording, medical records	4 months (epilepsy)	No	No	-
6	Febrile seizure (1), afebrile seizure, convulsion, fit or spell (2), paroxysmal events (3, 4, 5 & 7), epilepsy diagnosis (9)	Epilepsy (focal non-motor seizures with impaired awareness, focal non-motor post ictal seizures) febrile seizures	USI	3 months (febrile seizures), 18 months (epilepsy)	Did not take part in EEG study	Yes, right hemisphere polymicrogyria (USI)	-
7	Febrile seizure (1), afebrile seizure, convulsion, fit or spell (2), paroxysmal events (3, 4 & 5), epilepsy diagnosis (9)	Epilepsy (GTCS), acute symptomatic seizure after neonatal hypoxic-ischaemic encephalopathy (focal motor, possibly to bileteral tonic-clonic), febrile seizures	USI, medical records	Newborn (acute symptomatic seizures), 21 months (febrile seizures), 108 months (epilepsy)	Did not take part in EEG study	Neonatal hypoxic-ischaemic encephalopathy (USI and medical records)	GTCS were nocturnal
8	Febrile seizure (1), paroxysmal event (8)	Febrile seizure	USI, EEG recording	48 months (febrile seizure)	No	No	-

9	Febrile seizure (1), afebrile seizure, convulsion, fit or spell (2), paroxysmal events (3)	Febrile seizure, acute symptomatic seizures (GTCS secondary to hypocalcaemia)	USI	96 months (acute symptomatic seizures), 168 months (febrile seizure)	Did not take part in EEG study	No	-
10	Febrile seizure (1)	Febrile seizures	USI, EEG recording	4 months (febrile seizures)	No	No	-
11	Febrile seizure (1)	Febrile seizure	USI, EEG recording	18 months (febrile seizure)	No	No	-
12	Febrile seizure (1), paroxysmal events (3 & 5)	Febrile seizure, possible neonatal seizures	USI, EEG recording, medical records	Newborn (possible neonatal seizures), 9 months (febrile seizure)	No	Mild hypertelorism (medical records)	-

13	Paroxysmal events (5)	Possible unprovoked seizures (non-motor absences), 'uncertain' epilepsy diagnosis	USI, EEG recording, medical records	72 months (possible non-motor absences)	No	No	Possible absence seizures, alternatively may be a manifestation of cognitive slowing, or hesistancy in responding
14	Febrile seizure (1), afebrile seizure, convulsion, fit or spell (2), paroxysmal events (5)	Febrile seizures	USI, EEG recording, medical records	12 months (febrile seizures)	No	No	-
15	Febrile seizure (1)	Febrile seizure	USI	12 months (febrile seizure)	Did not take part in EEG study	No	-
16	Febrile seizure (1)	Febrile seizure	USI	14 months (febrile seizue)	Did not take part in EEG study	No	-
17	Febrile seizure (1), paroxysmal events (3)	Febrile seizure	USI, EEG recording	30 months (febrile seizure)	No	No	-

18	Paroxysmal events (5)	Possible unprovoked seizure (non-motor absences), 'uncertain' epilepsy diagnosis	USI, EEG recording	36 months (possible non-motor absences)	Yes, brief generalised and left-hemisphere spike-and-slow wave discharges during sleep and shortly after awakening	No	-
19	Afebrile seizure, convulsion, fit or spell (2), epilepsy diagnosis (9)	Single unprovoked seizure (GTCS)	USI, EEG recording, medical records	Newborn (single unprovoked seizure)	No	Electrographic seizures during clinical EEG recordings as a newborn	-
20	Afebrile seizure, convulsion, fit or spell (2), paroxysmal events (3)	Febrile seizures	USI, EEG recording	7 months (febrile seizure)	No	No	Did not report febrile seizure in the ESQ, however was diagnosed with a febrile seizure by the epileptologist after review of the USI data

21	Febrile seizure (1), afebrile seizure, convulsion, fit or spell (2), paroxysmal event (5)	Febrile seizure, acute symptomatic seizures (GTCS secondary to hypocalcaemia)	USI, EEG recording	Newborn (acute symptomatic seizures seconary to hypocalcaemia), 24 months (febrile seizure)	No	No	-
22	Febrile seizure (1)	Febrile seizures	USI, medical records	Newborn (febrile seizures)	Did not take part in EEG study	No	-
GTCS, generalised tonic-clonic seizure, USI, Unusual Spell Interview							

Table 5-5 describes the rates of different seizure aetiologies in young people with 22q11.2DS. The rate of acute symptomatic seizures was 77.3% (17/22), with all participants in this group having a febrile seizure. Two of these individuals (9.1%, 2/22) also had hypocalcaemia-induced seizures (GTCS in both cases). Another individual had an acute symptomatic seizure (focal motor) shortly after cardiac surgery (4.5%, 1/22). An additional individual had an acute symptomatic seizure as a neonate (focal motor, possibly to bilateral tonic-clonic) after hypoxic-ischaemic encephalopathy (4.5%, 1/22). The rate of unprovoked seizures was 45.5% (10/22). In 60% of these individuals, no clear structural aetiology was reported and so these were categorised as having a 'primary genetic' origin by epileptologist. In the remaining 40%, unprovoked seizures were linked to structural aetiologies, which included right hemisphere polymicrogyria, perinatal subdural haematoma, neonatal hypoxic-ischaemic encephalopathy and possible post pneumococcal meningitis (information about these structural aetiologies was obtained from the Unusual Spell Interview in three of these four individuals, with the remaining individual providing relevant medical records).

Table 5-5. Rates of different seizure aetiologies in young people with 22q11.2DS.

Seizure aetiology	n/n with epileptic seizures (%)
Acute symptomatic seizure	17/22 (77.3)
Febrile seizure with no other acute symptomatic seizures	13/22 (59.1)
Febrile seizure with other acute symptomatic seizures	4/22 (18.2)
Unprovoked	10/22 (45.5)
Primary genetic	6/22 (27.3)
Structural	4/22 (18.2)
22q11.2DS, 22q11.2 deletion syndrome	

Table 5-6 displays salient characteristics of febrile and unprovoked seizures in deletion carriers. All febrile seizures were GTCS, with a median length of 150 seconds (2.5 minutes). On average febrile seizures began within the first two years of life, although one individual experienced their first febrile

seizure aged 14 years. My sample of young people with 22q11.2DS had on average more than one febrile seizure, with one individual having experienced seven. The vast majority of unprovoked seizures were generalised. The median age of onset was within the first three years of life. On average, unprovoked seizures lasted for 135 seconds (just over two minutes). At their most frequent, unprovoked seizures occurred one-to-four times a month in the majority of deletion carriers. However, a notable proportion of young people with 22q11.2DS experienced unprovoked seizures on at least a daily basis (30%).

Table 5-6. Characteristics of febrile and unprovoked seizures in young people with 22q11.2DS.

Seizure aetiology	n	Seizure semiology (%)		Mean age of onset in months (range)[a]	Median seizure length in seconds[b]	Median number of seizures	Frequency (%)[c]			
		Generalised	Focal				less than once a month	one to four times a month	more than four times a month but less than once a day	once a day or more
Febrile	17	17 (100)	0 (0)	14 (0-168)	150 (10-900)	2 (1-7)	- -	-	-	-
Unprovoked	10	8/10 (80)	2/10 (20)	33.0 (0-108)	135 (3-330)	-	3 (30)	3 (30)	1 (10)	3 (30)

[a] For deletion carriers with multiple types of unprovoked seizure, the earliest age when seizures appeared was used. Within the range, '0' refers to deletion carriers who had their first seizure during the first month of life.

[b]For deletion carriers with seizures of differing lengths, the time of the longest seizure was used. One primary caregiver was unable to provide data about the length of their child's febrile seizure.

[c]Primary caregivers were asked to indicate how often seizures occurred in their child, when they were at their most frequent

5.4.4 Findings from the 24-hour case-control EEG study

44 young people with 22q11.2DS and 23 control siblings took part in the 24-hour EEG study. The overall mean recording length was 13:32:49 (s.d. = 05:08:33), ranging from 02:51:09-22:01:58). Table 5-7 displays descriptive statistics about the recording length separately for deletion carriers and controls, as well as the rate of epileptiform discharges in these two groups. 9.1% (4/44) of deletion carriers who underwent an EEG assessment showed brief generalised spike-and-slow-wave discharges of atypical morphology (control siblings 0%, 0/23 p=0.290). By 'atypical morphology', I refer to the fact that these discharges consisted of only one initial spike, followed by one or several after-going slow waves. By contrast, in a more 'typical' generalised spike-and-slow wave discharge, the spike-wave pattern repeats, usually at 3Hz[34]. These waveforms occurred during sleep and/or shortly after awakening in the morning. Two of the four deletion carriers additionally showed these epileptiform discharges in the left hemisphere, and one deletion carrier had these in their right hemisphere. No clear correlates were seen on the simultaneous video-monitoring (in addition, those deletion carriers showing abnormalities shortly after awakening were away from the camera). Figure 5-1 shows examples of these abnormalities during sleep (top panel) and awakening (bottom panel). These epileptiform discharges were equally likely to occur in deletion carriers who were diagnosed with epileptic seizures by the epileptologist (15.4%, 2/13) and those who were not (or who screened negative on the ESQ, 2/31, 6.5%, p=0.570). None of the deletion carriers or control siblings showed any background abnormalities.

Table 5-7. Results from the 24-hour case-control EEG study.

Deletion status	n	Mean length of recording (s.d.)	Range of recording lengths	Epileptiform discharge (%)
22q11.2DS	44	13:23:25 (05:11:59)	03:26:10-22:01:58	4/44 (9.1)
Control sibling	23	13:50:49 (05:08:00)	02:51:09-20:42:50	0/24 (0)
22q11.2DS, 22q11.2 deletion syndrome, EEG, electroencephalography				

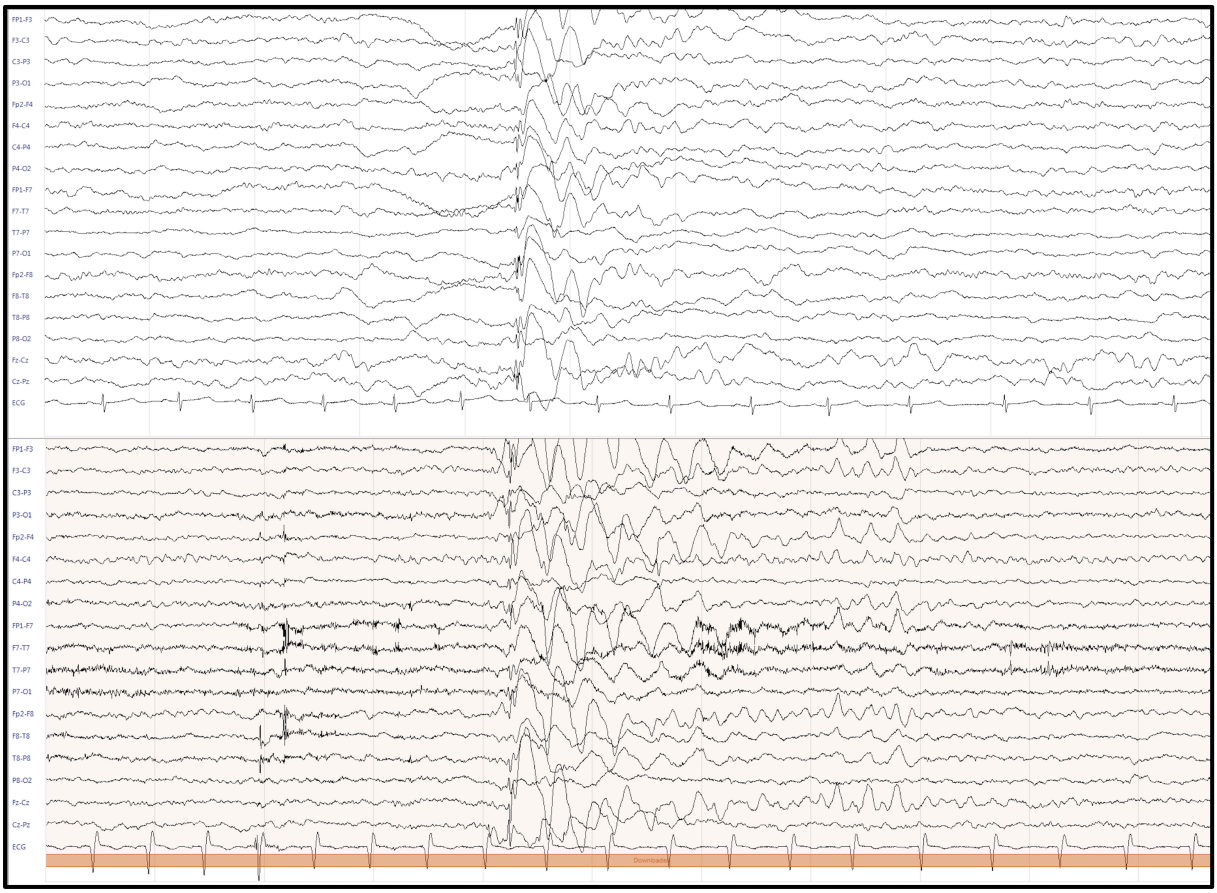

Figure 5-1. Examples of the brief generalised spike-and-slow wave discharges, seen in four young people with 22q11.2DS. Top panel: abnormality occurring during sleep. Bottom panel: abnormality occurring shortly after awakening.

5.4.5 Family history of epileptic seizures and epilepsy in young people with 22q11.2DS

Details about a family history of epileptic seizures and epilepsy were available for 37 young people with 22q11.2DS. Six deletion carriers had a positive family history of seizures or epilepsy. Only one deletion carrier having a first-degree relative with a positive family history. Table 5-8 provides further details about the family history in each of the six deletion carriers.

A greater proportions of deletion carriers with a family history of seizures or epilepsy than without were diagnosed with an epileptic seizure by the epileptologist (50%, 3/6 and 35.7%, 10/28, respectively). However, this difference did not reach significance (p=0.653).

Table 5-8. Details of the family history of epileptic seizures and epilepsy in six young people with 22q11.2DS.

Deletion carrier	Side of family	Relation (to deletion carrier)	Details of history
1	Father's	Great-uncle	Severe epilepsy and passed away whilst having a seizure when young
2	Mother's	Cousin	Diagnosed with epilepsy aged 8
3	Mother's	Aunt	Treated for 'suspected' epileptic seizures associated with amphetamine misuse
4	Mother's	Grandmother	Diagnosed with epilepsy as an adult (2014), MRI revealed congenital polymicrogyria
5	Father's	Uncle	Febrile seizures as a child
6	Mother's	Mother	Possible epileptic seizures (e.g. waking up with a clenched jaw). Abnormal EEG, suggestion from neurologist of treatment with medication, however mother was pregnant at the time so medication was not taken. These events were not clinically followed-up

5.4.6 Associations of epileptic seizures and epilepsy with neurodevelopmental problems in 22q11.2DS

I did not observe any significant differences in the performance IQ and the rates of ADHD, indicative ASD and indicative DCD between deletion carriers with or without any epileptic seizure. (Table 5-9). I also did not find any significant differences in any of these variables between deletion carriers with or without an epilepsy diagnosis. I did however observe a trend toward a greater proportion of deletion carriers diagnosed with epilepsy during the second assessment stage having ADHD (57.1% versus 28.3%, p=0.193) and indicative ASD (85.7% versus 57.1%, p=0.224). Interestingly, the rates of indicative DCD were equal between those who were or were not diagnosed with any epileptic seizure during the second assessment stage (75 versus 75.9%, p=1) but was slightly lower in those diagnosed with epilepsy (71.4% versus 76.2%, p=1). Concordant with these findings, when focusing only on screen-positive deletion carriers and comparing those who were and were not diagnosed with non-epileptic events, I observed a non-significant trend toward a greater proportion of deletion carriers with non-epileptic events having indicative DCD (86.7% versus 75%, p=0.672).

Table 5-9. Details of the family history of epileptic seizures and epilepsy in six young people with 22q11.2DS.

		Any epileptic seizure				
Measure	**n**	**No**	**Yes**	**W**	**OR**	**p-value**
PIQ	53	70	71	347	-	0.921
Neurodevelopmental problem	**n affected/total n**	**No (%)**	**Yes (%)**	**χ^2**	**OR**	**p-value**
ADHD	17/53	9 (29)	8 (36.4%)	0.32	1.39	0.573
Indicative ASD	30/49	17 (60.8)	13 (61.9)	0.01	1.05	0.933
Indicative DCD	37/49	22 (75.9)	15 (75)	-	0.96	1
		Epilepsy diagnosis				
Measure	**n**	**No**	**Yes**	**W**	**OR**	**p-value**
PIQ	53	70	72	158	-	0.948
Neurodevelopmental problem	**n affected/total n**	**No (%)**	**Yes (%)**	**χ^2**	**OR**	**p-value**
ADHD	17/53	13 (28.3)	4 (57.1)	-	3.3	0.193
Indicative ASD	30/49	24 (57.1)	6 (85.7)	-	4.39	0.224
Indicative DCD	37/49	32 (76.2)	5 (71.4)	-	0.79	1
		Non-epileptic event				
Measure	**n**	**No**	**Yes**	**W**	**OR**	**p-value**
PIQ	37	71	69	164	-	0.914
Neurodevelopmental problem	**n affected/total n**	**No (%)**	**Yes (%)**	**χ^2**	**OR**	**p-value**
ADHD	15/37	8 (36.4)	7 (46.7)	0.39	1.5	0.531
Indicative ASD	22/35	13 (61.9)	9 (64.3)	0.02	1.1	0.886
Indicative DCD	28/35	15 (75)	13 (86.7)	-	2.12	0.672

PIQ, performance IQ, ADHD, attention-deficit hyperactivity disorder, ASD, autism spectrum disorder, DCD, developmental coordination disorder

5.5 Discussion

In this second stage of assessment, I sought to confirm the epilepsy diagnoses, febrile seizures and afebrile seizure or paroxysmal events, reported in the ESQ, in a sub-group of my sample of young people with 22q11.2DS and controls siblings. In addition, I aimed to better delineate salient characteristics of epileptic seizures in young people with 22q11.2DS, such as their length and frequency. I also wanted to assess the extent to which a family history of epileptic seizures and epilepsy may confer risk for epileptic seizures in deletion carriers. I therefore completed interviews with the primary caregiver and child, obtained relevant medical-records about these events and asked the primary caregiver to record how often these events occurred in a two-month diary. I also conducted a 24-hour ambulatory EEG assessment with the child and asked the primary caregiver about a family history of epileptic seizures and/or epilepsy. All data from these measures were subsequently reviewed by a consultant epileptologist and consultant neurophysiologist. This study constitutes the first 'direct', systematic assessment of epileptic seizures and epilepsy in young people with 22q11.2DS.

5.5.1 Testing for bias in the sub-sample of deletion carriers and control siblings

I observed that deletion carriers who took part in the second stage of assessment were significantly older (14.62 years) than those that didn't (12.39 years). This may be explained by the fact that some of the measures used in this study would likely have been more difficult to tolerate for younger deletion carriers (e.g. the 24-hour EEG assessment). One limitation of this study may therefore be that I was unable to accurately represent epileptic seizures, epileptiform discharges and epilepsy in some of the younger children with 22q11.2DS. I did not observe any difference in mean FSIQ score and the rate of any psychiatric diagnosis. The cognitive and psychiatric profile of the sub-sample of deletion carriers who did take part in the second stage is therefore representative of my whole sample of young people with 22q11.2DS (described in Chapter 4).

Control siblings who took part in the second stage of assessment had a FSIQ score that on average was 14 points higher than those not taking part (114.05 versus 105.97). This is unlikely to mean that epileptic seizures, epileptiform discharges and epilepsy were underrepresented in the sub-sample of control siblings in this study however. These phenomena show associations intellectual disability (ID, FSIQ <70)27 and none of the control siblings who did not take part in the second stage met criteria for ID.

5.5.2 Agreement between ESQ-reports and epileptologist diagnoses

Around eighty percent (5/6) of deletion carriers reporting an epilepsy diagnosis in the ESQ, who were subsequently available for interview had their diagnosis confirmed by the epileptologist. This suggests that if all individuals with an ESQ-reported epilepsy diagnosis (n=12) had been available for interview, a high rate of agreement between the ESQ-reports and the epileptologist diagnoses would have been likely. In addition, two individuals who did not report a history of an epilepsy diagnosis in the ESQ were diagnosed by the epileptologist in this study. However, medical records were available for one of these individuals and suggested that they may have already received a clinical diagnosis of epilepsy (despite not reporting this in the ESQ), suggesting that the ESQ simply wasn't sensitive for epilepsy in this particular individual. Two further individuals (again with no history of a diagnosis) were labelled with an 'uncertain' epilepsy diagnosis (both diagnosed with recurrent 'possible' absence seizures). These findings suggest that the 11.1% rate of ESQ-reported epilepsy diagnoses in our total sample of young people with 22q11.2DS may actually be a conservative estimate of the true prevalence of epilepsy in this population. Regardless, the 11.1% rate is in line both with prior estimates of epilepsy in people with 22q11.2DS (4.4-36.8%) and in young people with this syndrome specifically (7-15%)[1-4,6,7].

Given the highly novel finding of the 21.1% rate of ESQ-reported febrile seizures in the absence of an epilepsy diagnosis (Chapter 4), I also chose to examine the agreement between the ESQ and epileptologist diagnosis for febrile seizures. The majority of deletion carriers with ESQ-reported febrile seizures were available for interview (14/20, 70%). Of those interviewed, the report of a febrile seizure was confirmed by the epileptologist in all but one case. This means that the rate of febrile seizures I observed in deletion carriers using the ESQ is likely to be accurate. These findings therefore support the conclusion that the 22q11.2 deletion confers significant risk for febrile seizures during childhood. As outlined in section 4.5.1 , the rate of febrile seizures I have observed in 22q11.2DS is considerably higher than in previous studies (2-6%[3-5]). One likely reason for this is the fact that prior studies have been based on historical medical record review, with different medical centres and clinicians varying in the quality and extent of their medical notes. This may have meant that certain cases with febrile seizures were not recorded in medical notes, or notes about a seizure were not sufficiently detailed for reviewers to determine the aetiology as febrile. By contrast, I directly interviewed the parents of deletion carriers with febrile seizures and took notes according to a set of questions that were systematically applied across all cases. By directly interviewing families, I may also have detected cases of febrile seizures which were not seen clinically and therefore would not have been included in medical record reviews.

Agreement between the ESQ and epileptologist diagnoses was poorer for the group of deletion carriers reported with an afebrile seizure or paroxysmal event (in the absence of an epilepsy diagnosis or febrile seizure) on the ESQ. Of those deletion carriers available for interview (n=17), two (11.8%) were diagnosed with 'possible' epileptic seizures by the epileptologist, with the rest having non-epileptic events. The findings from my sub-sample therefore suggest that the majority of deletion carriers reported with an afebrile seizure or paroxysmal event (in the absence of an epilepsy diagnosis and febrile seizure) in the ESQ are having non-epileptic events, rather than epileptic seizures. In addition, all control siblings reported with an afebrile seizure or paroxysmal event in the ESQ, who were available for interview (n=4) were diagnosed with non-epileptic events by the epileptologist. This suggests that the paroxysmal event items have poor specificity for epileptic seizures when used with a population with high rates of cognitive and psychiatric impairment. This is similar to the findings from the validation study of the ESQ, conducted by Ottman et al.[10]; individuals with and without unprovoked seizures were equally likely to respond positively to the paroxysmal event items . However, the paroxysmal event items do appear to increase the sensitivity of the ESQ for epileptic seizures; in my study two deletion carriers who had only screened positive for paroxysmal events on the ESQ (with the events being 'daydreams/stares into space more than other children' in both cases) were diagnosed with possible absence seizures. Similarly, in the Ottman et al. validation study, inclusion of the paroxysmal event items increased the sensitivity of the ESQ for epilepsy patients by 3% (from 91% to 94%)[10].

The methodology used in this chapter (interview, medical record review, diary, EEG, epileptologist diagnosis) allowed me to better assess the reported events in the ESQ. However, it is important to highlight some of the methodological limitations in this chapter. Firstly, the epileptologist did not directly assess each participant. They therefore did not have the freedom to ask salient questions which may have aided the diagnostic process. However, I underwent a significant amount of training in order to be able to ask diagnostically relevant questions and present the epileptologist with a clear, coherent picture of a given unusual spell (e.g. by shadowing epilepsy clinics, see Section 3.3.1). In addition, I used an interview that has been employed in previous studies of individuals with epilepsy and has been shown to provide valid and reliable classification of seizure categories[30,31,35,36]. One potential limitation of the Unusual Spell Interview however is that it has only ever been used to classify epileptic seizures in individuals with epilepsy; it has never been used to collate descriptions of unusual spells in individuals with no history of epilepsy, as in this study. This limitation may be offset by how I obtained medical records (n=11) to corroborate the interview data and how the final diagnoses were made by an epileptologist, who used data from all of the measures in this second stage of assessment.

5.5.3 Characteristics of epileptic seizures

In this direct assessment of seizures and epilepsy in young people with 22q11.2DS, I observed that this population are at an increased risk for both acute symptomatic seizures and unprovoked seizures (77% and 46% of deletion carriers diagnosed with epileptic seizures, respectively). These findings are in line with estimates from medical record reviews in this population[2-6,9].

5.5.3.1 Acute symptomatic seizures

My results suggest that young people with 22q11.2DS are at risk for several different types of acute symptomatic seizures during childhood, predominantly febrile seizures (77.3%), but also hypocalcaemia-induced seizures (9.1%) and those associated with surgery (4.5%) and hypoxic-ischaemic encephalopathy (4.5%). These findings indicate that the 22q11.2 deletion leads to a reduced seizure threshold for many triggers including fever, hypocalcaemia, ischaemia and the impact of prolonged or complex surgical procedures on the central nervous system (CNS). During adulthood, the study by Wither et al. indicated that the main trigger appears to be psychotropic drug use (65.6% of deletion carriers with epileptic seizures%)[4]. Of interest is that the rate of hypocalcaemia-induced seizures I observed in my sample of young people in 22q11.2DS (9.1%) was lower than prior estimates in children with this syndrome (43-67.7% of those with epileptic seizures[3-5]). This may be because of the relatively smaller number of deletion carriers with data about a lifetime history of hypocalcaemia available in this study (n=37) compared to previous medical record reviews (348 in Kao et al.[3]). In addition, the rate of hypocalcaemia in my sample of deletion carriers (35%, 13/37 deletion carriers with this data available) was lower than that typically seen in 22q11.2DS (~50%)[37], which may explain the lower rates of hypocalcaemia-induced seizures I observed. However, the rate of acute symptomatic and unprovoked seizures I observed (77.3% and 45.5%) were similar to those obtained from prior studies(e.g. 68% and 32% of deletion carriers with epileptic seizures in the study by Kao et al., respectively)[3], so perhaps previous medical record reviews have overestimated the prevalence of hypocalcaemia-induced seizures in children with 22q11.2DS.

In Chapter 4, I discussed the need to better characterise the febrile seizure phenotype in 22q11.2DS. Febrile seizures affecting only one side of the body ('complex') or those lasting 30 minutes or more ('febrile status epilepticus) have been associated with substantially increased risk of epilepsy (6-49%), as well as neurological injury and cognitive impairment([21-24]). The febrile seizures characterised in my sub-sample of young people with 22q11.2DS more closely resembled the 'simple' phenotype[23] in that they lasted for 15 minutes or less (on average 2.5 minutes, ranging from 10 seconds-15 minutes) and were all generalised. However, it is important to note that one of the criteria for the 'simple' phenotype in the general population is no previous history of neurologic problems, and these are

common in 22q11.2DS [9,23]. Regardless, my findings suggest that febrile seizures in 22q11.2DS are not complex or prolonged and therefore will be less likely to lead to poor neurodevelopmental outcomes. This conclusion is supported by my findings from Chapter 4, namely that febrile seizures reported in the ESQ showed no significant association with cognition, psychiatric health, sleep quality and motor functioning (see Sections 4.4.2.1 and 4.4.2.2). However, this does not mean that febrile seizures in 22q11.2DS are not important. They can cause significant distress to the child and to primary caregivers (particularly if they have never seen an epileptic seizure before) and slightly increase the risk of developing epilepsy (~2% after a first simple febrile seizure, whereas the prevalence of epilepsy in the general population is 0.5-1%[23,26,38]). In addition, my data suggest that young people with 22q11.2DS may be at particular risk for recurrent febrile seizures; deletion carriers had on average two febrile seizures during their lifetime, with one participant having seven. In the general population a salient risk factor for recurrent febrile seizures is an onset of less than 18 months of age[39]. In my sub-sample of young people with 22q11.2DS, febrile seizures had a median age of onset of 14 months. Importantly, there is evidence to suggest that recurrent febrile seizures significantly increase the risk of afebrile seizures[40]. It is therefore essential that future studies carefully monitor the association of febrile seizures with epilepsy risk and neurodevelopmental trajectories in young people with 22q11.2DS.

In sections 1.7.3.1 and 4.5.1, I discussed how the elevated rate of hypocalcaemia in 22q11.2DS may also increase the risk of febrile seizures in 22q11.2DS, namely by increasing neuronal excitability[41] and potentially lowering the seizure threshold. In this study, I observed that 35.3% (6/17) of deletion carriers diagnosed with febrile seizures during the second stage of assessment had hypocalcaemia. Low calcium may predispose to febrile seizures in 22q11.2DS, but my findings suggest there may be other, perhaps more salient risk factors, which future studies should aim to better elucidate.

5.5.3.2 Unprovoked seizures

The majority (60%) of unprovoked seizures in this sample of young people with 22q11.2DS were 'primary genetic' (i.e. thought to be a direct result of the 22q11.2 deletion). In addition, the vast majority were generalised (80%). This is in line with previous findings, which observed a strong association between 22q11.2DS and GGE[2-4,6,15]. Interestingly, my finding of a greater proportion of generalised than focal seizures contrast with those of Mudigoudar et al.[9] , who reviewed all published cases of unprovoked seizures and found that focal epilepsy was the most common epilepsy phenotype in 22q11.2DS, (44%, 39/88), followed by GGE (27%, 24/88). This discrepancy may be due to the relatively small number of patients with unprovoked seizures in my study (10), compared to Mudigoudar's review (88).

In this study, I was also interested in better delineating the length and frequency of unprovoked seizures in young people with 22q11.2DS, given the association of these seizure characteristics with poor developmental outcomes[16,17,42]. Unprovoked seizures lasted on average for just over two minutes in my sample of deletion carriers. In convulsive status epilepticus, the time point after which seizures are considered to be abnormally prolonged (and treatment should be initiated to end the seizure) is five minutes. The time point after which convulsive seizures may lead to serious long-term developmental consequences, such as neuronal injury and death, is 30 minutes[16]. On average then, the young people with 22q11.2DS in this study did not meet either of these two criteria. Only two participants would be categorised as having abnormally prolonged seizures; one participant had a GTCS lasting for five minutes and another had a focal to bilateral tonic-clonic seizure lasting for five-and-a-half minutes. The frequency of unprovoked seizures in deletion carriers was variable; in the majority, seizures occurred one-to-four times a month, or less than once a month, when they were at their most frequent. In around a third of deletion carriers with unprovoked seizures however (3/10), they were occurring once a day or more when at their most frequent. These three individuals were having focal motor seizures (post-ictal left-sided numbness upon awakening), possible non-motor absences and GTCS seizures with non-motor absences, respectively. In the general population, having weekly seizures significantly increases the risk of developing drug-resistant epilepsy[18]. The three deletion carriers with daily unprovoked seizures in this study may therefore be at significant risk of a more severe epilepsy phenotype. One limitation of my data on the length and frequency of unprovoked seizures is that they were acquired through the Unusual Spell Interview, meaning primary caregivers had to provide retrospective estimates if seizures were no longer ongoing. To overcome this, future studies could use a seizure diary, in which primary caregivers record how often seizures occur, in addition to the length of each seizure. I used a two-month Unusual Spell Diary in this study, however, only two individuals with unprovoked seizures met criteria for the diary. In one case no seizures (nocturnal GTCS) occurred over the two-month period and in the other (possible non-motor absence seizures) the diary was not returned. To avoid these issues, future studies could ask families to complete the diary over a longer period (e.g. six months) to increase the chances of recording seizures, although this may also increase the risk of a family not completing the diary. This may be overcome through offering incentives to families for completing the diary (e.g. vouchers).

In this study, unprovoked seizures had a median age of onset of 33 months. This finding conforms with estimates of the average age of onset of unprovoked seizures in medical record reviews of young people with 22q11.2DS, which range from 8 months to 5.99 years[2,3]. When considered alongside the median age of onset of febrile seizures observed in my study (14 months), these findings suggest that young people with 22q11.2DS are at significant risk for both acute symptomatic and unprovoked

seizures during the early years of life. Young children diagnosed with 22q11.2DS should arguably be closely monitored for epileptic seizures by clinicians from early in life. In addition, young children presenting with febrile seizures and/or unprovoked seizures, in combination with congenital heart defects, palatal abnormalities and developmental delay should arguably be referred for genetic testing for the 22q11.2 deletion.

5.5.4 Findings from the 24-hour case-control EEG study

I did not observe any background EEG abnormalities in my sample of young people with 22q11.2DS. To some extent, this contrasts with findings from prior EEG studies in deletion carriers, which have observed focal, generalised and diffuse slowing, particularly in those with epilepsy[1-4,6]. One possible explanation for this discrepancy is that many of the deletion carriers in my study with a more severe neurological phenotype did not take part in the EEG assessment. For example, I had EEG data for only 28.6% (2/7) of deletion carriers diagnosed with epilepsy, although all were asked to take part in the EEG assessment. In addition, none of the deletion carriers diagnosed with unprovoked seizures with a structural aetiology took part in the EEG assessment (all were invited to take part). Despite these limitations, evidence does suggest that background abnormalities can be relatively uncommon in 22q11.2DS: in some previous studies the majority of deletion carriers with epileptic seizures did not show background abnormalities[2,4] (however, see Daniella Andrade and colleague's EEG findings from 19 adults with 22q11.2DS[1]).

I did however observe epileptiform discharges in 9.1% of deletion carriers, although the rate was not significantly elevated relative to the control siblings (0%, p=0.290). These were brief generalised spike-and-slow discharges of atypical morphology, occurring during sleep and/or shortly after awakening. As described in the results section 'atypical morphology' refers to the way in which these waveforms were composed of an initial spike, followed by one or several after-going slow waves (Figure 5-1). By contrast, in typical generalised spike-and-slow-wave discharges, the spike-wave pattern repeats, usually at 3Hz. One possible explanation for the unusual morphology is that some of these waveforms I observed may be 'epileptiform K-complexes', which are composed of an initial spike, followed by a K complex. K-complexes are normal sleep phenomena which are thought to be responsible for suppressing arousal responses to non-threatening stimuli[34]. They typically occur during lighter sleep stages (Stage 2), but can be seen in deep slow wave sleep. Interestingly, examination of the background activity seen when these atypical spike-wave discharges occurred suggested the deletion carriers were lighter sleep stages, although it is important to note I did not formally score sleep stages. Epileptiform K-complexes are thought to be symptomatic of a broader dysfunction in which epileptiform activity is elicited in response to arousal, known as 'dyshormia', a term coined by Ernst

Niedermeyer[43]. This may explain why two individuals also displayed similar waveforms in the morning, shortly after awakening, which is a period of post-sleep drowsiness and sleep-wake transition. Importantly, epileptiform K-complexes are a common feature of GGE, a type of epilepsy the 22q11.2 deletion is thought to be strongly associated with[2-4,6,9,15]. This discussion about the reason for the atypical nature of these waveforms is at present speculative, however. Future studies could attempt to test some of these claims, for example experimenters could produce a noise whilst deletion carriers are in stage 2 sleep and observe whether the subsequent K-complex is preceded by a spike.

In this study, epileptiform discharges were not elevated in deletion carriers diagnosed with epileptic seizures relative to those who were not (p=0.570). These findings suggest that epileptiform discharges may not index epilepsy risk in 22q11.2DS and instead may simply be associated with the impaired cognition and psychopathology conferred by the deletion. However, I did observe a descriptive trend toward a greater proportion of deletion carriers with epileptic seizures having these epileptiform discharges (15.4%, 2/13), than those without epileptic seizures (6.5%, 2/31). The non-significant difference could be affected by lack of power caused by the relatively small number of deletion carriers showing epileptiform discharge in this study (n=4). As mentioned previously, I was only able to acquire EEG recordings from 2/7 deletion carriers diagnosed with epilepsy during the second stage of assessment. For the remaining five individuals, reasons given by the primary caregiver for the child not taking part in EEG were often that they would poorly tolerate this measure due to ongoing cognitive, behavioural and psychiatric difficulties. Although there were no significant differences in the cognitive or psychiatric profile between all deletion carriers who took part in the second assessment and those that didn't see (Section 5.5.1), there may have been a selection bias towards a milder phenotype in the EEG study specifically, particularly in those reporting an epilepsy diagnosis. Several of the five individuals with epilepsy who were interviewed by did not take part in EEG may have shown epileptiform discharges. Future studies should explore the rate of epileptiform discharges in a larger sample of individuals with 22q11.2DS and should attempt to conduct EEG recordings with as many screen-positive individuals as possible. In addition, longitudinal EEG and epilepsy assessments may help to better elucidate whether early epileptiform discharges predict future risk of epileptic seizures in people with 22q11.2DS.

One potential limitation of the review procedure for the EEG data in this study is that I was responsible for screening the EEG traces, with the epileptologist only reviewing those cases that I identified as potentially containing an abnormality. It is possible therefore that I may have missed salient background and epileptiform abnormalities in the children. However, I mitigated this risk through the extensive training in clinical EEG review that I undertook prior to this study (see Section 3.3.2). In addition, as outlined in Section 2.3.8, at the beginning of the EEG review process the epileptologist

and I together reviewed the entire EEG trace for five deletion carriers and five sibling controls. The epileptologist subsequently allowed me to screen the EEG data after he was confident in my ability to accurately detect normal variants, background abnormalities and epileptiform discharges. Despite this however, clinical EEG review is a complex process and requires many years of training to become proficient in, so future studies should have an EEG technician, neurophysiologist or epileptologist to review all of the EEG data in full.

Another potential limitation is that on average I was unable to record EEG for the full 24-hour period in both deletion carriers (where the mean recording length was around 13.5 hours) and control siblings (where the mean recording length was around 14 hours), meaning epileptiform discharges may have been missed. However, on average the length of EEG recordings for both deletion carriers and controls would have captured the sleep period and the wake-sleep/sleep-wake transitions in the morning and evening, when epileptiform discharges are particularly likely to occur[44].

5.5.5 Family history of epileptic seizures and epilepsy in young people with 22q11.2DS

No significant association was observed between a positive family history and the presence of epileptic seizures and/or epilepsy and/or epileptiform discharges in deletion carriers. This contrasts with findings in the general population, in which a family history is known to associate with an increased risk of epilepsy and abnormal EEG findings[25]. In this sample I had data about family history in a sub-set of deletion carriers (37/108) and only six deletion carriers reported a positive family history, so my findings may be affected by a lack of power. In addition, only one deletion carrier reported a positive family history from a first-degree relative. Interestingly, I did observe a non-significant trend toward a greater percentage of those with a positive family history being diagnosed with epileptic seizures by the epileptologist. One possible future direction would be to collect family history data on all 108 deletion carriers, in order to better assess whether a positive family history confers additional risk for epilepsy in 22q11.2DS.

5.5.6 Associations of epileptic seizures, epileptiform discharges and epilepsy with neurodevelopmental problems in 22q11.2DS

In this chapter, I did not observe any associations between any epileptic seizures and PIQ, ADHD, indicative ASD and indicative DCD in young people with 22q11.2DS. In addition, these neurodevelopmental variables did not show significant relationships with an epilepsy diagnosis in this population. This study therefore fails to replicate the associations of the ‘any positive’ ESQ summary variable with lower PIQ scores, and higher rates of ADHD, indicative ASD and indicative DCD, that I

observed in Chapter 4. Importantly however, I did observe trends toward a greater proportion of deletion carriers diagnosed with epilepsy having ADHD and indicative ASD than those not diagnosed. This may suggest that the significant associations of 'any positive' on the ESQ with ADHD and indicative ASD, observed in Chapter 4, are the most likely to be valid (i.e. reflect an association with true positives). In addition, in this chapter I observed that a greater proportion deletion carriers diagnosed with non-epileptic events during the second assessment stage had indicative DCD, although this trend did not reach significance. One the one hand, this may suggest that the association between 'any positive' on the ESQ and indicative DCD, observed in Chapter 4, is driven by screen-positive individuals who are actually having non-epileptic events (false-positives). However, it is important to note that the rate of indicative DCD was also very high (75%) in deletion carriers who not diagnosed with non-epileptic events (i.e. who were diagnosed with epileptic seizures). From the findings in this chapter, it therefore remains unclear whether the true-positives or false-positive are driving the significant association of 'any positive' with indicative DCD. Due to the small sample sizes (e.g. 22 deletion carriers were diagnosed with epileptic seizures, seven diagnosed with epilepsy and 15 were only diagnosed with non-epileptic events), these analyses are underpowered to address the question of the validity of the significant overlap between 'any positive', PIQ, a ADHD, indicative ASD and DCD, observed in Chapter 4. Future studies of larger number of deletion carriers diagnosed with epileptic seizures and epilepsy are required to better explore the association of these phenomena with neurodevelopmental trajectories and better assess the validity of the relationships I observed in Chapter 4. One immediate future direction would be to ensure all individuals screening positive on the ESQ take part in the second stage of assessment. Until larger studies are conducted, the associations of the 'any positive' ESQ summary variable with poorer neurodevelopmental outcomes should be interpreted with caution.

In conclusion then, the findings from this chapter reinforce that young people with 22q11.2DS are at an increased risk for epileptic seizures and epilepsy. They suggest that the rate of reported epilepsy I observed in Chapter 4 is accurate. They provide some support to the idea that epileptic seizures may be missed in some deletion carriers during routine clinical care, and an epilepsy diagnosis may be overlooked, although not in as many individuals as the findings from Chapter 4 initially suggested. Notably, these findings suggest that the majority of individuals reported with an afebrile seizure or paroxysmal event in Chapter 4 (in the absence of epilepsy or a febrile seizure) are having non-epileptic events. In addition, further investigation is warranted for those deletion carriers diagnosed with 'possible' epileptic seizures in this second stage of assessment, for example through direct assessment by an epileptologist. My findings reinforce that the 22q11.2 deletion confers significant risk for acute symptomatic seizures in childhood, mainly febrile seizures, suggesting a reduced seizure threshold in

deletion carriers. Whilst prevalent, these febrile seizures do not present with 'complex' features and do not meet criteria for febrile status epilepticus. They are therefore are less likely to associate with severe developmental impairment. Despite this, febrile seizures in 22q11.2DS should be closely monitored as they are recurrent and generally start within the first 18 months of life, potentially increasing the risk of subsequent afebrile seizures. The findings from this study also support an increased risk for unprovoked seizures in 22q11.2DS, notably generalised seizures with a genetic aetiology. The unprovoked seizures in my sample did not meet criteria for status epilepticus, but in some deletion carriers they were highly frequent, potentially conferring risk for a more severe epilepsy phenotype and therefore warranting close attention from clinicians. I did not observe significant associations of epileptic seizures and epilepsy with PIQ, ADHD, indicative ASD and indicative DCD in young people with 22q11.2DS, although trends toward a greater proportion of deletion carriers with epilepsy having ADHD and indicative ASD were observed. In addition, I observed a non-significant trend toward a greater proportion of deletion carriers with non-epileptic events having indicative DCD. Future research should attempt to incorporate all deletion carriers screening positive on the ESQ into the second stage of assessment, to better assess the validity of the relationships of the 'any positive' ESQ summary variable with poorer neurodevelopmental outcomes, that were observed in Chapter 4. Further studies are also required to assess whether the epileptiform discharges observed here associate with epilepsy risk in young people with 22q11.2DS, as well as whether a family history of epileptic seizures and/or epilepsy confers significant risk for epileptic seizures in this syndrome.

5.6 References

1 Andrade, D. M., Krings, T., Chow, E. W., Kiehl, T.-R. & Bassett, A. S. Hippocampal malrotation is associated with chromosome 22q11. 2 microdeletion. *The Canadian Journal of Neurological Sciences* **40**, 652-656 (2013).

2 Kim, E.-H. *et al.* Epilepsy and Other Neuropsychiatric Manifestations in Children and Adolescents with 22q11. 2 Deletion Syndrome. *Journal of Clinical Neurology* **12**, 85-92 (2016).

3 Kao, A. *et al.* Increased prevalence of unprovoked seizures in patients with a 22q11. 2 deletion. *American Journal of Medical Genetics Part A* **129**, 29-34 (2004).

4 Wither, R. G. *et al.* 22q11. 2 deletion syndrome lowers seizure threshold in adult patients without epilepsy. *Epilepsia* (2017).

5 Ryan, A. *et al.* Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *Journal of Medical Genetics* **34**, 798-804 (1997).

6 Strehlow, V. *et al.* Generalized Epilepsy and Myoclonic Seizures in 22q11. 2 Deletion Syndrome. *Molecular Syndromology* **7**, 239-246 (2016).

7 Olsen, L. *et al.* Prevalence of rearrangements in the 22q11.2 region and population-based risk of neuropsychiatric and developmental disorders in a Danish population: a case-cohort study. *The Lancet Psychiatry*, doi:https://doi.org/10.1016/S2215-0366(18)30168-8.

8 Pohlmann-Eden, B., Beghi, E., Camfield, C. & Camfield, P. The first seizure and its management in adults and children. *BMJ: British Medical Journal* **332**, 339 (2006).

9 Mudigoudar, B., Nune, S., Fulton, S., Dayyat, E. & Wheless, J. W. Epilepsy in 22q11. 2 Deletion Syndrome: A Case Series and Literature Review. *Pediatric Neurology* (2017).

10 Ottman, R. *et al.* Validation of a brief screening instrument for the ascertainment of epilepsy. *Epilepsia* **51**, 191-197 (2010).

11 Niarchou, M. *et al.* Psychopathology and cognition in children with 22q11. 2 deletion syndrome. *The British Journal of Psychiatry* **204**, 46-54 (2014).

12 Schneider, M. *et al.* Psychiatric disorders from childhood to adulthood in 22q11. 2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11. 2 Deletion Syndrome. *American Journal of Psychiatry* (2014).

13 Chez, M. G. *et al.* Frequency of epileptiform EEG abnormalities in a sequential screening of autistic patients with no known clinical epilepsy from 1996 to 2005. *Epilepsy & Behavior* **8**, 267-271 (2006).

14 Hughes, J. R., DeLeo, A. J. & Melyn, M. A. The Electroencephalogram in Attention Deficit–Hyperactivity Disorder: Emphasis on Epileptiform Discharges. *Epilepsy & Behavior* **1**, 271-277, doi:https://doi.org/10.1006/ebeh.2000.0073 (2000).

15 Lal, D. *et al.* Burden Analysis of Rare Microdeletions Suggests a Strong Impact of Neurodevelopmental Genes in Genetic Generalised Epilepsies. (2015).

16 Trinka, E. *et al.* A definition and classification of status epilepticus–Report of the ILAE Task Force on Classification of Status Epilepticus. *Epilepsia* **56**, 1515-1523 (2015).

17 Leidy, N. K., Elixhauser, A., Vickrey, B., Means, E. & Willian, M. Seizure frequency and the health-related quality of life of adults with epilepsy. *Neurology* **53**, 162-162 (1999).

18 Sillanpää, M. & Schmidt, D. Early seizure frequency and aetiology predict long-term medical outcome in childhood-onset epilepsy. *Brain* **132**, 989-998 (2009).

19 Waruiru, C. & Appleton, R. Febrile seizures: an update. *Archives of Disease in childhood* **89**, 751-756 (2004).

20 Verity, C. M., Greenwood, R. & Golding, J. Long-term intellectual and behavioral outcomes of children with febrile convulsions. *New England Journal of Medicine* **338**, 1723-1728 (1998).

21 Lewis, D. V. *et al.* Hippocampal sclerosis after febrile status epilepticus: the FEBSTAT study. *Annals of neurology* **75**, 178-185 (2014).

22 Martinos, M. M. *et al.* Early developmental outcomes in children following convulsive status epilepticus: a longitudinal study. *Epilepsia* **54**, 1012-1019 (2013).

23 Graves, R. C., Oehler, K. & Tingle, L. E. Febrile seizures: risks, evaluation, and prognosis. *American family physician* **85** (2012).

24 Martinos, M. M. *et al.* Recognition memory is impaired in children after prolonged febrile seizures. *Brain* **135**, 3153-3164 (2012).

25 Babtain, F. A. Impact of a family history of epilepsy on the diagnosis of epilepsy in southern Saudi Arabia. *Seizure* **22**, 542-547, doi:https://doi.org/10.1016/j.seizure.2013.04.002 (2013).

26 Rai, D. *et al.* Epilepsy and psychiatric comorbidity: A nationally representative population-based study. *Epilepsia* **53**, 1095-1103 (2012).

27 Kirby, A. *et al.* Benign childhood epilepsy with centrotemporal spikes (BECTS) and developmental co-ordination disorder. *Epilepsy & Behavior* **72**, 122-126 (2017).

28 Bowley, C. & Kerr, M. Epilepsy and intellectual disability. *Journal of Intellectual Disability Research* **44**, 529-543 (2000).

29 Parisi, P., Moavero, R., Verrotti, A. & Curatolo, P. Attention deficit hyperactivity disorder in children with epilepsy. *Brain and Development* **32**, 10-16 (2010).

30 Ottman, R., Hauser, W. A. & Stallone, L. Semistructured interview for seizure classification: agreement with physicians' diagnoses. *Epilepsia* **31**, 110-115 (1990).

31 Ottman, R. *et al.* Reliability of seizure classification using a semistructured interview. *Neurology* **43**, 2526-2526 (1993).

32 Angold, A. *et al.* The child and adolescent psychiatric assessment (CAPA). *Psychological medicine* **25**, 739-753 (1995).

33 Smith, P. The bare essentials: epilepsy. *Practical neurology* **8**, 195-202 (2008).

34 Britton, J. W. *et al.* (American Epilepsy Society, Chicago, 2016).

35 Phenome, E. & Consortium, E. K. De novo mutations in epileptic encephalopathies. *Nature* **501**, 217-221 (2013).

36 Shain, C. *et al.* Polymicrogyria-associated epilepsy: A multicenter phenotypic study from the Epilepsy Phenome/Genome Project. *Epilepsia* **54**, 1368-1375 (2013).

37 McDonald-McGinn, D. M. *et al.* 22q11. 2 deletion syndrome. *Nature Reviews Disease Primers* **1**, 15071 (2015).

38 Sanders, J. & Shorvon, S. Epidemiology of the epilepsies. *J Neurol Neurosurg Psychiatry* **61**, 433-443 (1996).

39 Berg, A. T. *et al.* Predictors of recurrent febrile seizures: a prospective cohort study. *Archives of pediatrics & adolescent medicine* **151**, 371-378 (1997).

40 Verity, C. & Golding, J. Risk of epilepsy after febrile convulsions: a national cohort study. *Bmj* **303**, 1373-1376 (1991).

41 Han, P., Trinidad, B. J. & Shi, J. Hypocalcemia-induced seizure: demystifying the calcium paradox. *ASN neuro* **7**, 1759091415578050, doi:10.1177/1759091415578050 (2015).

42 Sillanpää, M. *et al.* Incidence of Febrile Seizures in Finland: Prospective Population-Based Study. *Pediatric Neurology* **38**, 391-394, doi:https://doi.org/10.1016/j.pediatrneurol.2008.02.006 (2008).

43 Niedermeyer, E. Epileptiform K complexes. *American journal of electroneurodiagnostic technology* **48**, 48-51 (2008).

44 Matos, G., Andersen, M. L., do Valle, A. C. & Tufik, S. The relationship between sleep and epilepsy: evidence from clinical trials and animal models. *Journal of the neurological sciences* **295**, 1-7 (2010).

6 General discussion

6.1 Overview

Epileptic seizures and epilepsy are highly salient neurological disturbances that are associated with poor neurodevelopmental outcomes. Case reports and larger studies have sought to explore the prevalence of epileptic seizures and epilepsy in 22q11.2 deletion syndrome (22q11.2DS), but these have primarily relied on historical medical record review, rather than first-hand assessment[1-10]. Differences between clinicians and medical centres in clinical documentation and diagnostic systems may mean that estimates of the prevalence of epileptic seizures and epilepsy in this population are less than accurate. Crucially, these reviews may have failed to account for seizures that have not yet been clinically detected, for example brief, non-convulsive seizures such as absences. These seizures normally have to occur several times before the affected individual and their family become concerned and seek clinical help[11]. In addition, there has been very little research into the associations of epileptic seizures and epilepsy with neurodevelopmental outcomes in 22q11.2DS[2,12]. In this thesis, I sought to go beyond the limitations of a medical record review approach to conduct a systematic, 'first-hand' assessment of epileptic seizures and epilepsy in a cohort of young people with 22q11.2DS. To do this, 108 young people with 22q11.2DS were recruited from the Experiences of Children with Copy Number Variants (ECHO) study. As a control group, 60 unaffected biological siblings, closest in age to the person with 22q11.2DS, were also recruited. I used the following measures with this sample:

First stage of the assessment of epileptic seizures and epilepsy (Chapter 4, deletion carriers = 108, control siblings =60):

- Epilepsy Screen Questionnaire (ESQ)[13], to assess the lifetime history of an epilepsy diagnosis, seizures and paroxysmal events (behaviours which could be clinical manifestations of unrecognised seizures). This was completed by the primary caregiver.

Second stage of the assessment of epileptic seizures and epilepsy (Chapter 5, deletion carriers = 56, control siblings =23):

- Unusual Spell Interview[14,15] to acquire further detailed information about the events reported in the ESQ. This was completed with the primary caregiver. A supplementary section was also conducted with the child, where appropriate (see Section 2.3.2.1).
- Review of any medical records related to the events reported in the ESQ.

- Review of any video-recording of the events reported in the ESQ (however, none were provided)
- I also explored the possibility of obtaining further information about seizure frequency by asking the primary caregivers to complete the Unusual Spell Diary over a two-month period.
- 24-hour ambulatory EEG assessment with the children, in the family home (the 'case-control EEG study'). I invited deletion carriers and control siblings who screened positive or negative on the ESQ to take part, so that I could compare the brain activity of children with and without epileptic seizures and assess to what extent epileptiform discharges were associated with epilepsy risk in 22q11.2DS (however, see Section 2.3.7.2 for the potential pitfalls of interpreting epileptiform discharges in individuals with neurodevelopmental disorders).
- Review of family history of epileptic seizures or epilepsy (primary caregiver report).

All of the data from these measures was then reviewed by a consultant epileptologist (with a consultant neurophysiologist providing additional support to the EEG review process), who made diagnoses of epilepsy, epileptic seizures and epileptiform discharges according to the most recent International League Against Epilepsy guidelines[16-18].

The ECHO study seeks to phenotype the developmental outcomes of young people with 22q11.2DS (see Section 2.1). Given this, I was also able to explore the association of epileptic seizures and epilepsy with the following neurodevelopmental domains (the results of which are described across Chapters 4 and 5):

- Cognition. The child completed the Weschler Abbreviated Scale of Intelligence (WASI[19]), which provided me with estimates of the full-scale IQ (FSIQ), verbal IQ (VIQ) and performance IQ (PIQ).
- Psychopathology. The primary caregiver (and where appropriate, the child) completed the semi-structured Child and Adolescent Psychiatric Assessment (CAPA) Interview[20]. This provided me with information about the rates of DSM-IV-TR psychiatric disorder[21] (ADHD and anxiety disorder) in my sample. The Social Communication Questionnaire (SCQ)[22] was used to screen for autism spectrum disorder (ASD) and was completed by the primary caregiver.
- Sleep disturbance. The sleep disturbance section of the CAPA was used to screen for sleep problems (e.g. insomnia and somnambulism).
- Problems with motor coordination. The Developmental Coordination Disorder Questionnaire[23,24] was used to screen for Developmental Coordination Disorder and was completed by the primary caregiver .

In Chapter 4, I found that significantly more deletion carriers than control siblings screened positive to at least one item in the ESQ ('any positive', 63.9% and 13.3%, respectively, $p<0.001$). Whilst 11.1% of deletion carriers were reported as having an epilepsy diagnosis, nearly half (48.7%) were reported as having an afebrile seizure, convulsion fit or spell or paroxysmal events in the absence of an epilepsy diagnosis or a febrile seizure. These findings suggested that epilepsy may be under-diagnosed in some young people with 22q11.2DS. Further systematic assessment of these events was warranted to determine whether they represented 'true' epileptic seizures. I also observed that 21.1% of deletion carriers were reported as having febrile seizures (in the absence of an epilepsy diagnosis, 24.3% when cases with epilepsy were included), which provided support to the idea that the 22q11.2 deletion leads to a reduced seizure threshold. Finally, I found that young people with 22q11.2DS with 'any positive' had significantly lower PIQ and higher rates of attention-deficit hyperactivity disorder (ADHD), indicative autism spectrum disorder (ASD) and indicative developmental coordination disorder (DCD). This may suggest common neurobiological risk pathways for these deficits, one of which may be aberrant synaptic plasticity[25-29]. Additionally or alternatively, these associations may be explained through deleterious effects of epileptic seizures on cognitive, psychiatric and motor development in 22q11.2DS[25,30,31]. However, it is important to highlight that these associations with neurodevelopmental problems were found using an epilepsy screening questionnaire. The broad nature of the paroxysmal event items may have led to false-positives (e.g. behaviours associated with the impaired cognition and/or psychopathology in 22q11.2DS[32]). Please see Section 6.2.3 below for a full discussion.

In Chapter 5, I found that 5/6 of the deletion carriers reported with epilepsy in the ESQ were confirmed as having epilepsy by the epileptologist during the second assessment stage, suggesting the 11.1% of ESQ-reported epilepsy diagnoses is accurate. Interestingly, two deletion carriers who were not reported with epilepsy in the ESQ were subsequently diagnosed with epilepsy by the epileptologist, although in one case medical record review suggested they had in fact been given a clinical diagnosis of epilepsy prior to taking part in the study. In addition, two of the deletion carriers who only reported paroxysmal events on the ESQ were subsequently newly diagnosed with 'possible' non-motor absence seizures by the epileptologist during the second assessment stage. I therefore provide some evidence in this thesis that epileptic seizures may not be recognised in some young people with 22q11.2DS during routine clinical care and that an epilepsy diagnosis may be overlooked. This conclusion is limited by the 'possible' nature of of some of these diagnoses, brought about by the limitations inherent in the epileptologist 'remotely' assessing epilepsy (i.e. through data that I had collated). Further direct epileptologist assessment is therefore warranted to confirm these diagnoses. It is also important to note that remaining 88.2% of deletion carriers who had screened positive on the afebrile

seizure or paroxysmal event ESQ-variable (in the absence of an epilepsy diagnosis or a febrile seizure) were diagnosed with non-epileptic events (15/17, 88.2%). This suggests that the 'any positive' screen definition on the ESQ may be sensitive, but not specific, for epileptic seizures in 22q11.2DS. In Chapter 5, the epileptologist confirmed the ESQ-reported febrile seizures in all but one case (of those taking part in the second stage of assessment). This suggests that the 21.1% rate for febrile seizures (in the absence of an epilepsy diagnosis, 24.3% when epilepsy cases were included) that I observed in Chapter 4 was accurate and provides further evidence to support an increased risk for febrile seizures in young people with 22q11.2DS. Whilst these febrile seizures did not present with 'complex' features and did not meet criteria for febrile status epilepticus (phenotypes associated with poor neurodevelopmental outcomes in the general population[33-36]), they were recurrent. In the general population, recurrent febrile seizures associate with an increased risk of future afebrile seizures [37]. Unprovoked seizures in my sub-sample of deletion carriers from Chapter 5 were mostly generalised with a 'primary genetic' aetiology, providing further support for the robust association between 22q11.2DS and genetic generalised epilepsy (GGE) observed in previous studies[1,2,4,5,38]. I also observed that of those deletion carriers with unprovoked seizures, around a third were having them at least once a day at some point in their lifetime, which in the general population significantly increases the risk of drug-resistant epilepsy[39]. Brief spike-and-slow-wave epileptiform discharges of atypical morphology were observed during sleep and/or shortly after awakening in 9% of deletion carriers, but this rate was not significantly elevated relative to controls and no significant association was found with epileptic seizures, so it is currently not clear to what extent they are associated with epilepsy risk in 22q11.2DS. I did not provide evidence that a family history of epileptic seizures and/or epilepsy increases the risk of epileptic seizures in young people with 22q11.2DS. Finally, in Chapter 5, I did not observe any significant associations of epileptic seizures and epilepsy with PIQ, ADHD, indicative ASD and indicative DCD, suggesting that the relationships of these neurodevelopmental deficits with the 'any positive' summary variable, observed in Chapter 4, should be interpreted with caution. However, I did observe trends toward deletion carriers diagnosed with epilepsy being more likely to have ADHD and indicative ASD, respectively. I also observed that deletion carriers diagnosed with non-epileptic events were more likely to have indicative DCD. These analyses may ultimately have been limited by a lack of power brought about by the relatively small of deletion carriers diagnosed with epileptic seizures (n=22), epilepsy (n=7) and non-epileptic events (n=15).

6.2 Themes

Below I discuss the most salient findings from this thesis. At the end of this section, I discuss the plausible genetic and neurobiological mechanisms in 22q11.2DS that may explain these findings.

6.2.1 Young people with 22q11.2DS show high rates of epileptic seizures and epilepsy

In this thesis I have provided evidence showing that the 22q11.2 deletion confers significant risk for different types of epileptic seizures, as well as for epilepsy. This was most pronounced in the findings from Chapter 4, in which 63.9% of deletion carriers were reported as having an epilepsy diagnosis and/or seizures and/or paroxysmal events, compared to only 13.3% of control siblings ($p<0.001$). A more in-depth assessment of these events however showed that at least 21.7% (15/69) of the deletion carriers screening positive on the ESQ were having non-epileptic events. Despite this, during the second stage of assessment I still observed rates of acute symptomatic seizures and isolated or repeated unprovoked seizures in line with previous studies (77.3% and 45.5% of my sample of deletion carriers with epileptic seizures, respectively)[1-3,5,6,40]. 9.1% of deletion carriers also showed epileptiform discharges during a 24-hour EEG assessment, although these were not significantly more common than in the control siblings (0%, $p=0.290$) or more prevalent in deletion carriers with epileptic seizures (15.4%) than those without (6.5%, $p=0.570$).

A question discussed throughout this thesis is whether epilepsy is under-diagnosed in 22q11.2DS. In Chapter 4 I provided preliminary evidence for this. When excluding deletion carriers reported with an epilepsy diagnosis or a febrile seizure, 48.7% of the remaining deletion carriers were reported as having an afebrile seizure, convulsion, fit or spell or a paroxysmal event. During the second stage of assessment in Chapter 5, I provided further evidence to suggest that epileptic seizures may not be recognised in some young people with 22q11.2DS, and an epilepsy diagnosis overlooked. Two deletion carriers reporting afebrile seizures on the ESQ, but not epilepsy, were subsequently diagnosed with epilepsy by the epileptologist in this study. One of these deletion carriers was diagnosed with epilepsy based on a history of 'staring spells' (labelled as non-motor absences by the epileptologist), with the primary caregiver reporting that a paediatrician had labelled these as only 'possible' absence seizures and had not given an epilepsy diagnosis. This individual also showed an epileptiform discharge during the 24-hour EEG assessment. Medical records were available for the other deletion carrier and suggested that they had already received a clinical diagnosis of epilepsy prior to taking part in this study (despite the primary caregiver not reporting this in the ESQ or during the Unusual Spell Interview), suggesting that primary-caregiver report with the ESQ may sometimes lead to under-reporting of an epilepsy diagnosis. Two additional deletion carriers only reported as having

paroxysmal events in the ESQ (and who had not been seen clinically for these events) were diagnosed with repeated 'possible' absence seizures by the epileptologist in this study, leading to an 'uncertain' epilepsy diagnosis in both cases. Epileptiform discharges were also observed in one of these individuals, during the 24-hour EEG assessment.

Despite these findings, the conclusion that epileptic seizures and epilepsy may be overlooked in some young people with 22q11.2DS has significant caveats. Firstly, the epileptologist could only diagnose 'possible' absence seizures in two deletion carriers during the second stage of assessment. This was likely associated with the methodological limitations of the 'remote' epileptologist assessment employed during this stage (discussed below in Section 6.4.1). In addition, further findings from the second stage of assessment showed that 88.2% (15/17) of the deletion carriers reported with an 'afebrile seizure convlusion, fit or spell' or a paroxysmal event (in the absence of epilepsy or a febrile seizure) were having non-epileptic events. This suggests the 'any positive' screen definition on the ESQ may be sensitive, but not specific, for epileptic seizures and epilepsy in 22q11.2DS. To better address the question of whether epilepsy is under-diagnosed in 22q11.2DS, future studies should use direct epileptologist assessment with deletion carriers, particularly in those only reporting paroxysmal events during a screening stage.

6.2.2 Evidence for a reduced seizure threshold in young people with 22q11.2DS

Wither et al.[4] suggested the idea of a reduced seizure threshold in adults with 22q112.DS, after observing that, in deletion carriers with a seizure history, 71% had acute symptomatic seizures, typically associated with hypocalcaemia and psychotropic drug-use. More specifically, they observed that 17.6% of individuals exposed to psychotropic drugs had acute symptomatic seizures. By contrast, these seizures only occur in 1.6% of individuals from the general population who are exposed to psychotropic drugs[41]. In this thesis, I observed a high rate (21.1%) of ESQ-reported febrile seizures in deletion carriers, in the absence of an epilepsy diagnosis (rising to 24.3% when epilepsy cases were included). This was far higher than previous estimates from medical record reviews in this syndrome (2-6%[1,3,4]), likely because my direct interview method could have detected cases not seen clinically, or those which were but were not adequately detailed in sparse/missing medical records (see Section 5.5.2). All but one of the deletion carriers with ESQ-reported febrile seizures who took part in the second stage of assessment were confirmed as having febrile seizures (with a non-complex phenotype) by the epileptologist, suggesting the high rate from the first stage is likely to be accurate. Whilst this high rate of febrile seizures points toward a reduced seizure threshold, an alternative

explanation for this finding could be that deletion carriers are simply experiencing more infection-related high fevers than neurotypical individuals, brought about by the immunological problems strongly associated with this syndrome[42]. Indeed, I observed that recurrent infections were significantly more prevalent in deletion carriers (68.9%) relative to controls (10.9%) in my sample ($p<0.001$). Similarly, of those deletion carriers with febrile seizures, a greater percentage had recurrent infections (80.8% versus 19.2%), however this difference was not significant ($p=0.257$). There were however limitations to these analyses, namely that deletion carriers were not asked about a lifetime history of recurrent infections. As discussed in Section 4.5.1, the lack of a significant association between febrile seizures and infections in 22q11.2DS suggests that there may be additional mechanisms that contribute to the high rate of febrile seizures I observed, one of which could be an increased likelihood to have epileptic seizures during a high fever (i.e. a reduced seizure threshold) in some young people with this syndrome. Hypocalcaemia may plausibly contribute to this reduced threshold for febrile seizures, given that it irritates the central nervous system[43] and enhances neuronal excitability[44], thereby disturbing neuronal excitation-inhibition balance. Whilst hypocalcaemia is one of the most important risk factors for febrile seizures in the general population[45], during the second stage of assessment I observed that only 35.3% of deletion carriers diagnosed with febrile seizures had hypocalcaemia. Therefore, whilst hypocalcaemia may be important for febrile seizure risk in 22q11.2DS, other factors may also contribute to the lower seizure threshold and the increased risk for febrile seizures in this syndrome.

6.2.3 Epileptic seizures and epilepsy may associate with poorer neurodevelopmental outcomes in young people with 22q11.2DS

In Chapter 4, I observed that deletion carriers who screened positive on the ESQ ('any positive') were significantly more likely to have ADHD, indicative ASD and indicative DCD, as well as a lower performance PIQ. These findings have several implications:

1. One on the one hand, these findings replicate the association of epilepsy with poorer neurodevelopmental outcomes seen in the general population[25,46-49] and may point to shared neurobiological risk pathways (see Section 6.2.4 below). These relationships may additionally or alternatively represent deleterious effects of seizures on cognitive, psychiatric and motor development. As discussed in Section 4.5.2, there is evidence from the general population that epileptic seizures result in neurobiological changes that can lead to impaired cognition and ASD-related behaviours[25], that seizures can alter the functional organisation of motor control in the brain[30] and that paroxysmal epileptic activity can impair performance on tests assessing attentional processes[31].

2. One the other hand, the broad nature of the paroxysmal event questions in particular may have meant that in some cases the ESQ was detecting behaviours associated with the cognitive impairment and psychopathology in 22q11.2DS[32], rather than true epileptic seizures. These hypothetical false-positives could have been driving the association of 'any positive' with poorer neurodevelopmental outcomes. During the second stage of assessment in the sub-sample of deletion carriers (Chapter 5), I did not replicate these associations in deletion carriers diagnosed with epileptic seizures and epilepsy by the epileptologist. I did however observe non-significant trends toward a greater proportion of those diagnosed with epilepsy having ADHD and indicative ASD, respectively, suggesting the relationships between 'any positive' with ADHD and indicative ASD in Chapter 4 be the most likely to be valid (i.e. reflect a genuine association with true-positives). Notably, I also observed that a greater proportion of deletion carriers diagnosed with non-epileptic events had indicative DCD, although this trend was non-significant. It is also important to note that the rate of indicative DCD in individuals who were diagnosed with epileptic seizures was also high, so it is inconclusive from these analyses whether false-positives could solely account for the relationship of 'any positive' with indicative DCD. Analyses of the relationships of epileptic seizures, epilepsy and non-epileptic events with poorer neurodevelopmental outcomes (Chapter 5) were likely limited by a lack of power (e.g. only 7 deletion carriers were diagnosed with epilepsy in the second stage of assessment). These analyses therefore cannot fully address the validity of the relationships of the 'any positive' ESQ-variable with poorer neurodevelopmental outcomes. Future research should focus on incorporating all of the deletion carriers reported with 'any positive' into the second stage of assessment, so that the validity of these relationships can be accurately assessed. Until then, the significant overlap between a positive screen on the ESQ and ADHD, indicative DCD and indicative ASD in 22q11.2DS should be interpreted with caution.

In Chapter 5, I observed that febrile seizures in 22q11.2DS were recurrent and that some deletion carriers had experienced daily unprovoked seizures at some point during their lifetime. These features are known to associate with poorer neurodevelopmental outcomes in the general population, increasing the risk for afebrile seizures and drug-resistant epilepsy, respectively[37,39].

6.2.4 Plausible genetic and neurobiological mechanisms underpinning epilepsy risk in 22q11.2DS

What are the genetic and neurobiological mechanisms which could confer increased risk for wide-ranging epileptiform phenomena, a reduced seizure threshold and the possible association with comorbid cognitive impairments, ADHD, ASD and motor problems in young people with 22q11.2DS? As discussed in the Section 1.7.3.2, the deletion appears to confer risk for a range of structural brain abnormalities that are associated with epileptic seizures. These include diffuse cerebral atrophy, polymicrogyria, grey and white matter heterotopia and focal cortical dysplasia[6]. In Chapter 5 of this thesis, 40% of deletion carriers with unprovoked seizures reported structural aetiologies (in the Unusual Spell Interview and/or medical records) including right hemisphere polymicrogyria, perinatal subdural haematoma, neonatal hypoxic-ischaemic encephalopathy and possible post pneumococcal meningitis. In addition to these specific abnormalities, people with 22q11.2DS may have broader neuronal deficits, such as aberrant synaptic plasticity and a pervasive imbalance in neuronal excitation-inhibition, which in the general population been associated with epileptic seizures, ASD, impaired cognition, ADHD behaviours and problems with motor coordination[25-29,50]. Mouse models of 22q11.2DS have demonstrated aberrant synaptic plasticity. For example, hemizygosity for the microRNA biogenesis gene *Dgcr8* leads to enhanced short and long-term synaptic plasticity within hippocampal CA3-CA1 synapses, coinciding with spatial memory deficits, as well as enhanced short-term depression in the prefrontal cortex. Mice that are haploinsufficient for the mitochondrial function gene *Mrpl40* also show abnormal short-term potentiation within the hippocampus and co-occurring working memory deficits. The aberrant synaptic plasticity in these mouse models is mediated by dysregulation of presynaptic calcium levels and neurotransmitter release (e.g. enhanced glutamate activity)[51-53]. In addition, haploinsufficiency of *DCGR6* and *DCGR6L* may affect the expression levels of the $GABA_{B1}$ receptor subunit, leading to a reduction in neuronal inhibition (this mechanism has been suggested to underpin the robust association between 22q11.2DS and genetic generalised epilepsy, see Section 1.7.3.2)[5,54].

6.3 Implications

6.3.1 Young people with 22q11.2DS should be closely monitored for epileptic seizures from early in life

In this thesis I have observed elevated rates of both acute symptomatic seizures (notably febrile seizures) and repeated unprovoked seizures (i.e. epilepsy) in deletion carriers. Interestingly, both of these seizure types had a median age of onset within the first few years of life (14 and 33 months, respectively). These findings suggest that the early years of life represent a period of significant risk for epileptic seizures in young people with 22q11.2DS. Clinicians should arguably closely monitor deletion carriers for seizures during these years. This is particularly important given the associations I observed of 'any positive' on the ESQ with poorer neurodevelopmental outcomes (although see Section 6.2.3 for a discussion of the limitations of these relationships). In addition, febrile seizures were recurrent and in some deletion carriers unprovoked seizures were occurring at least once a day when at their most frequent, both of which increase the risk of a more severe epilepsy phenotype in the general population[37,39]. Where present, early intervention and treatment of epileptic seizures (e.g. fever reduction for febrile seizures, anti-epileptic drugs for repeated unprovoked seizures) may significantly improve long term outcomes in young people with 22q11.2DS.

6.3.2 Genetic testing for the 22q11.2 deletion

My findings highlight the prevalence of epileptic seizures and epilepsy in young people with 22q11.2DS. If young people present with these phenomena, in addition to other typical features of 22q11.2DS (e.g. congenital heart disease, palatal abnormalities, cognitive impairment and psychopathology), they should arguably be referred for genetic testing for the 22q11.2 deletion.

6.3.3 22q11.2DS is a useful model for exploring the aetiology of epileptic seizures and epilepsy and their relationship with neurodevelopmental trajectories

This thesis has highlighted the invaluable opportunity 22q11.2DS provides to explore the aetiology of epileptic seizures and epilepsy, as well as their interaction with neurodevelopmental trajectories, in individuals with a homogenous genetic lesion. Throughout this thesis I have shown that this deletion confers risk for different types of epileptic seizures, as well as for epilepsy. In particular, this thesis has provided evidence that the 22q11.2 deletion confers substantial risk for febrile seizures, thereby better elucidating the genetic aetiology of these seizures. My findings also suggest that epileptiform phenomena may show associations with impaired cognition and motor coordination, as well as ASD

and ADHD in this syndrome. This therefore provides insight into a potential genetic aetiology underlying the comorbidity of these conditions that is seen in the general population[46-49,55-57].

6.4 Limitations

6.4.1 Remote epileptologist assessment

The limitations of the specific measures I used to assess epileptic seizures, epilepsy and epileptiform discharges in 22q11.2DS have been discussed in Chapters 4 and 5. An important limitation with the general design of my study is that it relied on 'remote', rather than 'direct' assessment by an epileptologist. Throughout this thesis I have described my systematic assessment of seizures and epilepsy in 22q11.2DS as 'first-hand'. This is true with respect to how I obtained data from questionnaires, interviews and a 24-hour EEG assessment, rather than solely through retrospective medical record review. However, by 'remote' epileptologist assessment I mean that the epileptologist made diagnoses of epileptic seizures and epilepsy for each individual based on a report of the data that I had collected, rather than directly assessing these individuals himself. This limitation was most clearly evidenced when the diagnosis from the epileptologist was inconclusive, for example with the two deletion carriers diagnosed with 'possible' absence seizures. I attempted to mitigate this limitation by using validated instruments to collect data about epileptic seizures and epilepsy (however, see Sections 4.5.1 and 5.5.2 for a discussion of the limitations of these measures). In addition, as described in Chapter 3, I shadowed epilepsy clinics and attended a well-known paediatric epilepsy training course in order to ask more focused, diagnostically relevant questions during the interview. I acquired medical records relevant to an individual's unusual spells and obtained prolonged EEG recordings in a portion of my overall sample of deletion carriers and controls, to corroborate the report in the ESQ and/or the Unusual Spell Interview. However, it must be noted that direct epileptologist assessment might have improved the quality of my findings. For example, in the deletion carriers with 'possible' absence seizures the epileptologist would have been able to ask further questions that could have provided a more definitive diagnosis. To further mitigate the limitations of a 'remote' epileptologist assessment, I attempted to acquire video-recordings of children's unusual spells, as well as to capture these spells during simultaneous video-EEG recordings (the gold standard for diagnosis of an epileptic seizure[58]). I was however unsuccessful in both of these endeavours; primary caregivers did not provide any video-recordings of unusual spells and these spells were not captured during video-EEG (although interictal epileptiform discharges were observed, see Section 5.5.4).

6.4.2 Lack of power

A lack of power must also be considered as another limitation in my study. My total sample consisted of over 100 deletion carriers and around 60 control siblings, but this still may not have been large enough to detect important relationships. For example, a lack of power may explain the lack of significant associations between positive screens on the ESQ and anxiety disorder and sleep disturbance, as epilepsy is known to associate with these conditions in the general population[47,59]. In particular, the second stage of assessment was only conducted with a sub-sample of deletion carriers and controls. This may explain why, for example, a family history of epileptic seizures and epilepsy did not increase the risk of these phenomena in young people with 22q11.2DS, or why epileptiform discharges were not significantly elevated in deletion carriers relative to the control siblings.

6.4.3 Multiple comparisons

The findings in this thesis have not been corrected for multiple comparisons. The rationale for this is discussed in Section 2.7. The statistically significant relationships presented across chapters should therefore be viewed with caution and need to be replicated by future studies in order to confirm their validity and reliability.

6.4.4 Ascertainment bias

The ECHO study, from which my sample of deletion carriers was drawn, may be subject to ascertainment bias. As described in Section 2.1, one the main streams of recruitment in the ECHO study is via UK genetic clinics. Individuals with more medical problems may have been more likely to be referred for genetic testing in these clinics, and subsequently into the ECHO study. This may have meant that I conducted my systematic assessment of epileptic seizures and epilepsy with a more severely affected population of young people with 22q11.2DS and therefore perhaps obtained less accurate and representative estimates of the rates of these phenomena in deletion carriers.

6.5 Future directions

6.5.1 Direct epileptologist assessment, with review of video-recordings of unusual spells

As discussed in Section 6.4.1, ‘direct’ assessment of individuals with 22q11.2DS by an epileptologist may overcome some of the limitations in this study, for example, the ‘possible’ diagnoses. Studies should also strive to obtain video recordings of unusual spells, ideally with simultaneous EEG recordings, particularly when direct epileptologist assessment is not possible. This would provide a

gold-standard means of diagnosing epileptic seizures and epilepsy, overcoming some of the limitations of witness report associated with the Unusual Spell Interview used in this thesis (for example, use of imprecise terminology such as “shaking” or “fitting” when describing an unusual spell).

6.5.2 Longitudinal assessment

Future studies should endeavour to conduct longitudinal assessments of epileptic seizures, epilepsy and epileptiform discharges in people with 22q11.2DS, as well as concurrently assessing the development of other neurodevelopmental trajectories over time (e.g. cognition, psychiatric health). Doing so would shed light on a number of questions that have been raised by the findings in this thesis. For example, prospective monitoring of epileptic seizures and epilepsy in deletion carriers presenting with epileptiform discharges (but no prior history of epileptic seizures or epilepsy) could help to determine whether these waveforms act as a biomarker for future risk of epileptic seizures and epilepsy in 22q11.2DS. Similarly, longitudinal assessment could help to determine whether epilepsy predicts poorer cognitive, psychiatric and motor outcomes at future time points. Such findings in this clinical high-risk population could also have important implications for better understanding epilepsy, and it’s relationship with neurodevelopmental trajectories, in the general population.

6.5.3 Assessment of epileptic seizures, epilepsy and epileptiform discharges in larger 22q11.2DS cohorts

To avoid the limitations associated with a lack of power discussed in Section 6.4.2, future studies should conduct assessments of epileptic seizures and epilepsy, as well as their association with neurodevelopmental trajectories, in larger cohorts of people with 22q11.2DS. This could for example be implemented within the International Brain and Behaviour Consortium in 22q11.2 Deletion Syndrome (‘22q IBBC’). As described in the introduction to this thesis, the 22q IBBC has conducted a study of over 1,000 people with 22q11.2DS (children, adolescents and adults), drawn from 15 different sites across the globe[60]. Implementing a standardised protocol for the assessment of epileptic seizures and epilepsy across sites would provide far greater power. This would allow questions arising from this thesis to be more comprehensively addressed, for example whether epilepsy associates with anxiety and sleep disturbance in 22q11.2DS, as it does in the general population[47,59]. Conducting this study within the 22q IBBC framework would also allow for a comprehensive assessment within adults with 22q11.2DS. As outlined in the introduction, Andrade et al.[40] and colleagues conducted a first-hand assessment of epilepsy, epileptiform discharges and MRI findings in adult deletion carriers,

however this was only with a small sample (n=19). Adding a longitudinal component to this supposed 22q IBBC epilepsy assessment protocol would provide the benefits outlined in Section 6.5.2.

6.5.4 Population studies of 22q11.2DS

As outlined in Section 6.4.4, recruiting the sample of young people with 22q11.2DS from UK genetic clinics could have resulted in ascertainment bias in my study, with deletion carriers with more severe medical problems being more likely to be referred for genetic testing. One way to overcome this would be to identify individuals with 22q11.2DS by genotyping a sample drawn from the general population. The population-based sample would have to very large however. Olsen et al.[61] genotyped a random population sample of around 26,000 individuals from Denmark and observed the 22q11.2 deletion in only seven individuals. Incorporating epilepsy assessments into the 22q IBBC framework therefore seems a more plausible short-term solution for assessing epileptic seizures and epilepsy in a large cohort of people with 22q11.2DS, despite the risks of ascertainment bias. Very large population cohorts with available information about genotype do exist however, such as the UK Biobank, which contains 500,000 individuals. These individuals are aged between 40-69 years however, meaning they could only provide insight into epileptic seizures and epilepsy in adult deletion carriers.

6.6 Conclusion

In this thesis, I went beyond the limitations of medical record reviews and conducted a 'first-hand' assessment of epileptic seizures and epilepsy in young people with 22q11.2DS. This thesis replicates findings from previous studies of individuals with 22q11.2DS, for example that the deletion confers an increased risk of epilepsy and epileptic seizures. I also provide further evidence for the idea that the 22q11.2 deletion leads to a reduced seizure threshold. The novel contributions of this thesis to the literature include evidence to suggest that epileptic seizures may not be recognised in some deletion carriers during routine clinical care, and an epilepsy diagnosis may be overlooked. In addition, I observed a far higher rate of febrile seizures than previous estimates in this population, which provides further support for a reduced seizure threshold in this syndrome. Finally, I found some preliminary evidence for an overlap of epileptiform phenomena with higher rates of ADHD, ASD and motor problems, as well as a lower PIQ, in this syndrome, although this particular finding has significant limitations. In addition, my study is the first to shed light on several important characteristics of epileptic seizures in this syndrome, such as the phenotype of febrile seizures (simple) and the length and frequency of unprovoked seizures.

Future longitudinal studies of large cohorts of people with 22q11.2DS, involving direct epileptologist assessment and review of video-recordings of unusual spells are warranted to overcome the limitations of this thesis. These studies have the potential to provide greater insight into the prevalence of epileptic seizures and epilepsy in 22q11.2DS, as well as their relationship with neurodevelopmental outcomes. This may lead to clinicians more closely monitoring epileptic seizures in people with 22q11.2DS, providing earlier intervention and improving long-term outcomes for these individuals. These studies can also shed light on the genetic aetiology of epileptic seizures and epilepsy and provide more precise insights into their interaction with other neurodevelopmental domains, which may benefit individuals in the general population.

6.7 References

1 Kao, A. *et al.* Increased prevalence of unprovoked seizures in patients with a 22q11. 2 deletion. *American Journal of Medical Genetics Part A* **129**, 29-34 (2004).

2 Kim, E.-H. *et al.* Epilepsy and Other Neuropsychiatric Manifestations in Children and Adolescents with 22q11. 2 Deletion Syndrome. *Journal of Clinical Neurology* **12**, 85-92 (2016).

3 Ryan, A. *et al.* Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. *Journal of Medical Genetics* **34**, 798-804 (1997).

4 Wither, R. G. *et al.* 22q11. 2 deletion syndrome lowers seizure threshold in adult patients without epilepsy. *Epilepsia* (2017).

5 Strehlow, V. *et al.* Generalized Epilepsy and Myoclonic Seizures in 22q11. 2 Deletion Syndrome. *Molecular Syndromology* **7**, 239-246 (2016).

6 Mudigoudar, B., Nune, S., Fulton, S., Dayyat, E. & Wheless, J. W. Epilepsy in 22q11. 2 Deletion Syndrome: A Case Series and Literature Review. *Pediatric Neurology* (2017).

7 El Tahir, M., Kerr, M. & Jones, R. Two cases of generalized seizures and the Velocardiofacial syndrome–a clinically significant association? *Journal of Intellectual Disability Research* **48**, 695-698 (2004).

8 Roubertie, A. *et al.* Neurological presentation of three patients with 22q11 deletion (CATCH 22 syndrome). *Brain & Development* **23**, 810-814 (2001).

9 Tonelli, A. R., Kosuri, K., Wei, S. & Chick, D. Seizures as the first manifestation of chromosome 22q11.2 deletion syndrome in a 40-year old man: a case report. *J Med Case Rep* **1**, 167, doi:10.1186/1752-1947-1-167 (2007).

10 Lemke, J. R. *et al.* Juvenile myoclonic epilepsy with photosensitivity in a female with Velocardiofacial syndrome (del(22)(q11.2))--causal relationship or coincidence? *Seizure* **18**, 660-663, doi:10.1016/j.seizure.2009.07.008 (2009).

11 Pohlmann-Eden, B., Beghi, E., Camfield, C. & Camfield, P. The first seizure and its management in adults and children. *BMJ: British Medical Journal* **332**, 339 (2006).

12 Cheung, E. N. *et al.* Neonatal hypocalcemia, neonatal seizures, and intellectual disability in 22q11.2 deletion syndrome. *Genet Med* **16**, 40-44, doi:10.1038/gim.2013.71 (2014).

13 Ottman, R. *et al.* Validation of a brief screening instrument for the ascertainment of epilepsy. *Epilepsia* **51**, 191-197 (2010).

14 Ottman, R., Hauser, W. A. & Stallone, L. Semistructured interview for seizure classification: agreement with physicians' diagnoses. *Epilepsia* **31**, 110-115 (1990).

15 Ottman, R. *et al.* Reliability of seizure classification using a semistructured interview. *Neurology* **43**, 2526-2526 (1993).

16 Fisher, R. S. *et al.* Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* **46**, 470-472 (2005).

17 Fisher, R. S. *et al.* ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* **55**, 475-482 (2014).

18 Fisher, R. S. *et al.* Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. *Epilepsia* **58**, 522-530 (2017).

19 Weschler, D. Weschler abbreviated scale of intelligence (WASI). *London: Psychological Corporation* (1999).

20 Angold, A. *et al.* The child and adolescent psychiatric assessment (CAPA). *Psychological medicine* **25**, 739-753 (1995).

21 Association, A. P. & Association, A. P. *Diagnostic and statistical manual-text revision (DSM-IV-TRim, 2000)*. (American Psychiatric Association, 2000).

22 Rutter, M., Bailey, A. & Lord, C. *The social communication questionnaire: Manual*. (Western Psychological Services, 2003).

23 Wilson, B. N. The Developmental Coordination Disorder Questionnaire 2007. *Physical & occupational therapy in pediatrics* **29**, 182-202 (2012).

24 Wilson, B. N. *et al.* Psychometric properties of the revised developmental coordination disorder questionnaire. *Physical & occupational therapy in pediatrics* **29**, 182-202 (2009).

25 Brooks-Kayal, A. Epilepsy and autism spectrum disorders: are there common developmental mechanisms? *Brain and Development* **32**, 731-738 (2010).

26 Piochon, C. *et al.* Cerebellar Plasticity and Motor Learning Deficits in a Copy Number Variation Mouse Model of Autism. *Nature communications* **5**, 5586-5586, doi:10.1038/ncomms6586 (2014).

27 VanGuilder, H. D. *et al.* Hippocampal dysregulation of synaptic plasticity-associated proteins with age-related cognitive decline. *Neurobiology of disease* **43**, 201-212 (2011).

28 Selemon, L. D. A role for synaptic plasticity in the adolescent development of executive function. *Translational psychiatry* **3**, e238 (2013).

29 Lesch, K.-P. *et al.* Molecular genetics of adult ADHD: converging evidence from genome-wide association and extended pedigree linkage studies. *Journal of neural transmission* **115**, 1573-1585 (2008).

30 Woodward, K. E. *et al.* Recent seizure activity alters motor organization in frontal lobe epilepsy as revealed by task-based fMRI. *Epilepsy Res* **108**, 1286-1298, doi:10.1016/j.eplepsyres.2014.06.015 (2014).

31 Aldenkamp, A. & Arends, J. The relative influence of epileptic EEG discharges, short nonconvulsive seizures, and type of epilepsy on cognitive function. *Epilepsia* **45**, 54-63 (2004).

32 Schneider, M. *et al.* Psychiatric disorders from childhood to adulthood in 22q11.2 deletion syndrome: Results from the international consortium on brain and behavior in 22q11.2 deletion syndrome. *American Journal of Psychiatry* **171**, 627-639, doi:http://dx.doi.org/10.1176/appi.ajp.2013.13070864 (2014).

33 Graves, R. C., Oehler, K. & Tingle, L. E. Febrile seizures: risks, evaluation, and prognosis. *American family physician* **85** (2012).

34 Lewis, D. V. *et al.* Hippocampal sclerosis after febrile status epilepticus: the FEBSTAT study. *Annals of neurology* **75**, 178-185 (2014).

35 Martinos, M. M. *et al.* Recognition memory is impaired in children after prolonged febrile seizures. *Brain* **135**, 3153-3164 (2012).

36 Martinos, M. M. *et al.* Early developmental outcomes in children following convulsive status epilepticus: a longitudinal study. *Epilepsia* **54**, 1012-1019 (2013).

37 Verity, C. & Golding, J. Risk of epilepsy after febrile convulsions: a national cohort study. *Bmj* **303**, 1373-1376 (1991).

38 Lal, D. *et al.* Burden Analysis of Rare Microdeletions Suggests a Strong Impact of Neurodevelopmental Genes in Genetic Generalised Epilepsies. (2015).

39 Sillanpää, M. & Schmidt, D. Early seizure frequency and aetiology predict long-term medical outcome in childhood-onset epilepsy. *Brain* **132**, 989-998 (2009).

40 Andrade, D. M., Krings, T., Chow, E. W., Kiehl, T.-R. & Bassett, A. S. Hippocampal malrotation is associated with chromosome 22q11. 2 microdeletion. *The Canadian Journal of Neurological Sciences* **40**, 652-656 (2013).

41 Lee, K. C., Finley, P. R. & Alldredge, B. K. Risk of seizures associated with psychotropic medications: emphasis on new drugs and new findings. *Expert opinion on drug safety* **2**, 233-247 (2003).

42 McDonald-McGinn, D. M. *et al.* 22q11. 2 deletion syndrome. *Nature reviews. Disease primers* **1**, 15071 (2015).

43 Castilla-Guerra, L., Fernández-Moreno, M. d. C., López-Chozas, J. M. & Fernández-Bolaños, R. Electrolytes disturbances and seizures. *Epilepsia* **47**, 1990-1998 (2006).

44 Han, P., Trinidad, B. J. & Shi, J. Hypocalcemia-induced seizure: demystifying the calcium paradox. *ASN neuro* **7**, 1759091415578050, doi:10.1177/1759091415578050 (2015).

45 Delpisheh, A., Veisani, Y., Sayehmiri, K. & Fayyazi, A. Febrile seizures: etiology, prevalence, and geographical variation. *Iranian journal of child neurology* **8**, 30-37 (2014).

46 Bowley, C. & Kerr, M. Epilepsy and intellectual disability. *Journal of Intellectual Disability Research* **44**, 529-543 (2000).

47 Rai, D. *et al.* Epilepsy and psychiatric comorbidity: A nationally representative population-based study. *Epilepsia* **53**, 1095-1103 (2012).

48 Parisi, P., Moavero, R., Verrotti, A. & Curatolo, P. Attention deficit hyperactivity disorder in children with epilepsy. *Brain and Development* **32**, 10-16 (2010).

49 Kirby, A. *et al.* Benign childhood epilepsy with centrotemporal spikes (BECTS) and developmental co-ordination disorder. *Epilepsy & Behavior* **72**, 122-126 (2017).

50 Edden, R. A., Crocetti, D., Zhu, H., Gilbert, D. L. & Mostofsky, S. H. Reduced GABA concentration in attention-deficit/hyperactivity disorder. *Archives of general psychiatry* **69**, 750-753 (2012).

51 Devaraju, P. *et al.* Haploinsufficiency of the 22q11. 2 microdeletion gene Mrpl40 disrupts short-term synaptic plasticity and working memory through dysregulation of mitochondrial calcium. *Molecular psychiatry* (2016).

52 Earls, L. R. *et al.* Age-dependent microRNA control of synaptic plasticity in 22q11 deletion syndrome and schizophrenia. *Journal of Neuroscience* **32**, 14132-14144 (2012).

53 Fénelon, K. *et al.* Deficiency of Dgcr8, a gene disrupted by the 22q11. 2 microdeletion, results in altered short-term plasticity in the prefrontal cortex. *Proceedings of the National Academy of Sciences* **108**, 4447-4452 (2011).

54 Schuler, V. *et al.* Epilepsy, hyperalgesia, impaired memory, and loss of pre-and postsynaptic GABAB responses in mice lacking GABAB (1). *Neuron* **31**, 47-58 (2001).

55 Arshad, S. *et al.* Epilepsy and intellectual disability: Does epilepsy increase the likelihood of co-morbid psychopathology? *Research in developmental disabilities* **32**, 353-357 (2011).

56 Saemundsen, E., Ludvigsson, P., Hilmarsdottir, I. & Rafnsson, V. Autism Spectrum Disorders in Children with Seizures in the First Year of Life—A Population-Based Study. *Epilepsia* **48**, 1724-1730 (2007).

57 Clarke, D. F. *et al.* The prevalence of autistic spectrum disorder in children surveyed in a tertiary care epilepsy clinic. *Epilepsia* **46**, 1970-1977 (2005).

58 Smith, P. The bare essentials: epilepsy. *Practical neurology* **8**, 195-202 (2008).

59 van Golde, E. G., Gutter, T. & de Weerd, A. W. Sleep disturbances in people with epilepsy; prevalence, impact and treatment. *Sleep medicine reviews* **15**, 357-368 (2011).

60 Schneider, M. *et al.* Psychiatric disorders from childhood to adulthood in 22q11. 2 deletion syndrome: results from the International Consortium on Brain and Behavior in 22q11. 2 Deletion Syndrome. *American Journal of Psychiatry* (2014).

61 Olsen, L. *et al.* Prevalence of rearrangements in the 22q11.2 region and population-based risk of neuropsychiatric and developmental disorders in a Danish population: a case-cohort study. *The Lancet Psychiatry*, doi:https://doi.org/10.1016/S2215-0366(18)30168-8.

Appendices

Interview conducted with primary caregiver. Adapted from the interview developed by Ottman et al[1,2]. and the Epilepsy Phenome-Genome Project[3]

How are you completing this interview?

- o With participant present
- o With participant via telephone

Caregiver ID:

Relationship of caregiver to child:

Child ID:

Child DOB:

Child age:

Child gender (M/F):

Date of interview:

Key

()= the interviewer needs to choose from one of the options inside the brackets

SMALL CAPS=instructions to interviewer

Part 1: Febrile seizures

NOTES FOR INTERVIEWER

IF CHILD HAS SCREENED POSITIVE ON QUESTION 1 OF EPILEPSY SCREEN, COMPLETE THE FOLLOWING SECTION. OTHERWISE, SKIP TO PART 2: 'OTHER SEIZURES'.
From the questionnaire we sent you asking about seizures and epilepsy, you said that □ *(Name of child)* had a seizure or convulsion caused by a high fever □ *(Name of child)* possibly had a seizure or convulsion caused by a high fever
INTERVIEWER READS THE FOLLOWING I'd now like to ask some more questions about the seizure or convulsion caused by a high fever that (*name of child*) has had.

Q2.	Please think back to a specific time (*name of child*) had one of these event s. It could be the first time (s/he) had one, the time (s/he) had one most recently, or the time you feel you can describe the best. I would like to get a detailed description of that event

	Q2A.	What would you say is the very first thing that happened when (*name of child*) had this event ? **PROBE TO SPECIFY DETAILS** **PROBE FOR WHETHER CHILD HAD ANY INFECTIONS IN THE AROUND THE TIME OF THE FEBRILE SEIZURE. IF YES, WHAT INFECTIONS.**
	Q2B.	What is the next thing that happened? **PROBE TO SPECIFY DETAILS**
	Q2C.	How did *(name of child)* feel, what happened, or what did *(s/he)* do did during the rest of this event ? **PROBE TO SPECIFY DETAILS**

	Q2D.	How long would you say this event lasted? Would you say…..	Less than five minutes □ 5-15 minutes □ More than 15 minutes □ Don't know □ Not applicable □
			IF MORE PRECISE ESTIMATE, NOTE DOWN HERE
	Q2E.	How did *(name of child)* feel, what happened, or what did *(s/he)* do <u>afterwards</u>? **PROBE TO SPECIFY DETAILS**	

		Q2Ei.	How long did (*name of child*) (*feel that way/do that for*)?	One hour or less □ More than one hour □ Don't know □ Not applicable □
				IF MORE PRECISE ESTIMATE, NOTE DOWN HERE

	Q2F.	(*Is/was*) this event always the same when it happens?	Yes **[GO TO Q3]** □ No □ Don't know **[GO TO Q3]** □ Not applicable □

		Q2Fi.	What differ*(s/ed)*? **PROBE TO SPECIFY DETAILS**	

Q3.	Has (*name of child*) ever had two or more of these event s within the same 24 hour period, or during the same period of illness?	Yes □ No □ Don't know □ Not applicable □	
Q4.	How old was (*name of child*) the first time (s/he) had one of these event s? **ENTER IN MONTHS IF CHILD WAS YOUNGER THAN 2 YEARS, YEARS IF OLDER. ENTER IN DAYS IF CARER REPORTS THIS EVENT OCCURRED WITHIN THE FIRST MONTH OF LIFE. CHECK DAYS, MONTHS OR YEARS.**	age	days □ months □ years □
Q5.	How old was (*name of child*) the last time (s/he) had one of these event s?	age	days □ months □ years □

Q6.	How many of these event s has (*name of child)* had in (*his/her*) lifetime?	One □ Between 2 and 4 □ Between 5 and 10 □ More than 10 □ Don't know □ Not applicable □
		IF MORE PRECISE ESTIMATE, NOTE DOWN HERE

Q7.	Has (*name of child*) ever been prescribed medication to control these event s?	Yes □ No [**GO TO Q8**.] □ Don't know [**GO TO Q8**.] □ Not applicable □
	Q7A. Which medications has *(name of child)* been prescribed?	**RECORD NAMES OF MEDICATIONS HERE** **1.** **2.** **3.** **4.** **5**.
Q8.	During the period of life when (*name of child*) had these event s, did she ever have a seizure, convulsion, fit or event without a fever?	Yes □ No **[GO TO PART 2]** □ Don't know **[GO TO PART 2]** □ Not applicable □
	Q8A. What would you say is the very <u>first thing</u> that happened when (*name of child*) had this event ? **PROBE TO SPECIFY DETAILS**	

	Q8B. What is the next thing that happened? **PROBE TO SPECIFY DETAILS**
	Q8C. How did (*name of child*) feel, what happened, or what did (s/he) do did during the rest of this event ? **PROBE TO SPECIFY DETAILS**
	Q8D. How did *(name of child)* feel, what happened, or what did *(s/he)* do afterwards? **PROBE TO SPECIFY DETAILS**

Supplementary: Febrile seizure probes

With this event….

Did (*name of child*) vomit or foam at the mouth?

Did (*name of child*) wet or soil themselves?

Was there any twitching of the face, arms or legs?

- Both sides, one side?

Did *(name of child*) lose consciousness?

Were they sleep or confused afterwards?

- How long were they like this more (one hour or more)?

Did they have any weakness of the limbs afterwards?

- One side/both sides?

Part 2: Other unusual spells

Section A: Description of unusual spells

NOTES FOR INTERVEWER

IF COMPLETED THE FEBRILE SEIZURE SECTION, INTERVIEWER READS THE FOLLOWING

Thank you for talking to me about *(name of child's)* other seizures. In this next section, I'd like to talk to you about any other unusual spells that (*name of child*) has experienced.

In the Epilepsy Screen Questionnaire that we sent you, you said that:

LIST ALL OF THE POSITIVE RESPONSES FROM THE EPILEPSY SCREEN (EXCLUDING Q1 FEBRILE SEIZURES)

INTERVIEWER READS THE FOLLOWING:

Many people have more than one 'type' of unusual spell. What we mean by a 'different type 'is that (*name of child*) feels different during the unusual spell, or if what happens before, during or after the unusual spell is different from (*his/her*) other types.

Q1.	With this in mind, how many 'different types' of unusual spell*s* would you say (*name of child)* has had?	# different types

Q2.	Could you give me a name to use for each type of unusual spells, to make it easier to describe each one? Please give me the names in order, from the type (*name of child*) has had most frequently, to the type *s(he)* has had least frequently.	1. 2. 3. 4. 5.

INTERVIEWER READS THE FOLLOWING
Now I'd like to ask you some questions about (*this/these*) unusual spells that (*name of child*) has experienced.

IF MULTIPLE TYPES OF SEIZURE/UNUSUAL SPELL

Let's start with the first type:

Let's move onto the next type:

REPEAT Q1-Q18A AND THE SYMPTOM TABLE FOR EACH TYPE OF UNUSUAL SPELL THE CARER HAS IDENTIFIED

Q3.	Please think back to a specific time when (*name of child*) had one of these *(name of spell)*s- it could be the first time (*s/he*) had one, the time (*s/he*) had one most recently, or the time you feel you can describe the best. I would like to get a detailed description of that *(name of spell)*.

	Q3A.	What would you say is the very first thing that happened when (*name of child*) had this *(name of spell)*? **PROBE TO SPECIFY DETAILS**
	Q3B.	What is the next thing that happened? **PROBE TO SPECIFY DETAILS**
	Q3C.	How did *(name of child)* feel, what happened, or what did *(s/he)* do did during the rest of this *(name of spell)*? **PROBE TO SPECIFY DETAILS**
	Q3D.	**ASK ONLY IF CARER REPORTS CHILD BEING DIZZY OR LIGHT-HEADED, OTHERWISE GO TO Q3E** What do you mean by (*dizzy/light-headed*)? **PROBE TO DIFFERENTIATE VERTIGO FROM FEELING LIGHT-HEADED OR FAINT**

	Q3E.	How did *(name of child)* feel, what happened, or what did *(s/he)* do <u>afterwards</u>**?** **PROBE TO SPECIFY DETAILS**

Q4.	How long did the *(name of spell)* last? Would you say…	Less than 15 seconds □ 15 seconds to 5 minutes □ More than 5 minutes □ Don't know □ Not applicable □
		IF MORE PRECISE ESTIMATE, NOTE DOWN HERE

Q5.	During the *(name of spell)*, which of the following best describes *(name of child)*'s awareness of the surroundings?	Fully aware □ Somewhat aware, but less than usual □ Fully unaware □ Don't know □ Not applicable □
Q6.	During this *(name of spell)*, was *(name of child)* able to communicate as *(s/he)* normally does?	Yes □ No □ Don't know □ Not applicable □
Q7.	Was *(name of child)* tired or confused afterwards?	Yes □ No **[[GO TO Q8]** □ Don't know **[[GO TO Q8]** □ Not applicable □

	Q7A. For how long was *(name of child)* tired or confused? Would you say…	Less than 10 seconds □ 10 seconds- 20 minutes □ More than 20 minutes □ Don't know □ Not applicable □
		IF MORE PRECISE ESTIMATE, NOTE DOWN HERE

Q8.	How old was *(name of child)* the first time (*s/he)* had this *(name of spell)* **ENTER IN MONTHS IF CHILD WAS YOUNGER THAN 2 YEARS, YEARS IF OLDER. ENTER IN DAYS IF CARER REPORTS THIS UNUSUAL SPELL OCCURRED WITHIN THE FIRST MONTH OF LIFE. CHECK DAYS, MONTHS OR YEARS.**		days □ months □ years □
Q9.	What month and year was the <u>last</u> time *(name of child)* had one of these *(name of spell)*s?	/	
Q10.	How old was *(name of child)* at that time? **ENTER IN MONTHS IF CHILD WAS YOUNGER THAN 2 YEARS, YEARS IF OLDER. ENTER IN DAYS IF CARER REPORTS THIS UNUSUAL SPELL OCCURRED WITHIN THE FIRST MONTH OF LIFE. CHECK DAYS, MONTHS OR YEARS.**		days □ months □ years □

Q11.	About how many times in *(name of child)*'s life would you say (*s/he*) experienced these *(name of spell)*s like the one you just described? Would you say…	Less than 10 □ Between 10 and 100 □ More than 100 □ Don't know □ Not applicable □

		IF MORE PRECISE ESTIMATE, NOTE DOWN HERE
Q12.	At the time in *(name of child)*'s life when (*s/he*) had this type of unusual spell <u>most frequently</u>, how often did (s/he) have them? Would you say…	Less than once a month □ 1 to 4 times a month □ More than 4 times a month but less than once a day □ Once a day or more □ Don't know □ Not applicable □
		IF MORE PRECISE ESTIMATE, NOTE DOWN HERE

Q13.	How old was *(name of child)* at that time? Would you say… **PROBE FOR BEST ESTIMATE; READ AGE CATEGORIES ONLY UP TO CHILD'S CURRENT AGE**	<1 year old □ 1-5 years old □ 6-10 years old □ 11-19 years old □ 20 years old or older □ Don't know □ Not applicable □	**PROBE FOR PRECISE AGE IF KNOWN**
Q14.	Has this *(name of spell)* occurred whilst (*name of child*) is awake or asleep? Would you say….	Only while *(name of child)* is asleep [**GO TO Q16**] □ Mostly while *(name of child)* is asleep, but not always □ Equally during sleeping and waking □ Mostly while *(name of child)* is awake, but not always □ Only while *(name of child)* is awake Don't know [**GO TO Q16**] □ Not applicable □	

Q15.	Of these *(name of spell)*s that *(name of child)* has while *(s/he)* is awake, when do most of them occur? Would you say…		Within one hour of waking up in the morning or after a nap □ More than 1 hour after waking up but not while falling asleep □ While falling asleep □ Don't know □ Not applicable □
			IF MORE PRECISE ESTIMATE, NOTE DOWN HERE
Q16.	Does (*name of child*) ever have a warning or aura that this *(name of spell)* is about to happen?		Yes □ No **[GO TO Q17]** □ Don't know **[GO TO Q17]** □ Not applicable □
	Q16A.	Could you tell me more about that? **PROBE TO SPECIFY DETAILS**	

	Q16.B	Does (*name of child*) ever experience the (*warning/aura*) you described previously, without it leading to the rest of this *(name of spell)*?		Yes □ No **[GO TO Q17]** □ Don't know **[GO TO Q17]** □ Not applicable □
		Q16Bi	Could you tell me more about that? **PROBE TO SPECIFY DETAILS**	
Q17.	Is this *(name of spell)* always the same when it happens?			Yes **[GO TO Q18.]** □ No □ Don't know **[GO TO Q18.]** □ Not applicable □
	Q17A.	What differs? **PROBE TO SPECIFY DETAILS**		

Q18.	**ASK THIS QUESTION ONLY IF CHILD HAD CHANGE IN LEVEL OF AWARENESS** Has *(name of child)* ever had an *(name of spell)* with similar symptoms except that (*s/he*) remained fully aware of the surroundings?		Yes □ No [**GO TO SYMPTOM TABLE]** □ Don't know [**GO TO SYMPTOM TABLE]** □ Not applicable □
	Q18A.	Could you tell me more about that? **PROBE TO SPECIFY DETAILS**	

SEIZURE/UNUSUAL SPELL SYMPTOMS To make sure we get the same information on everyone, now I would like to ask you about <u>specific</u> symptoms that may occur with the *(name of spell)* that we have just spoken about. With this *(name of spell)*:				
Symptom **IF NO, SKIP TO NEXT SYMPTOM**	**Does this happen before, during or after the (*name of spell)*)?** **PROBE TO SPECIFY DETAILS**	**Which parts of the body are involved?** **PROBE TO SPECIFY DETAILS**	**When they have this, is it…**	**Can you tell me more about this?** **PROBE TO SPECIFY DETAILS (E.G. WHAT IS THIS SYMPTOM LIKE)?**

A.	Does *(name of child)* have uncontrollable shaking or stiffening of part or all of *(his/her)* body? Yes □ No □ Don't know □ Not applicable □			Always on the left side □ Always on the right side □ Always on the same side, but Don't know which side □ Sometimes left side, sometimes right side □ On both sides □ Don't know □ Not applicable □	

B.	Does *(name of child)* have sudden and unexpected jerking or twitching movements of part of *(his/her)* body? **[IF NO, SKIP TO C]** Yes □ No □ Don't know □ Not applicable □ **IF NO OR DON'T KNOW, SKIP TO C**			Always on the left side □ Always on the right side □ Always on the same side, but Don't know which side □ Sometimes left side, sometimes right side □ On both sides □ Don't know □ Not applicable □	

	B1. Has *(name of child)* ever said the jerking felt like an <u>electric shock</u> going through *(his/her)* body? Yes □ No □ Don't know □ Not applicable □				

	B2. Has this type of jerking occurred as a result of lights shining in *(name of child)*'s eyes—for example, strobe lights, video games, reflections, or sun glare? Yes □ No □ Don't know □ Not applicable □				

C.	Does *(name of child)*'s head or other body parts turn toward one side? Yes □ No □ Don't know □ Not applicable □				
D.	Do *(name of child's)* eyes roll to the back of their head? Yes □ No □ Don't know □ Not applicable □				

E.	Does *(name of child)* bite their cheek or the side of their tongue? Yes □ No □ Don't know □ Not applicable □				
F.	Does *(name of child)* pass urine? Yes □ No □ Don't know □ Not applicable □				

G.	Does *(name of child)* experience any frothing at the mouth? Yes □ No □ Don't know □ Not applicable □				
H.	Does *(name of child)* experience any change in skin colour? Yes □ No □ Don't know □ Not applicable □				

I.	Does (*name of child*) have noisy, heavy or harsh breathing? Yes □ No □ Don't know □ Not applicable □				

J.	Does *(name of child)* have numbness, tingling, pain, or other unusual feeling in part or all of *(his/her)* body? Yes □ No □ Don't know □ Not applicable □			Always on the left side □ Always on the right side □ Always on one side, but Don't know which side □ Sometimes left side, sometimes right side □ On both sides □ Don't know □ Not applicable □	

K.	Does *(name of child)* have sudden weakness of <u>part of *(his/her)*</u> <u>body</u>, causing (him/her) to fall or drop things? Yes □ No □ Don't know □ Not applicable □			Always on the left side □ Always on the right side □ Always on the same side, but Don't know which side □ Sometimes left side, sometimes right side □ On both sides □ Don't know □ Not applicable □	

L.	Does *(name of child)* have sudden weakness of *(his/her)* whole body, or drop to the ground uncontrollably? Yes □ No □ Don't know □ Not applicable □ **IF NO OR DON'T KNOW, SKIP TO M.**				

	L1. Does *(name of child)* have any jerking movements just before the whole body weakness? Yes □ No □ Don't know □ Not applicable □			Always on the left side □ Always on the right side □ Always on the same side, but Don't know which side □ Sometimes left side, sometimes right side □ On both sides □ Don't know □ Not applicable □	

	L2. During the weakness or drop attack, which of the following best describes *(name of child)*'s awareness of *(his/her)* surroundings? Would you say… Fully aware □ Somewhat aware, but less than usual □ Fully unaware □ Don't know □ Not applicable □				

M.	Does *(name of child)* have other changes in the way *(his/her)* body feels, such as shortness of breath, chest tightness, rising abdominal sensation, a feeling of a wave going through their head, a feeling that their arms or leg are bigger or small then they are or feeling hot or cold? Yes □ No □ Don't know □ Not applicable □				
N.	Does *(name of child)* behave in unusual ways such as involuntary laughter, smacking				

	(his/her) lips, touching *(his/her)* clothes ,making cycling movements or doing any other repetitive or unusual things without intending to? Yes □ No □ Don't know □ Not applicable □			

O.	Does *(name of child)* have a brief lapse of awareness – lasting 15 seconds or less -- without any strange sensation or feelings? Yes □ No □ Don't know □ Not applicable □ **IF NO OR DON'T KNOW, SKIP TO P.**				
	O1. Does this brief lapse of awareness occur with eyelid fluttering? Yes □ No □ Don't know □ Not applicable □				

P.	Does *(name of child)* have an unusual taste or smell? Yes □ No □ Don't know □ Not applicable □				
Q.	Does *(name of child)* have a change in *(his/her)* hearing, or hearing sounds that aren't there? Yes □ No □ Don't know □ Not applicable □				

R	Does *(name of child)* have a change in *(his/her)* vision, or seeing things that aren't there? Yes □ No □ Don't know □ Not applicable □				
S.	Does *(name of child)* have changes in *(his/her)* ability to speak or understand words while still awake and largely aware of what				

	is going on around (*him/her*)? Yes □ No □ Don't know □ Not applicable □			

T.	Does *(name of child)* have sudden unexplained changes in *(his/her)* emotional state that occurred for no apparent reason -- such as fear, anxiety, sadness, anger, happiness, laughter, or other emotions? **PROBE TO DISTINGUISH REASONABLE REACTIONS FROM PRIMARY EMOTIONAL OR PSYCHIC AURAS; DO NOT CODE REASONABLE REACTIONS** Yes □ No □ Don't know □ Not applicable □				

U.	Does *(name of child)* have déjà vu--that is, a feeling that (s/he) has experienced something before when it is really the first time it has happened? Yes □ No □ Don't know □ Not applicable □				
V.	Does *(name of child)* have jamais vu - that is, things that are familiar to them seem strange or foreign? Yes □ No □ Don't know □ Not applicable □				

W.	Does *(name of child)* have a feeling that things around (him/her) are not real or that (s/he) is not real, or an "out of body experience?" Yes □ No □ Don't know □ Not applicable □				

X.	Does *(name of child)* have changes in *(his/her)* thoughts such as speeding up, *(his/her)* mind "racing," or slowing down of thoughts? Yes □ No □ Don't know □ Not applicable □				
Y.	Does *(name of child)* have any other feelings, experiences, or symptoms				

	that we haven't spoken about? Yes □ No □ Don't know □ Not applicable □ **RECORD SYMPTOM BELOW (VERBATIM)**			

Section B: Seizure triggers

Have any of the unusual spells we have discussed been triggered by any of the following stimuli:

ASK ALL OF A-Q, AND PROBE FOR FURTHER DESCRIPTION OF TRIGGER FOR EACH 'YES' RESPONSE

	Yes	No	Don't know	Which of *(name of child's)* unusual spells does this trigger?	Could you tell me more about that? **PROBE TO SPECIFY DETAILS**
A. Sounds such as the phone ringing, music playing, or someone speaking?					
B. Being startled?					
C. Any particular movements *(name of child)* makes?					
D. Being touched?					
E. Reading?					
F. Flashing or blinking lights?					
G. Writing or thinking?					
H. Standing up?					
I. Poor sleep?					
J. Stress?					
M. Exercise (e.g. after a P.E. lesson)?					
N. Missing a meal (e.g. missing breakfast, lunch or dinner)?					

O. A high fever?					
P**. ASK ONLY IF CHILD IS OLDER THAN 13** Alcohol?					
Q. Any other triggers?					

Section C: Screen for status epilepticus, prolonged seizures or recurrent seizures

Q1.	Has *(name of child)* ever had an unusual spell lasting 10 minutes or more?	Yes ☐ No **[GO TO Q5]** ☐ Don't know **[GO TO Q5]** ☐ Not applicable ☐ **IF YES, PROBE FOR WHICH TYPE OF UNUSUAL SPELL**	
Q2.	How many unusual spells lasting 10 minutes or more has *(name of child)* had?		
Q3.	How long was the longest unusual spell *(name of child)* had? **RECORD LENGTH IN MINUTES**	minutes	
Q4.	How old was *(name of child)* when *(s/he)* had that unusual spell? **RECORD AGE IN MONTHS IF <2 YEARS, AND IN YEARS IF ≥2 YEARS;**		days ☐ months ☐ years ☐

Q5.	Has *(name of child)* ever had several unusual spells one right after the other?	Yes ☐ No **[GO TO PART 3]** ☐ Don't know **[GO TO PART 3]** ☐ Not applicable □ **IF YES, PROBE FOR WHICH TYPE OF UNUSUAL SPELL**	
Q6.	How old was *(name of child)* when that happened the first time?		days ☐ months ☐ years ☐
Q7	How many unusual spells did *(name of child)* have in a row?		seizures
Q8.	Did *(name of child)* recover fully between unusual spells?	Yes ☐ No ☐ Don't know ☐ Not applicable □	
Q9.	Could you tell me more about that? **PROBE TO DETERMINE HOW CLOSELY TOGETHER THE UNUSUAL SPELLS OCCURRED**		

Part 3: Further information

ASK THE FOLLOWING QUESTIONS ABOUT PART 1 (FEBRILE SEIZURES) AND PART 2 (OTHER UNUSUAL SPELLS)

Section A: Investigation and Medication

Q1. Has (*name of child*) ever been to a GP because of these unusual spells that they have had?			Yes □ No **[GO TO Q2]** □ Don't know **[GO TO Q2]** □ Not applicable □		Further details **PROBE FOR FURTHER DETAILS IF NEEDED**
	Q1A.	How old was (*name of child)* when they first saw a GP because of these (*seizures/*unusual spell*s*)?		days □ months □ years □	Further details **PROBE FOR FURTHER DETAILS IF NEEDED**
	Q1B.	How many times in the last 12 months has *(name of child)* visited this doctor about their unusual spells?			Further details **PROBE FOR FURTHER DETAILS IF NEEDED**

Q2. Has (*name of child)* ever seen a specialist because of these (*seizures/*unusual spell*s)* that they have had?			Yes □ No **[GO TO Q3]** □ Don't know **[GO TO Q3]** □ Not applicable □		Further details **PROBE FOR THE TYPE OF SPECIALIST (E.G. NEUROLOGIST/ PAEDIATRICIAN)**
	Q2A.	How old was (*name of child)* when they first saw a specialist because of these (*seizures/*unusual spell*s*)?		days □ months □ years □	Further details **PROBE FOR FURTHER DETAILS IF NEEDED**
	Q2B.	How many times in the last 12 months has *(name of child)* visited this specialist about their unusual spells?			Further details **PROBE FOR FURTHER DETAILS IF NEEDED**
Q3. Has a doctor or specialist ever suggested a potential cause for *(name of child)*'s unusual spells?			Yes □ No □		Further details

	Don't know □ Not applicable □	
Q4. Has *(name of child)* ever had their unusual spells investigated, for example, with an MRI scan, CAT scan or EEG scan?	Yes □ No □ Don't know □ Not applicable □	Further details **PROBE FOR WHEN, WHERE, RESULT?**
Q5. Has (*name of child*) ever taken any medication for these unusual spells?	Yes □ No [**GO TO Q7**] □ Don't know [**GO TO Q7**] □ Not applicable □	Further details **PROBE FOR FURTHER DETAILS IF NEEDED**

	Q5A.	How old was (*name of child)* when they first started taking medications for these unusual spell*s*?		days □ months □ years □	Further details **PROBE FOR FURTHER DETAILS IF NEEDED**
	Q5B.	Is (*name of child*) still taking medications for these unusual spells now?	Yes [**GO TO Q5C.]** □ No □ Don't know □ Not applicable □		Further details **PROBE FOR FURTHER DETAILS IF NEEDED**
		Q5Bi. How old was *(name of child)* when they stopped taking the medications?		days □ months □ years □	Further details **PROBE FOR FURTHER DETAILS IF NEEDED**
	Q5C.	**IF STILL TAKING MEDICATIONS** Which medications are they currently taking now for their unusual spells? **IF NO LONGER TAKING MEDICATIONS** Which medications did they previously take for their unusual spells?	Medications 1. 2. 3. 4. 5.		

Q6.		(Does/did) taking this medication reduce the frequency of (*name of child's*) unusual spells?	Yes □ No □ Don't know □ Not applicable □	Further details **PROBE FOR FURTHER DETAILS IF NEEDED**
Q7. Has *(name of child)* ever been diagnosed with hypocalcaemia? **PROMPT "LOW LEVELS OF CALCIUM IN THE BLOOD" IF CARER DOESN'T KNOW WHAT THE TERM MEANS**			Yes □ No [**GO TO SECTION B**] □ Don't know [**GO TO SECTION B**] □ Not applicable □	**PROBE FOR PRECISE BLOOD CALCIUM LEVELS IF KNOWN**
	Q7A.	How old was *(name of child)* when they received the diagnosis? **CHECK DAYS, MONTH OR YEARS DEPENDING ON CARER'S RESPONSE**		days □ months □ years □
	Q7B.	Has a doctor or specialist ever suggested that *(name of child's)* seizures were associated with *(his/her)* hypocalcaemia?"	Yes □ No □	Further details

			Don't know □	
	Q7C.	Did (*name of child*) continue to have seizures after their calcium levels returned to normal?	Yes □ No □ Don't know □	<u>Further details</u>

Section B: Wrap-up

So that brings us to the end of our questions.	
Q1.	Is there anything else that you would like to add?
Thank you very much for your participation! This research would not be possible without your help.	

INTERVIEWER'S IMPRESSIONS:

1.	**RESPONDENT'S UNDERSTANDING OF QUESTIONS**	Excellent □ Good □ Adequate □ Poor □
2.	**OTHER IMPRESSIONS**	

Supplementary interview conducted with the child

How are you completing this interview?

- ○ With participant present
- ○ With participant via telephone

Child ID:

Child DOB:

Child age:

Child gender (M/F):

Date of interview : ____ ____ /____ ____ /____ ____ ____ ____

D D M M Y Y Y Y

Section A: Description of Auras.

I'm going to talk to you about the *(seizures/unusual spells)* that you have experienced. I'd like to check a few things about them with you, if that's ok.

Your mother/father told us that you: **GIVE SUMMARY OF SEIZURE /UNUSUAL SPELL IDENTIFIED IN THE CAREGIVER INTERVIEW**	**Q1.** *a)* Can you tell me a little bit more about this *(name of spell)*? b) In your own words, can you tell me what happens, or how do you feel, before this *(name of spell)* starts?
1.	
2.	
3.	

4.	
5.	
6.	

Section B: Seizure triggers

Q3. Does anything seem to make you have, or set off, your *(seizures/unusual spells)*? **[AFTER RESPONSE, PROBE]:** Anything else? **[IF CHILD IS UNSURE, PROBE]:** such as flashing or blinking lights, reading or being touched?

References for Appendices

1 Ottman, R., Hauser, W. A. & Stallone, L. Semistructured interview for seizure classification: agreement with physicians' diagnoses. *Epilepsia* **31**, 110-115 (1990).

2 Ottman, R. *et al.* Reliability of seizure classification using a semistructured interview. *Neurology* **43**, 2526-2526 (1993).

3 Collaborative, E. The epilepsy phenome/genome project. *Clinical trials* **10**, 568-586 (2013).