Sleep problems in young people with 22q11.2 Deletion Syndrome: associations with the neurodevelopmental phenotype

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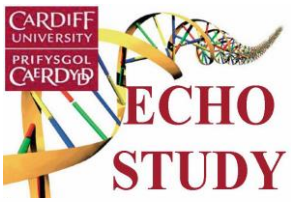

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Contributions

I carried out all literature reviews and summarised all background information for the projects contained within this thesis.

With regards to the sleep problems analysis from the Child and Adolescent Psychiatric Assessment (CAPA), I completed all of the analyses including the calculation of all scores and diagnoses. I carried out all analyses and interpreted the results.

Psychiatric and cognitive data was predominantly collected by members of the ECHO field team and as part of visits to family's homes and clinical visits at Cardiff. Sarah Law, Dr Samuel Chawner and Dr Jo Doherty were responsible for many of the wave 2 visits and Sinéad Morrison primarily the wave 3 visits. I conducted approximately 12 psychiatric interviews and six cognitive assessments. I derived and summarised all psychiatric and cognitive data and compiled these into usable variables for my analyses. I assisted in coding and double-coding many psychiatric assessments providing research diagnoses. Ali Baird, Rachael Adams, Jacqui Smith, Bethan Hughes, Vera Shroeter, Kali Barawi and Ffion Evans were responsible for collecting the adult data in **Chapter 3**. I carried out all of the statistical analyses reported in **Chapter 3**.

I wrote the ethics for the pilot study and the main EEG study along with Christopher Eaton (PhD student) and recruited all of the participants for both studies. Ulli, Chris and I designed the pilot study protocol in **Chapter 4** and then developed the main study protocol in **Chapter 5**.

In **Chapters 6** and **7**, for the sleep studies, there were 45 visits completed in which I conducted approximately 25 of these visits over weekends and during school holidays with students. The

other visits were led by Chris. Ullrich Bartsch also assisted in the sleep study overnight visits and myself, Chris and Ulli were responsible for the collection of the EEG data. Students including Rachel Tompkins, Stephen Naughton, Ciara Walker, Nadia Pantouw, Poppy Sloane, Kezzie Fish, Alice Walsh and Amy Isley assisted in the acquisition of data, actigraphy data and set-up of the overnight EEG. Sinéad Morrison, Sarah Law, Hayley Moss, Ali Baird, Jacqui Smith and Sophie Minton assisted in visits also, often when sleep study visits were combined with the collection of ECHO study psychiatric and cognitive data.

I interpreted all of the actigraphy data and carried out all of the statistical analyses in **Chapter 6**. This included both creating and developing the sleep diaries as well as the calibration of actigraphy watches, as well as interpreting the data and conducting analyses. The data was checked twice to ensure consistency of scoring.

In **Chapter 7**, Dr Nick Donnelly processed the EEG study data using the PREP pipeline. Once the EEGs were prepared, I scored the sleep EEG data for each person, and assimilated each of the files for further analyses. This included scoring 45 nights of sleep EEG, scoring for each stage of sleep and removing artefacts manually. Ulli and Nick provided MATLAB guidance and scripts. I have conducted all other analyses.

All work was carried out according to the guidance of my supervisors, Prof. Marianne van den Bree, Sir Prof. Michael Owen and Prof. Jeremy Hall, and the EEG data analyses were also overseen by Dr Ulli Bartsch, Dr Nick Donnelly and Prof. Matt Jones. Manuscripts arising from the work in **Chapter 4** were commented on by my supervisors along with Dr Ulli Bartsch, Prof. David Linden and Prof. Matt Jones.

Publications based on thesis

Hayley A Moulding, Ullrich Bartsch, Jeremy Hall, David E. Linden, Matthew Jones, Michael J Owen and Marianne B van den Bree. **Sleep problems and associations with psychopathology and cognition in young people with 22q11.2 deletion syndrome (22q11.2DS).** (accepted for publication in *Psychological Medicine* in April 2019)**.**

Summary

This is the first thesis to explore sleep problems in young people with 22q11.2 Deletion Syndrome. This thesis includes both subjective and objective methods of sleep assessment which have contributed to furthering academic knowledge of sleep problems in 22q11.2DS.

Firstly, the prevalence, nature and severity of sleep problems in a sample of young people with 22q11.2DS compared to their unaffected siblings was determined. The prevalence of sleep problems was high in young people with 22q11.2DS (~60%) and that the high preponderance remained stable in small subsamples of toddlers, adolescents and adults. The nature of sleep problems in the young people with 22q11.2DS was elucidated, showing that more sleep problems associated with poorer psychopathology and cognitive impairment.

Building on the foundations of the subjective assessment of sleep, objective measures were introduced to begin to explore the physiology of sleep in these young people. A pilot study with a small typically developing sample of young people was conducted to assess the feasibility of an overnight sleep study. Resultantly, an overnight sleep study was developed in a subsample of young people with 22q11.2DS and their unaffected siblings which included polysomnography (PSG), two-week actigraphy assessments with a sleep diary and validated sleep questionnaires. Actigraphy-derived sleep parameters were compared between 22q11.2DS and siblings showing some differences, in addition to differences in sleep parameters between the objective and subjective methods of assessment. Investigations into sleep architecture were few and small but provided a basis to build from when exploring the neurophysiology of sleep in young people with 22q11.2DS. Overall, the results from this thesis could have important implications for understanding sleep and its relationship with the neurodevelopmental phenotype in 22q11.2DS.

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Chapter 1 – Introduction: 22q11.2 Deletion Syndrome

1.1 Copy Number Variation

Variability in the human genome is fundamental for evolution. The DNA sequences of any two human individuals are nearly identical with 99.9% comparability (National Human Genome Research Institute, 2018), yet it is the variation of the genome that contributes to the vast gene pool and diversity of the human race. There are around 20,000 genes in the human genome, and several mechanisms that underlie individual differences in genomic structure and function.

Mutation is a term which can be interpreted both as a verb and a noun. As a verb, mutation can be a process of change whereas as a noun a mutation is used to define variants with a minor allele frequency of >1% (Loewe and Hill, 2010). Variants with a minor allele frequency of >1% include single nucleotide polymorphisms (SNPs). There are ~3 million SNPs identifiable in the human genome (Barreiro *et al.*, 2008). A SNP can occur in any position throughout the genome: the position of the SNP influences the effect that it can have on encoding aberrant proteins and increasing the susceptibility to developing a certain disease. An example of this is the ApoE encoding protein increasing likelihood of Alzheimer's disease (Hyman *et al.*, 1996). They can also be benign, for example a SNP in the melanocortin-1 receptor on chromosome 16 that gives rise to red hair.

Mutational events can also affect multiple genes; these multi-genic changes are also abundant across the genome. Variations of a multi-genic nature are more likely than mutations in a single gene to impact many different cell types, tissues and organ systems.

Copy number variation (CNV) is an example of multi-genic variation in the genome. A CNV refers to a duplication, deletion or translocation of a segment of DNA that is 1 kilobase (kb) or larger (Thapar and Cooper, 2013). The affected section of DNA appears at a variable copy number: there are either more or fewer sections of DNA compared to the baseline human genome. A CNV can be detected as a more simple duplication of a segment of DNA or as a more complex change such as deletions or duplications of homologous DNA sequences at different sites across the genome (Redon *et al.*, 2006). CNVs can be found in allosomes and autosomes alike, occurring in any of the 23 pairs of chromosomes in humans. For example where an additional 'X' allosome is present in the genome of males in Klinefelter Syndrome (Bhartia, Ramachandran and Ramachandran, 2012); an example of a CNV syndrome resulting in multi-system changes, especially in skeletal and endocrine systems. Changes to the genome including deletions and duplications tend to be detected less frequently than aneuploidies (Feuk, Carson and Scherer, 2006) (changes in the total number of chromosomes, for example trisomy 21 which is Down syndrome). However, CNV events are rarer in the human genome than smaller, individual nucleotide changes. The rarer events tend to have greater impacts such as aberrant protein expression and haploinsufficiency, where one chromosome in a chromosome pair is deleted and the remaining intact chromosome has to compensate (Prasad, Howley and Murphy, 2008).

CNVs are present in every human and can occur across the whole genome. There are areas of the genome known as 'mutation hotspots' where CNVs occur most frequently. Highly homologous sequences of DNA known as low copy repeats (LCRs) (Shaikh *et al.*, 2000) are present at these hotspots. When there are two copies of LCRs within 10 megabases of each other, there is a greater change of misalignment of paired chromosomes during meiosis.

When this occurs, typical genomic recombination is disrupted, resulting in deletion or duplications of the DNA region between the LCRs (Rosenberg and Pascual, 2015). This process is known as non-allelic homologous recombination (NAHR).

Some CNVs can result in elevated risk for debilitating disorders and are therefore considered pathogenic. For instance, the deletion on chromosome 3q29 is the largest known biological risk factor for the development of schizophrenia (Kirov *et al.*, 2012; Szatkiewicz *et al.*, 2014). Genetic syndromes caused by a CNV impact an individual unpredictably. This so-called pleiotropy suggests that the phenotypic outcome is modified by other genetic or environmental factors.

1.2 22q11.2 Deletion Syndrome Background

22q11.2 Deletion Syndrome (22q11.2DS) is a relatively common microdeletion syndrome, characterised by the hemizygous deletion of ~50 genes (Swillen and McDonald-McGinn, 2015) on the long (q) arm of chromosome 22. The prevalence of 22q11.2DS is uncertain with estimated rates varying from 1 in 2,000-4,000 live births (Wilson, Cross and Wren, 1994; Tezenas du Montcel *et al.*, 1996; Swillen and McDonald-McGinn, 2015). Some studies have reported rates as high as 1 in 992 in *in utero* testing (Grati *et al.*, 2015). 22q11.2DS can be tested for using array comparative genomic hybridisation (CGH) (Theisen, 2008). Array CGH is a method for detecting genomic CNVs using fluorescence marking, for example a green fluorochrome, of a DNA base. A reference DNA sample is tagged with the opposite fluorochrome (red fluorochrome) and therefore, once the DNA extract is processed, the arrays can be imaged and the fluorescence can be measured. Based on the ratio of the fluorescence, CNVs can be detected (Ahn *et al.*, 2015). Indicators for clinical testing could be

a new-born presenting with cardiac abnormalities prenatally or soon after birth (M.-Y. Lee *et al.*, 2014) or the presentation of cleft palate (Driscoll *et al.*, 1992). Another indication is developmental delay, which would generally be established somewhat later in childhood (Miller *et al.*, 2010).

Within the 22q11.2 region there are four LCRs in which deletions can occur. In ~86-90% of individuals with 22q11.2DS there is a 'typical' 3-megabase (Mb) deletion between LCR22A and LCR22B (Edelmann, Pandita and Morrow, 1999); there are approximately 7-10% that have a 1.5-Mb region (Amati *et al.*, 1999) and ~3% of individuals have an atypical deletion at the loci on chromosome 22 (Emanuel, 2008). The 1.5-Mb deletion has helped to define a 'critical region' for the behavioural phenotype in 22q11.2DS based on the 28 genes within this smaller region. The critical region could be smaller still but more work is needed to define this (Williams, 2011).

Individuals that have the smaller 1.5-Mb deletion have been shown to share a common proximal breakpoint with individuals with the 3-Mb deletion. However, it is the nested distal deletion breakpoint that distinguishes the deletion (Funke *et al.*, 1999). Evidence suggests that the shared critical region from both deletion sizes presents as a decreased gene dosage which is associated with craniofacial abnormalities, conotruncal heart defects and has shown to associate with learning disabilities (Funke *et al.*, 1999).

The majority of cases of 22q11.2DS, ~93%, are *de novo* (McDonald-McGinn *et al.*, 2001), occurring spontaneously during meiosis. The remaining 7% are inherited from a parent with 22q11.2DS. This does not necessarily suggest that the parent was aware that they had 22q11.2DS. The 22q11.2DS adult study at Cardiff University (https://www.cardiff.ac.uk/neuroscience-mental-health/research/projects/the-wellcome-trust-strategic-award-define-

grant) found that some adults did not receive their diagnosis until after their child was diagnosed with 22q11.2DS. Due to the variable phenotype, some adults might experience only minor features of 22q11.2DS and never realise they carry the CNV until they are screened because their child is diagnosed. The understanding of how varied the phenotype of 22q11.2DS is remains limited. Many adults may still remain without a diagnosis, especially if the individual was born before particular symptoms could be identified, which prompts for further testing (McDonald-McGinn *et al.*, 2001). For example, older individuals may have presented with congenital heart problems or developmental delay in their childhoods, however at the time of presentation, clinical understanding and capacity to refer these individuals to genetic clinics for genetic testing would have been limited. Techniques for the identification of CNVs would also have been limited. There was and currently is no routine testing for 22q11.2DS and existing clinical guidelines for treating individuals with 22q11.2DS are focussed on children, therefore prompting the development of adult-specific clinical practice guidelines (Fung *et al.*, 2015).

The varied phenotype has historically caused difficulty in diagnosing 22q11.2DS. There are over 180 distinct clinical manifestations that have been associated with 22q11.2DS (Furuya *et al.*, 2015). These variable outcomes amongst individuals contributes to confusion and to begin with different disorders were suspected. Initially certain clusters of outcomes that presented in conjunction with each other such as heart defects with facial dysmorphia were recognised. For instance, a collection of features including conotruncal heart defects (interrupted aortic arch and tetralogy of Fallot for example), calcium deficiency, immune suppression due to thymus gland defects and occasionally a cleft palate was described as 'DiGeorge syndrome' (Demczuk and Aurias, 1995). Conotruncal anomaly face syndrome (CTAF) featured typical

facial appearances in patients with conotruncal heart abnormalities; Velocardiofacial syndrome (VCFS) or Shprintzen syndrome linked CTAF diagnoses with DiGeorge syndrome diagnoses resulting in the commonly used term VCFS. When overlaps were subsequently found between VCFS and DiGeorge syndrome it became clear that most patients with VCFS had a deletion at 22q11.2 suggesting the same genotype to DiGeorge syndrome patients. This resulted in DiGeorge and VCFS becoming the same diagnosis, but other chromosome 22 deletion syndromes remained separate from this combined diagnosis. Other disorders unified by a 22q11.2 deletion include CTAF, autosomal dominant Opitz G/BBB syndrome, Cayler cardiofacial syndrome and Sedlackova syndrome. In the field of genetic research, the term 22q11.2DS is now used to represent a syndrome characterised by the underlying genetic lesion rather than the collection of symptoms (McDonald-McGinn *et al.*, 2015).

Inconsistent presentation of the syndrome and the number of different phenotypic features can still affect timely diagnoses. There are characteristic physical features that are most commonly used as the indicators for genetic testing at birth. Not all individuals have these features, yet those that do often have such physical manifestation as cleft palate, hypotonia, facial dysmorphisms and conotruncal cardiac defects, particularly tetralogy of Fallot to name but a few (McDonald-McGinn, Emanuel and Zackai, 1993).

Many individuals with 22q11.2DS have thickened keratinous features such as voluminous hair and rapidly growing nails. The extracellular differences in structures are also clear in the facial dysmorphisms and skeletal problems for which leg splints and walking aids are often needed. The facial features can include malar flatness, fullness of eyelids, tubular nose, round ears, broad nasal tip, thick helix and low-set ears (Skarsdttir *et al.*, 2008). Many of the physical manifestations such as cleft palate can be surgically repaired, usually during childhood and

early adolescence. Septal heart defects are often repaired as a neonate. The earlier the intervention, the earlier other associated traits such as speech and language development can be corrected.

In some individuals, the prevalence of physical manifestations is high suggesting that physical assistance might be required including wheelchairs and/or crutches, and continuous positive airway pressure (CPAP) machines. In other individuals, physical problems present in combination with a psychiatric phenotype, which can include developmental problems and possibly neurological conditions including seizures and epilepsy. Epilepsy and seizures are neurological features of 22q11.2DS with rates of childhood seizures above the typical rates (Vestergaard and Christensen, 2009). One study found that 7% of participants had unprovoked epileptic seizures (Kao *et al.*, 2004), but another reported a rate as high as 15.2% (Kim *et al.*, 2016). Seizures have a range of different underlying causes and many are present in 22q11.2DS: hypocalcaemia; adverse effects of antipsychotic drug use; hyperthyroidism and hypothalamic temperature dysregulation resulting in high fever.

Much of the work currently conducted on the neuropsychiatric manifestations of 22q11.2DS is focussed on longitudinal assessment. Following of children from a young age over time can show the development of pathologies and morbidities. These critical periods are vulnerable to increased risk of psychiatric morbidity, which through the investigation of the adolescent period, can lead to a greater understanding of the developmental trajectory of 22q11.2DS. Neurology and physiology of individuals with 22q11.2DS is under-represented in current research, although a large-scale neuroimaging study focusing on the use of fMRI has begun to reveal neuroanatomical changes (Sun *et al.*, 2018). More work in the field of neurophysiology and neuroanatomy will help to develop an understanding of the underlying

neural changes that are hypothesised as the foundation of the manifestation of some of the developmental, cognitive and psychiatric deficits seen in 22q11.2DS. Creating a complete description of the variable phenotype in 22q11.2DS can help in the understanding of individual variation and possible treatments and interventions throughout life.

1.3 The Neurodevelopmental Phenotype of 22q11.2DS

Neurodevelopment is a term which can broadly include a wide range of disorders and problems: there is much debate around what the term itself includes and defines. In this thesis, the phrase 'neurodevelopmental phenotype' is being used to define a broad group of disorders which involve disruption to brain development. The disorders which are namely defined by the term 'neurodevelopmental' are Attention-deficit hyperactivity disorder (ADHD), Autism spectrum disorders (ASD), Developmental coordination disorder (DCD) and learning disorders (cognitive impairments) (Thapar, Cooper and Rutter, 2017).

However, there is evidence to suggest that the term can be used more broadly to define other related problems. These include rare genetic syndromes and as a result of this, it is this perspective that has been adopted throughout this thesis. This thesis uses the phrase 'neurodevelopmental phenotype' to include ADHD, ASD, DCD and cognitive impairments, in addition to commonly occurring childhood psychiatric disorders including anxiety disorders, oppositional defiant disorder (ODD) and conduct disorder (CD) (Ollendick *et al.*, 2008).

The definition in this thesis recognises the psychiatric and cognitive morbidities common in 22q11.2DS as part of the 'neurodevelopmental phenotype' (ADHD, ASD, DCD, anxiety disorders, CD and ODD and intellectual disability).

It is important to add that I recognise that including the psychiatric morbidities in the definition of the neurodevelopmental phenotype is not necessarily a widely used definition. I have taken the approach to use 'neurodevelopmental phenotype' in a broader and wider sense to be able to use it consistently throughout this thesis in reference to the young people with 22q11.2DS.

Evidence derived from the paper by Mullin, A.P. et al. (Mullin *et al.*, 2013) explores the definition of neurodevelopmental disorders and the contributions from the genome and specifically the genotype which is of relevance to the 22q11.2DS population in this thesis. Further evidence explored by Thapar, A. et al. outlines that the term neurodevelopmental has been used to broadly define a group of disabilities which commonly have not been regarded as a neurodevelopmental disorder including neurological, psychiatric and cognitive problems (Thapar, Cooper and Rutter, 2017).

For this thesis, the definition has been formulated based on the underlying genetic predisposition to these morbidities and can be used to simplify matters when referring to the number of different morbidities which are often compared throughout this thesis.

There are many psychiatric morbidities in 22q11.2DS which often create a complex presentation. Psychiatric morbidities such as anxiety disorders can be coupled with developmental problems such as ADHD as well as cognitive impairments and learning disability. Comorbid diagnoses are common in individuals with 22q11.2DS with 54% of children meeting diagnostic criteria for one or more psychiatric disorders (Niarchou *et al.*, 2014).

The multitude of problems follow varying trajectories over time. There is evidence to suggest that the prevalence of mood disturbance increases in adolescence (Gothelf, Penniman, *et al.*, 2007), the incidence of ADHD decreases with age (Niarchou *et al.*, 2018) and that psychotic symptoms emerge in adolescence and early adulthood (Chawner *et al.*, 2019). Many of the following problems feature in the psychiatric phenotype of 22q11.2DS, often presenting comorbidly. This section outlines those psychiatric and neurodevelopmental morbidities with most relevance to sleep problems.

1.3.1 Anxiety Disorders

Anxiety disorders are a collection of a number of diagnoses including generalised anxiety disorder (GAD), obsessive compulsive disorder (OCD), specific phobias, social phobias and separation anxiety and panic disorder (Barton *et al.*, 2014). Rates of anxiety disorders in typically developing young people range from 9% to 32% (Essau and Gabbidon, 2012; Creswell, Waite and Cooper, 2014). Symptoms of anxiety can refer to significant worrying, unpleasant feelings and thoughts but will differ dependent on the diagnosis. Anxiety symptoms in research can be recorded from self-reports and semi-structured interviews (Angold *et al.*, 1995).

Anxiety disorders are commonly diagnosed in young people with 22q11.2DS with rates ranging from 40-76% (Niarchou *et al.*, 2014; Schneider *et al.*, 2014). Rates of anxiety disorders in 22q11.2DS were consistently higher than the unaffected sibling controls. A recent literature review identified that anxiety disorders in 22q11.2DS are found to have an average prevalence of ~39% of individuals. This review showed specific phobia as the most frequent type of anxiety disorder followed by generalised anxiety disorder (Bertrán, Tagle and Irarrázaval, 2018).

Anxiety and the propensity for mood changes can worsen with age and during puberty (Romeo, 2013). There is dysregulation of the endocrine system in 22q11.2DS shown by the elevated presence of hyperthyroidism and hypocalcaemia. These hormonal imbalances could potentially contribute to the elevated prevalence of neurodevelopmental disorders (NDDs) and anxiety for young people with 22q11.2DS (Swillen and McDonald-McGinn, 2015). There has also been evidence in the general population of pronounced gender differences in rates of internalising disorders which become pronounced during puberty, showing higher rates of affective disorders in females than males, suggesting hormonal changes could be a contributory factor (Hayward and Sanborn, 2002).

Young people with 22q11.2DS and comorbid anxiety have a six-times higher risk of schizophrenia when compared to young people with 22q11.2DS without a comorbid anxiety disorder (Schneider *et al.*, 2014; Bertrán, Tagle and Irarrázaval, 2018). Anxiety disorders in 22q11.2DS differ from schizophrenia as they are present at high rates in young people whereas the high preponderance of psychotic symptoms is apparent in adolescence/early adulthood. There is evidence showing that the level of anxiety symptoms in childhood is associated with continued anxiety and severe chronic mood disorder across the lifespan (Gothelf, Feinstein, *et al.*, 2007; Fabbro *et al.*, 2012). Anxiety symptoms can have an impact on well-being, educational attainment, daily routines and impairment of social performance and ability to function in a family and/or school setting (Debbané *et al.*, 2006). In individuals without 22q11.2DS, these symptoms are significant in themselves with anxiety causing substantial disruption to day-to-day routine and debilitating symptoms in typical individuals (Devine, Kempton and Forehand, 1994).

However, these problems are exacerbated in young people already at elevated risk for developmental delay, social and communication impairment and behavioural problems. One study of a small cohort of young people with 22q11.2DS (n=62) showed that anxiety was identified in 62.2% of individuals. Specific phobia was the highest represented anxiety disorder across all age groups and was diagnosable in 66.7% of adolescents in the study (Fabbro *et al.*, 2012).

In young people with 22q11.2DS, parents routinely report anxiety in their children. Assessing symptoms of anxiety against the DSM-IV-TR guidelines, many different anxiety disorders can be identified. These include GAD, specific phobias, social phobias and separation anxiety (Baker and Skuse, 2005; Niarchou *et al.*, 2014; Schneider *et al.*, 2014). The anxieties can manifest differently in young people with 22q11.2DS leading to outcomes such as withdrawal, avoidance, excessive worry or through self-harming including cases of trichotillomania and skin-picking (Tang *et al.*, 2015).

The aetiology of anxiety disorders in 22q11.2DS remains unknown. One problem that is often reported by parents is that young people with 22q11.2DS frequently go to hospital (Bassett *et al.*, 2011), may have to undergo one or more surgeries and have numerous medical tests and immunisations. This can contribute to unstable home and school lives. Anecdotal evidence suggests that there are two groups of young people with 22q11.2DS: those who have become familiar with hospital surroundings and seemingly do not mind having medical tests and those young people who have an overwhelming phobia of the sight of blood, injections and being in the vicinity of a hospital. These situations themselves can cause or exacerbate anxieties with physiological and psychological stress at a young age as a marker for later onset of psychological problems and behavioural problems (Bonn, 1994).

Furthermore, a longitudinal study across two time points showed that the presence of anxiety symptoms in childhood is associated with the later emergence of psychotic experiences in adolescence (Chawner *et al.*, 2019). Cross-sectional assessments outlined more about the comorbid nature of anxiety disorder diagnoses; 90% of young people with 22q11.2DS and an anxiety disorder diagnosis had comorbid Developmental Coordination Disorder (DCD) in (Cunningham *et al.*, 2017). In cross-sectional analysis, the young people with poorer scores on the DCD questionnaire also reported more anxiety symptoms (Cunningham *et al.*, 2017).

1.3.2 Attention-Deficit Hyperactivity Disorder (ADHD)

ADHD is one of the more common neurodevelopmental disorders and is considered to affect around 1.4-3% of children (Thapar and Cooper, 2016) in the general population. The diagnosis rate is often skewed dependent on geography with higher rates of diagnosis in western societies and also has a higher preponderance amongst boys (Taylor *et al.*, 2016). Despite ADHD being diagnosed most commonly in childhood, it can be a lifelong disorder and persists into adulthood (Watters *et al.*, 2017) with one study suggesting that 41% of individuals with ADHD experienced symptoms into young adulthood (Sibley *et al.*, 2017). There is evidence showing the continued presence of ADHD into adulthood despite a reduction in the symptom severity (Eyre and Thapar, 2014).

Previously portrayed as a behavioural manifestation of poor parenting and general insolence from a child seen as 'naughty' (Timimi and Leo, 2009), the perception of young people who are diagnosed with ADHD has changed in the 21st century. Gene-environment interactions were identified through behavioural genetic and molecular genetic studies (Faraone *et al.*, 2005) with evidence of genetic associations with ADHD in the dopaminergic, serotonergic and noradrenergic neurotransmitter systems. These studies suggested that genetic and non-

genetic factors (Faraone *et al.*, 2005) were important in the development of ADHD. Twin studies were used to explore the heritability of ADHD (Thapar *et al.*, 2000) with high heritability evidence from parent reports, which alongside teacher-rated categories, also provided evidence for shared environmental effects. Wider heritability studies ultimately provided evidence of the mean heritability estimate of 76% demonstrating ADHD was one of the most heritable psychiatric disorders (Faraone *et al.*, 2005).

On recognising that there was a genetic aetiology and disposition for the manifestation of ADHD, it was important to identify what genetic lesions were contributing to the manifestation of ADHD. Genome-wide analysis studies of ADHD showed large, rare copy number variants were identified in children with ADHD at a higher rate than typically developing controls (Williams *et al.*, 2010). This provided evidence for enrichment of genetic lesions in young people with ADHD but in particular rare genetic lesions as evidence for common genetic variants already existed and has continued to be built upon (Demontis *et al.*, 2017). Ongoing investigations have shown the importance of studying the gene-environment interactions and contributions from both genes and the environment (N. M. Williams *et al.*, 2012).

ADHD is characterised by three subtypes: 1) inattentive which includes difficulty in concentrating and organising tasks and making careless mistakes, 2) hyperactive-impulsive which includes restlessness and acts like they are 'driven-by-a-motor', 3) and combined, where the latter indicates the presence of symptoms of both the inattentive and hyperactive-impulsive subtypes (Graeta *et al.*, 2001). In 22q11.2DS, the rate of ADHD has been reported to range between 12-68% (Schneider *et al.*, 2014) and the inattentive subtype of ADHD is the

most prevalent in 22q11.2DS (Schneider *et al.*, 2014; Niarchou, J. Martin, *et al.*, 2015; Niarchou *et al.*, 2018).

The rates of ADHD in young people with 22q11.2DS were found to reach ~37.1% in the International Brain and Behaviour Consortium (IBBC) and continued incidence of ADHD into adulthood was shown, with evidence of 15.6% of adults in the consortium having diagnosable ADHD (Schneider *et al.*, 2014; Niarchou, J. Martin, *et al.*, 2015; Niarchou *et al.*, 2018). ADHD in 22q11.2DS shows a male preponderance (Schneider *et al.*, 2014) reflecting what is seen in the general population (Boat *et al.*, 2015)

In non-genotyped (idiopathic) ADHD, there is a high comorbid rate of conduct disorder (Boat *et al.*, 2015). This is not reflected in individuals with 22q11.2DS: fewer individuals have comorbid diagnoses of conduct disorder (CD) and oppositional defiant disorder (ODD) but despite this, there are higher rates of comorbid anxiety disorders (Niarchou, J Martin, *et al.*, 2015; Niarchou *et al.*, 2018) in young people with 22q11.2DS compared to the non-genotyped ADHD population. This distinction suggests that ADHD in 22q11.2DS presents differently to idiopathic ADHD. This can also be reflected in the treatments for ADHD in the idiopathic population compared to 22q11.2DS.

There are a small number of studies investigating the efficacy of methylphenidate medication for the symptoms of ADHD in 22q11.2DS. A randomised controlled trial in 34 young people with 22q11.2DS with a comorbid ADHD diagnosis showed that methylphenidate treatment could be safe and effective after a six-month treatment schedule. This trial showed a reduction of 40% in ADHD parent-reported symptomology of the young child with 22q11.2DS (Green *et al.*, 2011). Another study showed that behavioural and cognitive impairments improved substantially after treatment with methylphenidates, with minimal adverse effects

(Gothelf *et al.*, 2003). The study sample sizes and time-scales limit interpretation, suggesting further studies are required to examine the use of stimulants to treat ADHD symptoms in 22q11.2DS (Niarchou *et al.*, 2018).

A cross-sectional study showed that 41% of the 22q11.2DS cohort had an ADHD diagnosis (Niarchou *et al.*, 2014). An association between risk for psychopathology (including an ADHD diagnosis) and IQ was shown via a reduction in IQ to elevated risk of psychopathology. The longitudinal course of ADHD prevalence, showing a reduction in prevalence in individuals with 22q11.2DS with age, aligns with the typical idiopathic population (Langley *et al.*, 2010). Study into the long-term persistence of ADHD into the adult population with 22q11.2DS will help to develop more appropriate and targeted treatments.

1.3.3 Autism Spectrum Disorders (ASD)

Autism spectrum disorder (ASD) in the general UK population has an estimated prevalence of about 1.1% with a higher preponderance in males (Reid and Ayris, 2011). ASD covers a range of diagnoses that, before the release of The Diagnostic Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (Kocsis, 2013), were classified as separate NDDs (*DSM-5 Criteria | Autism Speaks*, 2013). The diagnosis is inclusive of pervasive developmental disorder and Asperger's syndrome and ties together the diagnoses because of their overlapping morbidity and symptomology. ASD is often considered to consist of subdomains which better explain the difficulties individuals have: social interaction impairment, communication deficits and restrictive and repetitive behaviours (Bishop, 2003). An ASD diagnosis can be determined by gold-standard diagnostic tools such as the Autism Diagnostic Interview-Revised (ADI-R) (Rutter *et al.*, 2005) and the Autism Diagnostic Observation Schedule (ADOS) (Lord *et al.*, 2002). These techniques involve a clinician or trained health professional interviewing the

parent of the child in the case of the ADI-R or observations of the child as well as interviews with the parents in the case of the ADOS. The tools are dependent on inter-rater consensus.

In 22q11.2DS, rates of ASD range from 10 to 50% (Niklasson *et al.*, 2001; Vorstman *et al.*, 2006). However, there is a debate in the literature about whether autism spectrum disorder traits in 22q11.2DS are indicative of an ASD diagnosis, or because of the wide-range of comorbidities in 22q11.2DS, the symptoms themselves could be associated with an ASD phenotype but not indicative of an ASD diagnosis. For example, poor communication could be indicative of ASD or other morbidities that present in 22q11.2DS such as intellectual disability, selective mutism and developmental delay (Fine *et al.*, 2005; Vorstman *et al.*, 2006; Antshel *et al.*, 2007; Ousley *et al.*, 2017).

One study that included the ADI-R showed that 42% of the 22q11.2DS sample (n=41) met diagnostic criteria for ASD (Antshel *et al.*, 2007) whereas another study with a sample size twice as large (n=98) showed elevated ASD symptoms in 20% of children with 22q11.2DS and only 11.2% (n=11/98) were deemed to have a clinically accurate diagnosis of ASD despite 14.3% (n=14/98) being established to have a diagnosis of autism from the ADI-R (Fine *et al.*, 2005).

One study showed that in young people with 22q11.2DS and ASD, 94% had a co-occurring psychiatric disorder compared to a rate of 60% in young people with 22q11.2DS without ASD (Antshel *et al.*, 2007). However, another study suggested that the high prevalence of ASD was inaccurate and that comorbidities like anxiety disorders need to be accounted for when diagnosing ASD. Furthermore, diagnostic variability among studies results in no common universal diagnostic tool or scale which is comparable and routinely used (Angkustsiri *et al.*, 2014).

Additionally, studies have investigated whether autistic traits in childhood could predict risk for development of psychotic traits in adulthood. A cohort of young people with 22q11.2DS (n=60) showed that 26.7% of those with ASD later developed psychotic symptoms (Vorstman *et al.*, 2006).

1.3.4 Developmental Coordination Disorder (DCD)

Developmental Coordination Disorder (DCD) is defined by the American Psychiatric Association (APA) as a disorder including features associated with impaired development of motor coordination (both gross and fine). Such impairments in motor movements can be identified through motor assessments undertaken by neurologists. There are particular indicators which can suggest that an individual could have DCD. Such indicators can include the absence of meeting particular milestones throughout development. Delayed milestones can include achieving standing and walking at a later age compared to a normative distribution of children (Kirby and Sugden, 2007). Presentations of impaired performance in activities requiring hand-eye coordination such as sporting activities, as well as poor handwriting can also be indicative of DCD. Problems pertaining to DCD can often manifest in impaired academic performance for the young person.

DCD rates in children have been shown to reach 4-5% in one population-based study involving the Movement ABC Battery (Wright and Sugden, 1996) and up to 6% when accounting for the APAs diagnostic criteria (Kirby and Sugden, 2007). Much like young people with 22q11.2DS, children with DCD have been shown to have a range of neurodevelopmental comorbidities such as ADHD (Kirby and Sugden, 2007).

DCD can be assessed by the Movement ABC Battery, an objective tool which is the most widely used. The Movement ABC identifies and defines impairments on motor performance

of children and adolescents with comparative norms. This is done by exploring the fine and gross movements of a child such as assessing their ability to catch a small beanbag on two feet, then introducing different parameters such as on one foot.

Functional MRI has shown anatomical changes in the brains of individuals with 22q11.2DS. There is evidence for widespread neurological changes in young people with 22q11.2DS that include increased cortical surface area in the sensorimotor cortex (Sun *et al.*, 2018) and reduced cerebellum size showing association with motor function. These changes could be associated with the motor deficit shown in 22q11.2DS individuals.

The parent-completed Developmental Coordination Disorder Questionnaire (DCDQ) is a screening tool used for DCD. This 15-item scale includes questions asking about whether the young person has reached milestones including tying their own show laces or fastening their buttons. The scale is reversed and therefore, scoring high on the DCDQ correlates with better coordination and fewer motor problems. In a cross-sectional study of indicative DCD symptoms, motor coordination problems were identified in 80% of young people with 22q11.2DS in this cohort (Cunningham *et al.*, 2017). It was also seen that DCD presents comorbidly with the neurodevelopmental phenotype showing that poorer coordination was associated with elevated symptoms of anxiety, ADHD and ASD. Looking at the young people with ADHD and 22q11.2DS, 100% met criteria for indicative DCD (Cunningham *et al.*, 2017). Poorer sustained attention was associated with motor coordination also, resulting in a complex presentation.

1.3.5 Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD)

Oppositional Defiant Disorder (ODD) is characterised by oppositional behaviour including rule breaking, disobedience, annoying behaviour, vindictive behaviours and promise breaking.

Oppositional behaviours tend to be relatively 'low-level' problems that can often be controlled. These behaviours include often and easily losing one's temper and deliberately annoying or upsetting people which is found in DSM-5 criteria (American Psychiatric Association, 2013). Violence is not common in ODD but can be indicative of Conduct Disorder (CD). CD is characterised by symptoms including deception and activities involving violence such as vandalism, arson or violence enacted towards someone or something such as fighting or cruelty to animals.

Rates of ODD and CD in young people in the general population are estimated to occur at a rate of below 4% for both disorders (Boat *et al.*, 2015). Cross-sectional analysis of a cohort of young people with 22q11.2DS showed that 18.8% of young people with 22q11.2DS had an ODD diagnosis compared to 0% with a CD diagnosis (Niarchou *et al.*, 2014). This reflects similar evidence suggesting the prevalence of CD is lower than ODD in typical young person populations without 22q11.2DS (National Institute for Health and Care Excellence, 2017).

ODD and CD can present comorbidly, with the individual demonstrating symptoms of both disorders (Costello *et al.*, 2003). Furthermore, individuals with ADHD can present comorbidly with CD and/or ODD (Biederman *et al.*, 2008). A study of young people with 22q11.2DS showed that 30% of the young people presenting with ODD also had symptoms of ASD suggestive of a diagnosis demonstrating comorbidity (Niarchou *et al.*, 2014). This is a high rate of comorbidity and sets the precedent for further evidence of the comorbid nature of ODD in 22q11.2DS.

Another ECHO study-based project looked at the clinical presentation of ADHD in 22q11.2DS and showed that 34% of young people with 22q11.2DS and ADHD had an ODD diagnosis yet none had a CD diagnosis. Compared to a clinical idiopathic (non-genotyped) ADHD sample,

both of these rates were lower with 46% of the clinical ADHD sample having an ODD diagnosis and 21% having a CD diagnosis (Niarchou, J. Martin, *et al.*, 2015). Consistently, there were fewer symptoms of ODD and CD in 22q11.2DS and ADHD compared to the clinical ADHD sample even when controlling for gender and IQ scores. However, there are elevated symptoms in 22q11.2DS than in population-based samples (Owens and Hoza, 2003).

1.3.6 Cognitive Deficits

Cognition involves high level processing of information such as through thinking, memory consolidation, perception, language processing and skilled motor movements. Learning and memory are fundamental processes in cognition, which assessment of these two domains through hippocampal memory tasks or abstract reasoning, can allow for recognition of cognitive deficits (Karpicke and Roediger, 2008). Impaired information processing, recall, ability to pay attention, initiation of speech and critical thinking can all indicate potential cognitive deficit (Trivedi, 2006).

Decline in cognitive development in children with 22q11.2DS has been shown in a 2012 study where a decline in full-scale IQ was found across time points between 5.5 years to 9.5 years. An absolute decline in cognitive scores was found in n=10 young people (15%) of the cohort (Duijff *et al.*, 2012). However, wide variability in cognitive function has been determined in young people with 22q11.2DS with divergent cognitive trajectories between studies (Swillen, 2016).

One study included assessments of working memory, executive function, attention and information processing and IQ allowing for investigation of cognitive deficits in young people with 22q11.2DS. Cognitive deficits are widely reported in young people with 22q11.2DS with the average IQ estimated to be ~30 IQ points lower than in typically developing children

(Niarchou *et al.*, 2014) and deficits in cognitive function including reaction time, sustained attention, processing speed, spatial working memory and executive functioning have also been documented (Hooper *et al.*, 2013; Niarchou *et al.*, 2014). ~31% of young people with 22q11.2DS have mild intellectual disability (ID) (53-69 full-scale IQ (FSIQ)), 31% borderline ID (70-79 FSIQ) and 39% an average IQ score (81-109 FSIQ) (Niarchou *et al.*, 2014). Longitudinal study of cognitive function in n=75 young people with 22q11.2DS showed deficits in all cognitive domains. However the overall decline over time from time point 1 to 2 showed a similar group trajectory to the sibling controls suggesting that the decline in cognitive function reflects a normative developmental change rather than a specific 22q11.2DS cognitive deficit of decline (Chawner *et al.*, 2017)

The following morbidities have been reported in young people with 22q11.2DS however they are not prevalent in the sample described in this thesis. This is as a result of the age range of the young people with the prevalence of schizophrenia and mood disorders increasing in later adolescence and early adulthood (Schneider *et al.*, 2014).

1.3.7 Schizophrenia

Reported at a lifetime prevalence of 1-2% in the general population, schizophrenia (SCZ) is considered to be one of the less common psychiatric disorders (Kirkbride *et al.*, 2012). A debilitating disorder, SCZ is associated with chronic disability and experiences of positive (hallucinations and delusions) and negative (social withdrawal) symptoms as well as impairment in cognitive function (Owen, Sawa and Mortensen, 2016). SCZ tends to present during late childhood, adolescence and early adulthood when there is great plasticity and

neurological changes happening (Kolb and Gibb, 2011). It is not often a feature recognised in childhood yet psychotic experiences and episodes can be reported (Poulton *et al.*, 2000).

SCZ is a polygenic disorder and 108 distinct genetic loci were linked to risk of SCZ in a genome-wide association study (Consortium *et al.*, 2014). 22q11.2DS is one of the strongest known biological risk factors for SCZ (Rees *et al.*, 2011, 2014; Kirov *et al.*, 2014) with a 25-30% elevated risk for the development of SCZ. This rate is substantially larger than the typically developing population, and the risk for psychotic disorder is even higher at around 38% and with an estimated rate of psychotic experiences reaching 50% (Schneider *et al.*, 2014). Rates of psychotic disorder can reach up to 41% in individuals over 25 years with 22q11.2DS (Bassett *et al.*, 2008; Schneider *et al.*, 2014).

A cross-sectional cohort of n=137 young people with 22q11.2DS showed an association between the total ADHD symptom score and overall levels of subthreshold psychotic symptoms, with inattentive symptoms at a higher prevalence in 22q11.2DS with subthreshold psychosis compared to non-deletion with subthreshold psychosis (Niarchou *et al.*, 2018). A longitudinal study showed that anxiety symptoms at the first time point were associated with the emergence of psychotic experiences at a later time point as well as differential development of cognitive domains such as the attention-executive domain (Chawner *et al.*, 2019).

1.3.8 Mood Disorders

Mood disorders are affective, emotional disorders. Depressive disorders are often characterised by loss of interest, low self-worth, disturbed sleep, tiredness and loss of concentration including many forms such as unipolar depression (World Health Organisation (WHO), 2018). Bipolar affective disorders typically include both depressive episodes and

manic episodes where elevated or irritable mood is exhibited alongside decreased need for sleep, over-activity and inflated self-worth (Lewis *et al.*, 2017). The World Health Organisation (WHO) states that there are 300 million people affected by depression and 60 million by bipolar affective disorder globally (World Health Organisation (WHO), 2018).

Mood disorders commonly present comorbidly in individuals with co-occurring psychiatric disorders such as anxiety disorder (Hirschfeld, 2001). One study showed that depression and anxiety disorder in young people with 22q11.2DS were predictors of later onset of psychiatric experiences with evidence of childhood depression and anxiety predicting 61% of the variance in the severity of psychosis assessed in later life (Gothelf, Feinstein, *et al.*, 2007). The same research group published another study showing mood disorders, in the overall sample group including young people and adults, were present in 15.1% of the cohort; rates of mood disorder in young people under 12 were 9%, with 4.5% having major depressive disorder. In adults with 22q11.2DS, 40.7% scored for major depressive disorder; a higher rate than psychotic disorder in that sample (32.1%) (Green *et al.*, 2009).

In young people with 22q11.2DS, mood disorders are shown to be amongst the least frequent psychopathologies. Nonetheless, they can have debilitating and significant effects especially with regards to symptomology such as suicidal ideation. Mood disorder can impact upon social and emotional communication as well as general functioning including educational attainment.

1.4 Summary

This chapter has comprehensively outlined the features of the neurodevelopmental phenotype in 22q11.2DS. It has demonstrated the multiple complexities of the syndrome and

the number of different problems that can present. 22q11.2DS is associated with a range of physical and neurodevelopmental problems, with elevated preponderance for ADHD, ASD, anxiety disorders and an increased risk for schizophrenia. Rates of mood disorders, CD and ODD are shown to have lower prevalence's in 22q11.2DS however there is great overlap in the shared symptomology. There is strong evidence of indicative DCD and learning disability, with impairments in cognition present in many individuals. The variability of the presentation in each individual complicates the understanding of 22q11.2DS further for medical professionals suggesting all hospital disciplines should be interested in the syndrome.

In the next chapter, I will discuss the overarching theme of this thesis and a common symptom reported in many of the developmental, psychiatric and cognitive problems addressed in this chapter: sleep problems.

Chapter 2 – An Introduction to Sleep

2.1 Key Principles of Sleep

A third of a human's life is spent asleep (National Institute of Neurological Disorders and Stroke, 2018), and is therefore is one of the most common activities undertaken by humans. Sleep has been preserved throughout evolution and as a result, these factors contribute to the suggestion that sleep serves an important role in human development (Lee and Rosen, 2012).

Sleep is an intricate and complex process that relies on the interaction between the brain and the body (Lu and Zee, 2006; Walker and Stickgold, 2006). Healthy sleep can be recognised through the detection of normative changes in the brain's electrical activity and subsequent changes in the body's activity. However, sleep can be vulnerable to disruptions that are acute or those that can become chronic. Chronic sleep disruption can have both detrimental physical and psychological impacts including elevated risk for type 2 diabetes and obesity (Gottlieb *et al.*, 2005) as well as depression and anxiety (Johnson, Roth and Breslau, 2006; Roenneberg and Merrow, 2016; Freeman *et al.*, 2017). Sleep deprivation impairs cognitive clarity and can impair daily tasks for example, driving a car and concentrating on work (Alhola and Polo-Kantola, 2007; Killgore, 2010).However, activities such as going to bed late in the evening resulting in tiredness can sometimes be unavoidable.

There is no single definitive reason for why we sleep however, there are four theories that have evidence to support their claims ('Why do we sleep?', 2000): 1) for rest and recuperation; 2) for recall, cognitive learning and memory consolidation (Gais *et al.*, 2000;

Maquet, 2000; Stickgold, James and Hobson, 2000); 3) it is an evolutionarily retained behaviour and 4) to undertake extracellular clearance in the brain, acting as a mechanism for removing toxic by-products assisted by the expansion of glial cells in the brain (Xie *et al.*, 2013).

This section of the thesis, *Key Principles of Sleep,* addresses sleep in the wider literature, paediatric literature and what we know about sleep with regards to the neurodevelopmental phenotype including neurodevelopmental disorders (NDDs) and psychiatric disorders. Sleep problems that have been identified in genetic syndromes are included. The focus of this chapter is to outline the neurophysiology of sleep and provide a background to what is currently known regarding brain activity during sleep in the neurodevelopmental phenotype, explained in **section 1.3 (page 8)**. This is in order to better understand **Chapter 7** later.

2.1.1 Background to the Sleep Literature

The sleep-wake cycle is a balanced mechanism regulated by two processes: process circadian (process C) and process sleep-wake homeostasis (process S) (Borbély, 1982; Borbély *et al.*, 2016), illustrated by **Figure 1**.

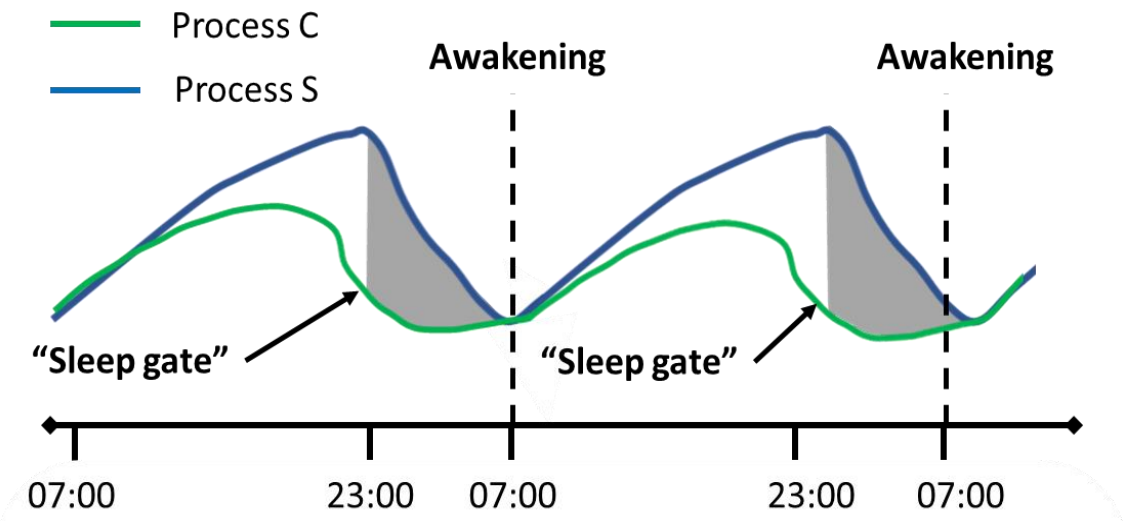


Figure 1 The two-process model of sleep adapted from the Borbély model published in 1982

Process S signifies the sleep-wake homeostatic pressure which increases throughout the day. Process S can be modified by the activities an individual undertakes during the day and by the substances ingested for example, caffeine (Silver and Rainbow, 2013). Contrastingly, process C is the circadian pattern which acts to counterbalance process S until the evening where there is a peak in the endogenous hormone melatonin. This is where the 'sleep-gate', a dominance of process S over process C, results in the increased pressure for sleep and the ultimate goal of sleep onset (Kotagal and Pianosi, 2006). Throughout the day and night, process C and S remain synchronous and balanced in a healthy individual. Changes to the balanced processes can result in clinical problems with the sleep phase. This refers to the timings of sleep onset and wake. For example, an advanced sleep phase results in an earlier than typical sleep onset time whereas a delayed sleep phase would be identified by a later sleep onset from the normative time (Barion and Zee, 2007). These processes are relevant to the later chapter (**Chapter 6**) where standardised sleep questionnaires have been adopted to try and acquire evidence of sleep phase disorders.

Sleep loss is evidenced to contribute to elevated risk for disease (Hanis *et al.*, 2016; Krysta *et al.*, 2017) but overall, the amount of sleep someone needs is dependent on the individual and their preferences for sleep timings (Vitale *et al.*, 2015). The amount of sleep needed changes across the life-course and new-borns do not establish a circadian rhythm until four months of age (Armstrong, Quinn and Dadds, 1994). In new-borns, brain plasticity and neurogenesis are vital and reflected in an average of 14-17 hours of sleep/day (Parmelee, Schulz and Disbrow, 1961). The architecture of sleep varies across a lifetime; there are differences in the percentage of time spent in the stages of sleep in young children compared to adolescences and adults (Baker *et al.*, 2016). Over the first year, a typical diurnal pattern of sleep-wake

starts to develop but with some daytime naps present and there is a shift in REM sleep dominance to non-REM (NREM) (Anders and Keener, 1985). For pre-school aged children, 10-13 hours is recommended, down to 9-11 for school age and 8-10 for teenagers. It isn't until 18 years old that a sleep duration of 7-9 hours is recommended and after the age of 65, sleep recommendations reduce to 7-8 hours (Berry *et al.*, 2016).

2.1.2 Sleep Problems in the Typically Developing Paediatric Population

Typically developing children experience sleep problems with reported rates ranging from 25-50% (Owens and Mindell, 2011). These problems are likely to be transient: rates of sleepwalking are ~40% and night terrors 1-7% in young children (Gertner *et al.*, 2002).

Given the four theories for sleep (see **section 2.1, pages 25-26**), the universal importance of sleep in brain development, metabolism and cognition is recognised, which suggests that it is essential for young children to maintain healthy sleep (Cirelli and Tononi, 2017). In younger children, there is a high degree of brain plasticity, neurological development and building of new neural circuits. This means that the consequences of sleep deprivation are more likely to be problematic in younger children than older individuals (Jan *et al.*, 2010; Bonuck and Grant, 2012). Having said that, sleep deprivation and poor sleep quality at any age can elevate risk of neuronal dysfunction, and impaired neurogenesis and memory (Bonnier, 2008). However, sleep problems from a young age can contribute to a heightened risk of cognitive impairment and poor psychiatric outcomes (Dorris *et al.*, 2008; Krystal, 2012) that can persist into adolescence and adulthood (Dahl, 2007; Kahn-Greene *et al.*, 2007; Limoges *et al.*, 2013; Rasch and Born, 2013; Krause *et al.*, 2017).

Sleep hygiene is a clinical tool that can be used to improve sleep behaviours especially surrounding the night time and sleep onset (Barion and Zee, 2007). These behaviours include

the development of a night time routine. Blue light however can be used in therapies to promote melatonin suppression in individuals with advanced sleep phases, depression or trouble with waking (Figueiro, Plitnick and Rea, 2014).

Sleep is a prevalent topic in the paediatric literature. There is evidence to suggest that studies demonstrating the preponderance of sleep problems in young people have contributed to changes in health services and the media, aiming to encourage better sleep in young people. Mobile phone applications providing self-help for poor sleep such as 'Sleepio' (Espie, 2016; Elison *et al.*, 2017) and parent-led monitoring of their child's sleep habits and patterns through 'Kids Dr Sleep' (Gringras, 2015), are leading the way in encouraging conversations about children's sleep and how to maintain healthy practice.

2.1.3 Sleep Problems and the Neurodevelopmental Phenotype

The neurodevelopmental phenotype as referred to in **Chapter 1, section 1.3 (page 8)** is being used to define the neurodevelopmental problems (Attention-deficit hyperactivity disorder, and Autism spectrum disorders predominantly) and other psychiatric morbidities (anxiety, conduct and oppositional defiant disorders and schizophrenia) based on the underlying genetic predisposition to these morbidities. This definition is derived from the paper by Mullin, A.P. et al. (Mullin *et al.*, 2013) which explores the definition of neurodevelopmental disorders and the contributions from the genome and specifically the genotype which is of relevance to the 22q11.2DS population in this thesis.

This thesis includes neurodevelopmental disorders (NDDs), psychiatric disorders and cognitive deficits. There are disorders discussed that can be defined as neurodevelopmental, psychiatric and possibly behavioural, such as Attention-deficit hyperactivity disorder (ADHD).

Throughout, reference to the collection of disorders as a group that is outlined in this section of the thesis will be done so as the 'neurodevelopmental phenotype'.

2.1.3.1 Sleep in Neurodevelopmental Disorders (NDDs)

NDDs are characterised by impaired emotional, psychological and physical development and can include communication disorders and learning disabilities. One of the most common features of NDDs is sleep problems, which have a reported prevalence as high as 86% (Wiggs, 2001; Malow *et al.*, 2006; Johnson and Malow, 2008; Reynolds and Malow, 2011). Sleep problems feature in the diagnostic criteria for developmental disorders, including Angelman syndrome (Owens and Palermo, 2008; Robinson-Shelton and Malow, 2016).

Features such as inattention can present as behavioural symptoms of NDDs, but could be a consequence of an underlying sleep problem, or both (Owens, 2009). It can be difficult to determine the aetiology of certain symptoms and to determine whether sleep problems are consequential or causative of behavioural problems.

A vicious circle of behavioural problems that are exacerbated by the sleep problems, which in turn themselves, further increase the risk of poor behaviour as a result of poor sleep (Corkum, Tannock and Moldofsky, 1998; Esbensen *et al.*, 2017). Behavioural and sleep problems are inherently intertwined, for example bedtime resistance and anxiety-related problems (Iwadare *et al.*, 2015) are prevalent in NDDs.

Attention-Deficit Hyperactivity Disorder

There is a large literature regarding sleep problems in ADHD with reported rates ranging from 25-50% (Wiggs, 2001; Robinson-Shelton and Malow, 2016) regardless of the particular subtype of ADHD whether symptoms of hyperactivity-impulsivity or inattentiveness are

dominant, or a combination of both presenting as the combined subtype. Parental reports, as well as objective assessment of sleep such as actigraphy, have been used in cohorts of young people with ADHD, with findings showing consistent disturbance (Corkum *et al.*, 2001; Owens, 2008): shorter total sleep times from these objective studies with evidence of wake after sleep onset and fragmentation of sleep have been shown. These symptoms and reports of sleep disorders in ADHD including restless legs syndrome and periodic limb movements, sleep-disordered breathing and hypersomnia (Silvestri *et al.*, 2009) suggest sleep interventions are required.

In ADHD, there is the added problem of controlling the hyperactive-impulsive behaviours through medication such as methylphenidates (O'Brien *et al.*, 2003) which adversely affect sleep by stimulating the young person and can mean that melatonin is prescribed in many children with ADHD to promote sleep (Van der Heijden *et al.*, 2007; Cortese *et al.*, 2013).

Autism Spectrum Disorders

Elevated rates of sleep problems of ~40-80% in young people with ASD (Souders *et al.*, 2009) have been identified since the nineteen-nineties (Richdale and Schreck, 2009) with problems including restless sleep parasomnias, bedtime resistance, insomnia and daytime sleepiness (Johnson and Malow, 2008; Souders *et al.*, 2009).

Different sleep problems required different treatments: melatonin is commonly used in ASD to mitigate sleep problems. In some young people with ASD, a deficient melatonin profile is identified (Melke *et al.*, 2007) leading to a chaotic sleep-wake cycle. Medication is the most effective treatment in these young people however not all individuals with ASD have a melatonin-deficiency and behavioural programmes and parent-based sleep education can prove to be effective (Malow *et al.*, 2014). Sleep hygiene is used as a behavioural intervention

for many children experiencing sleep problems. This intervention is shown to work in children with NDDs (Blackmer and Feinstein, 2016). The idea of sleep hygiene is that routines are developed around bedtime and an environment is constructed to be conducive to sleep such as no phone in the bedroom, a comfortable bed and a window kept ajar (Hiscock *et al.*, 2015).

Each child responds to treatment differently so the possibility of a range of options in young people with NDDs such as sleep hygiene practices, cognitive behavioural therapy and medication can give each child the best chance of improving their sleep problems.

2.1.3.2 Psychiatric Disorders and Sleep Problems

Studies of REM sleep in individuals with psychiatric disorders such as post-traumatic stress disorder have shown reactivation of memories and replaying traumatic events, but in a state of sleep where biochemical responses and stress hormone levels are supressed (Lipinska *et al.*, 2014). This theory suggests that physiological responses to the traumatic events can reduce over time (van der Helm *et al.*, 2011) but other studies suggest that sleep deprivation after a traumatic event prevents consolidation of the memory which is better for long-term health and recovery (Harvey, Talbot and Gershon, 2009; Pace-Schott, Germain and Milad, 2015). Nonetheless, there is substantial evidence linking sleep and emotion, and resultantly sleep to mental health. This section reviews sleep problems in psychiatric disorders.

Anxiety disorder and depression

Sleep problems are considered 'core symptoms' of depression (Nutt, Wilson and Paterson, 2008) with around 75% of adults with depression having insomnia, and there are reports of hypersomnia in up to 40% of young depressed individuals (Nutt, Wilson and Paterson, 2008). Depression often presents comorbidly with anxiety (Hirschfeld, 2001) with ~24-36% of individuals with insomnia and ~27-42% with hypersomnia having an anxiety disorder (Ford

and Kamerow, 1989; Breslau *et al.*, 1996; Staner, 2003). There is evidence of reduced latency to the first REM stage of sleep in depression and anxiety (Spiker *et al.*, 1978; Papadimitriou, Linkowski, *et al.*, 1988; Wilson and Argyropoulos, 2005) and prolonged REM sleep with more sleep fragmentation (Nutt, Wilson and Paterson, 2008). This can result in increased tiredness resulting in more daytime naps and sleepiness (Breslau *et al.*, 1996).

Conduct (CD) and oppositional defiant disorder (ODD)

As disruptive behavioural disorders, young people with CD and ODD have shown to have a high prevalence of sleep problems resulting in a reduction in total sleep time and reduced sleep efficiency when compared to age-matched children (Aronen *et al.*, 2014). Elevated prevalence and severity of externalising problems including physical aggression are characteristic of CD, and other symptoms such as temper tantrums that present in CD, associate with more sleep problems (Paavonen *et al.*, 2002; Lycett *et al.*, 2015). The reported frequency of other externalising problems indicative of CD such as bullying and aggressive behaviours have been shown to be elevated in individuals that are at an elevated risk for sleep-disordered breathing compared to young people not at risk for sleep problems. Associations between the conduct symptoms and restless legs syndrome and periodic limb movements, both pathological sleep disorders, was also demonstrated in the same study (Chervin *et al.*, 2003).

Schizophrenia

Insomnia-like sleep disturbance occurs in approximately 80% of individuals with a SCZ diagnosis (Wulff *et al.*, 2012) with experience of delayed sleep onset as well as heightened nocturnal activity (Foster and Kupfer, 1975). Profound circadian misalignment, circa-bidian

rhythms (around-48-hours) (Hofstetter, Lysaker and Mayeda, 2005) as well as other non-24-hour rhythms have been shown in SCZ (Pritchett *et al.*, 2012; Zanini *et al.*, 2013).

Sleep problems and symptoms of the neurodevelopmental phenotype are intertwined with a perpetual cycle of poor sleep exacerbating symptoms. Balancing the treatment of sleep and psychiatric, behavioural or cognitive symptoms is required for the optimal prognosis.

2.1.4 Copy Number Variants and Sleep Problems

Sleep problems can be one of the possible consequences of carrying a CNV associated with high risk of neurodevelopmental disorder. The sleep problems can be a direct consequence of aberrant protein expression caused by the genetic lesion, or an indirect result, where they are linked to the comorbidities arising from the genetic lesion. The literature on sleep problems in CNV carriers is quite sparse, however, some syndromes have received a degree of research attention.

2.1.4.1 Smith-Magenis Syndrome

Smith-Magenis syndrome (SMS) is characterised by a deletion or mutation on/at chromosome 17 at locus p11.2 and is associated with prominent sleep and circadian rhythm disruption (SCRD) reported in up to 98% of individuals (De Leersnyder, 2012). A missense mutation in the retinoic acid 1 gene (*RAI1*) disrupts circadian *CLOCK* gene function giving rise to an inversion of the melatonin circadian rhythm profile. Individuals with SMS do not entrain to their environment shown by a delay in the melatonin peak which is observable at 4 a.m. in typically developing individuals (De Leersnyder *et al.*, 2001).

2.1.4.2 Angelman Syndrome

Angelman syndrome (AS), caused by a number of different genetic abnormalities that occur on chromosome 15 at the loci q11-13 which is a locus susceptible to genomic imprinting (Clayton-Smith and Laan, 2003), is also associated with prominent sleep problems such as reduced total sleep time, disrupted sleep architecture with reduced REM sleep, and nocturnal limb movements (Goldman, Bichell, *et al.*, 2012; Robinson-Shelton and Malow, 2016). Atypical neurodevelopment in AS also suggests that dysregulation of GABA-mediated inhibition in thalamocortical interactions disrupts sleep-wake regulation in these individuals.

2.1.4.3 Williams Syndrome

Williams syndrome (WS) is characterised by the deletion of 26 to 28 genes at locus q11.23 on chromosome 7. Sleep problems present in up to 97% of young people with reports of greater bedtime resistance, sleep anxiety, nocturnal awakenings and daytime sleepiness as well as poorer sleep efficiency and more respiratory-related nocturnal arousals compared to children without pathogenic CNVs (Mason et al., 2011; Ashworth et al., 2013). Hyperhidrosis, excessive sweating during sleep, is also seen in these individuals (Santoro et al., 2016), as are abnormal melatonin and cortisol (defined as the 'stress hormone' is a glucocorticoid steroid) circadian profiles. These profiles show more shallow troughs of cortisol concentrations and dampened increases in melatonin concentrations at bedtime compared to controls, and higher cortisol levels at bedtime maintaining wakefulness (Sniecinska-Cooper et al., 2015).

These three examples show that sleep problems are a common feature of at least some pathogenic CNVs. For the majority of CNV syndromes, the exact reason for sleep problems remains unclear. Understanding the aetiology in CNV carriers affected by sleep problems is an important first step towards best practise for interventions and treatments.

2.2 Sleep Problems in 22q11.2DS

There are currently no published systematic assessments of sleep in 22q11.2DS. This is in leu of considerable anecdotal evidence, based on parental reports to doctors, to researchers and through charities and social media.

Mentioned in **section 1.2 (pages 3-8)** 22q11.2DS is commonly screened for due to the detection of cardiac abnormalities in utero and in new-borns (Lee *et al.*, 2014; McDonald-McGinn *et al.*, 2015) or as a result of developmental delay in later childhood (Bassett and Chow, 1999; Swillen and McDonald-McGinn, 2015). In childhood, these problems are dominant and families seek advice and guidance from numerous professionals to provide support for their child with many individuals requiring numerous assessments early in childhood (Bassett *et al.*, 2011).

In young people regardless of psychiatric disorders, sleep problems can be disruptive to the individual and family, acting negatively on daytime behaviours (Stores, Wiggs and Campling, 1998). However, having disturbed sleep in addition to a predisposition to a psychiatric disorder, cognitive impairment, behaviour difficulties and physical problems adds to a more complex phenotype. This section of the thesis aims to explore the scant literature regarding sleep problems in 22q11.2DS, highlighting the need for further understanding.

Anecdotally, parental reports have shown consistently high rates of sleep problems in 22q11.2DS, however the literature is scant other than report of sleep-related breathing disorders (SRBD), especially obstructive sleep apnoea (OSA) (Spruijt *et al.*, 2012; Crockett *et al.*, 2014; Kennedy *et al.*, 2014; Silvestre *et al.*, 2014a). The incidence of OSA is estimated at ~10% of young people with 22q11.2DS, compared to only 2-4% in typically developing

children (Chang and Chae, 2010; Kennedy *et al.*, 2014). OSA is characterised by an obstruction or occlusion in any part of the upper airway (Waters, Suresh and Nixon, 2013), reducing the capacity for air to enter the lungs and making breathing difficult, especially when lying down. Individuals with 22q11.2DS commonly have high arched palates and cleft palate that is present in 9-11% of individuals (Heike *et al.*, 2007; Crockett *et al.*, 2014; Kennedy *et al.*, 2014), and narrowed airways (Bassett *et al.*, 2011) elevating the risk for OSA (Kennedy *et al.*, 2014). Hypotonia can result in OSA, where there is reduced muscular tone leading to lack of support and stability in the neck and jaw (Crockett *et al.*, 2014). Hypoplasia and small occluded airways, as well as airways prone to collapse, also factor into the risk for OSA and all of which are common in 22q11.2DS. Another factor contributing to risk for OSA is the presence of hypertrophic adenoids and tonsils in children with 22q11.2DS (McDonald-McGinn, Emanuel and Zackai, 1993).

The physical phenotype of OSA leads to subsequent problems such as effected breathing during sleep. It is also important to understand how impaired breathing during sleep can influence the behaviour and cognitive phenotype of an individual, whether they are a young person with 22q11.2DS (Heike *et al.*, 2007; Kennedy *et al.*, 2014)or an individual without a genetic lesion (Bourke *et al.*, 2011). There is evidence of OSA and cognitive impairment in the wider literature that is not isolated to childhood (Lal, Strange and Bachman, 2012).

There is a literature base focussing on the behaviours and problems that manifest as a result of SRBD and OSA: ADHD-like symptoms are common with increased inattentive and excessive daytime sleepiness (Gaultney, Terrell and Gingras, 2005; Wiggs, Montgomery and Stores, 2005) for example. Often the problems which manifest from OSA can be interpreted as behavioural or neurodevelopmental problems with one study showing attentional deficits in

up to 95% of individuals with OSA and an incidence of 20-30% of OSA in clinical ADHD-diagnosed individuals, supporting the interlinking diagnoses and symptomology (Youssef *et al.*, 2011).

To assess sleep-related breathing, a gold-standard diagnostic sleep assessment called a polysomnography (PSG) is required. This requires an individual to attend a sleep clinic and have physiological measures such as pulse oximetry, limb movements and respiratory rate and pressure assessed. There have been a small number of studies that using PSG in individuals with 22q11.2DS. One study included 21 individuals with 22q11.2DS who underwent a PSG with the results showing that 48% scored for SRBD and within those individuals, 43% reporting sleepiness (Moraleda-Cibrian *et al.*, 2014). Similarly, another patient cohort study, including 26 individuals with 22q11.2DS, showed that young people with 22q11.2DS were at the highest risk for a positive OSA outcome compared to other individuals with similar syndromic cleft palate including Pierre Robin Sequence. 50% of the young people with 22q11.2DS scored for SRBD in the Paediatric Sleep Questionnaire (PSQ)-SRBD scale (Silvestre *et al.*, 2014a).

The only report of sleep problems in 22q11.2DS outside of OSA forms part of a wider study of the phenotype of 22q11.2DS where sleep problems were mentioned, yet there was no emphasis on the issue. Higher scores on the Child Behaviour Checklist (CBCL) (Achenbach, 1992) for sleep problems in girls compared to boys with 22q11.2DS were identified (Briegel, Schneider and Schwab, 2008) yet only four questions regarding sleep problems are included in the CBCL, so this must be taken with caution.

This thesis will be the first to identify and report sleep problems in these young people, hopefully instigating further research into sleep problems in 22q11.2DS.

2.3 The Neurophysiology of Sleep

The process of falling asleep can be defined as '*-a link between two general states of consciousness, wakefulness and sleep'* (pages 157-185) (Cvetkovic and Cosic, 2011). This quote suggests that there are different states of consciousness between daytime activity and sleep. Therefore, it is not naïve to derive that wakeful neurophysiology is different from sleep neurophysiology.

The state of sleep itself is comprised of two general stages defined as non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. NREM is composed of stages 1, 2 and 3 which are characterised by differences in the waveforms and physiology presented. During sleep, different percentages of time are spent in different stages and across a lifetime, the composition of a night's sleep changes. Neonates spend ~50% of time in NREM and 50% in REM sleep, with 50-minute sleep cycles whereas adults spend on average 18-25% of a night's sleep in REM with a full-sleep cycle equating to around 90-minutes (Foster *et al.*, 1976).

The following section will briefly highlight the fundamentals of typical sleep physiology. In **Chapter 7 (pages 202-214)** comprehensive descriptions of the detected neurophysiology that are required for the scoring and recognition of each stage of sleep is outlined to allow for interpretation of the results.

2.3.1 Wake

Sleep initiates with wake and the physiology of wake is important with regards to the transition into sleep. The sleep-wake cycle aligns with the light-dark cycle illustrating a circadian pattern of alignment to the environment based on biological cues such as light (Roenneberg and Merrow, 2016). The neurophysiology of wake is distinctive to sleep yet

there are overlapping waveforms present in both. Wake activity comprises of gamma, beta and alpha waveforms. Gamma is detectable at the highest frequency range (25 to 100 Hz), commonly at 40 Hz and originates from the thalamus but is traceable across the cortex (Başar-Eroglu *et al.*, 1996). Beta activity is often 13-25 Hz, with some literature reporting up to 30 Hz (Groppe *et al.*, 2013). This activity characterises a state of high alertness and focus in an individual during wake (Kamiński *et al.*, 2012). Alpha activity (8-14Hz) centralises to the occipital cortex and is present in quietened and relaxed wakefulness (Blume, 2006) and is the dominant waveform detected when eyes are closed. Alpha activity can continue into sleep as background activity.

2.3.2 Non-Rapid Eye Movement Sleep (NREM) - Stage 1

Stage 1 features high frequency and low voltage activity, but can be identified mostly by low-amplitude mixed frequency (LAMF) waveforms, slow eye movements and a transition from alpha activity into theta frequencies (4-8 Hz) (Biswal *et al.*, 2010). Theta waves are not isolated to sleep and have been evidenced in situations of stress in adults during wake however detection of theta is most prominent in stage 1 where the frequency proliferates (Singh *et al.*, 2015).

In addition, sharply contoured waves called 'vertex sharp waves' are seen in the transition to stage 1 from other stages of sleep and are dominant across centralised regions of the brain. This waveform acts as a marker for the transition of stages, a process that can be difficult to detect from an EEG (Stern *et al.*, 2011).

Stage 1 transition from wake at the beginning of the night can be defined as a 'hypnagogic' state. This first stage 1 of the night in children can feature 'hypnagogic-hypersynchrony' which is high-amplitude, sinusoidal, 3-4 Hz paroxysmal bursts of waves seen globally but mainly in

frontocentral regions of the cortex. This activity is only detectable in young children and as a result, highlights some differences in brain activity between adults and children, with the stabilisation of most wave forms occurring around 11 years old (Berry *et al.*, 2016).

2.3.3 NREM - Stage 2

Stage 2 of NREM sleep is characterised by the dominance of theta waveforms (4-8 Hz) and symbolic features called 'K-complexes' and 'sleep spindles' (Caporro *et al.*, 2012).

K-complexes in stage 2 are negative, sharp waves followed immediately by a positive induction. The positive induction is apparent from the background EEG and will occur in isolation of arousal or artefacts (Berry *et al.*, 2012). K-complexes are measured maximally across the frontal cortex and last for a duration of at least 0.5 seconds. K-complexes are particularly interesting to study with regards to evoked-potentials (Colrain and Campbell, 2007). K-complexes can contribute to the slow wave frequency band range (0.5-0.9 Hz) and the characteristic shape of K-complexes has been used to prove that they contribute to the 1-4 Hz frequency band and therefore the delta band range (Steriade and Amzica, 1998).

Sleep spindles are lengths of sinusoidal waveforms lasting for at least 0.5 seconds, detectable at 11-16Hz but most commonly at 12-14Hz (Iber, 2007; Berry *et al.*, 2012). Spindles are a defining feature of stage 2 sleep but can occur during stage 3 also however their presences can be obscured by slow-wave oscillations (Cox *et al.*, 2017).

Sleep spindles can be divided into two classifications: slow (~10 Hz) and fast spindles (~13 Hz) based on the theory that slow and fast spindles serve distinct functional roles (Tamaki *et al.*, 2008; Hoedlmoser *et al.*, 2014). Sleep spindles derive from the thalamus (Steriade *et al.*, 1987)

and slow spindles are detected more densely in the frontal cortex whereas fast spindles are seen more centrally and parietally.

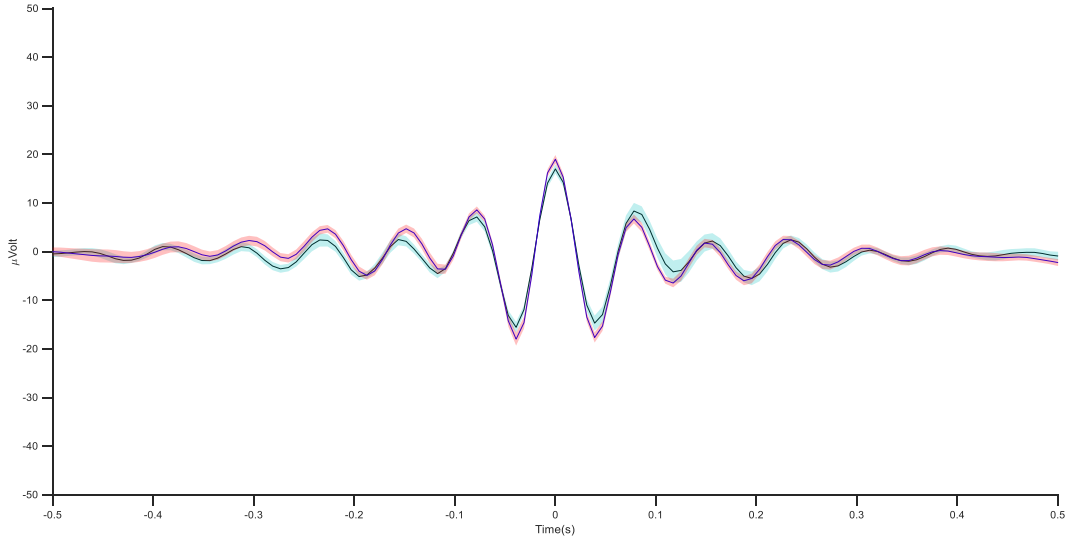


Figure 2 An example of a fast sleep spindle averaged from the central electrodes on an EEG

Sleep spindles are important in exploring the role of sleep in cognitive processing and learning with the detection of sleep spindles indicative of sleep-dependent learning (Gais *et al.*, 2002). By completing memory or motor tasks pre- and post-sleep can allow for spindles to be analysed in relation to the cognitive task performance (Walker *et al.*, 2002).

2.3.4 NREM - Stage 3

Stage 3 is characterised by slow-wave oscillations at frequencies ~0.5-2Hz and delta frequencies which include the slow-wave oscillation but include higher frequencies ranging from 0.5-4Hz. These oscillations can be best measured by EEG locally at the frontal cortex of the brain. Stage 3 of NREM sleep can be defined as the period of 'deep-sleep' (Fujitani and Hosogai, 1983) or consolidative sleep and is the stage characterised with the lowest frequency waveforms.

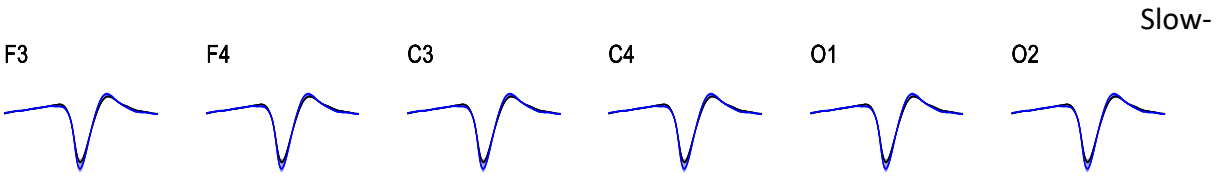


Figure 3 Examples of averaged slow-waves featuring delta across fronto-central-occipital regions of an EEG

waves oscillations originate from and are modulated in the neocortex (Achermann *et al.*, 1993). Synchronised coupling of slow-wave oscillations with sleep spindles provides evidence of sleep-dependent memory consolidation (Rasch and Born, 2013).

Across a single night, the duration of slow-wave sleep per 90-minute sleep cycle will depreciate (Achermann *et al.*, 1993). This is mirrored by the progressively increasing duration of REM sleep throughout the night with the longest time spent in REM in the early morning of the sleep period (Endo *et al.*, 1981).

2.3.5 Rapid Eye Movement (REM) Sleep

REM sleep is characterised by rapid eye movements and 'saw tooth waves' which present as sharply contoured trains of waves that appear serrated and occur at 2-6 Hz (Berry *et al.*, 2012). The neurophysiology of REM sleep is comparable to wake activity and can be difficult to detect on EEG traces when not coupled with other physiological markers (see **Chapter 7**).

The purpose of REM sleep remains unknown however one theory suggests that REM is responsible for emotional processing and disconnection. Communication between the amygdala and the ventromedial prefrontal and dorsolateral prefrontal cortex can contribute emotional disconnection in healthy humans, dampening emotional responses to memories and thoughts (Walker and van der Helm, 2009).

2.4 Neurophysiology of Sleep in the Neurodevelopmental Phenotype

There is limited work in the paediatric literature regarding sleep EEG. Where there is literature, the investigation of epileptiform activity dominates with scant literature regarding the significant of sleep physiology in the neurodevelopmental phenotype. This limits the understanding of interactions between brain mechanisms and sleep such as motor learning and sleep in young people (Walker *et al.*, 2002). There are however more studies regarding adults and their electrophysiology including sleep EEG in adults with NDDs.

Nonetheless, the small number of studies investigating sleep electrophysiology in the neurodevelopmental phenotype in young people are outlined in this section.

2.4.1 Neurophysiology of ADHD

The number of overnight EEG studies that have been conducted with young people with ADHD are limited. However, there are studies that include small samples of individuals where sleep-EEG was conducted to cross-validate other objective measures such as actigraphy or subjective measures of sleep in these individuals such as sleep questionnaires and sleep diaries/logs.

One study conducted overnight polysomnography (PSG) including sleep-EEG and additional cardiorespiratory measures in young people with ADHD (O'Brien *et al.*, 2003). This study included n=47 individuals with ADHD recruited from a clinic, n=53 individuals with ADHD recruited from a community sample and n=49 controls. The young people wore the sleep-EEG measures for 12 hours over a single night and showed that there were substantial differences between groups in the REM onset latency and the percentage of time spent in REM sleep. The clinical ADHD groups showed lower spontaneous arousals than both other groups and

there were a higher number of periodic limb movement arousals in the ADHD young people compared to the controls. Objective investigation of sleep in ADHD showed the prevalence of disturbance. This was further supported by other studies that investigated disturbed sleep in young people with ADHD with objective methods. A smaller n=55 investigation of young people with ADHD only (mean age of 8.9 years old), used overnight video-polysomnography coupled with a subjective behavioural assessment and structured sleep interview to assess sleep in ADHD. Many of the individuals had a hyperactive-impulsive or combined subtype of ADHD (n=39); n=16 with inattentive ADHD subtype. An abnormal EEG was evident for 23.6% (n=13) of the sample when assessing the overnight video-PSG, and 40% (n=22) of the sample were objectively assessed to have periodic limb movements during sleep (Silvestri *et al.*, 2009).

Crabtree et al. studied a sample of young people identified from two sources: a paediatric sleep medicine centre and a community survey sample in the USA. The young people that were sourced from the sleep centre completed a single night of PSG. From this sample of young people, those individuals identified to have periodic limb movement disorder (PLMD) were allocated into one group. Among this group, the individuals were further divided into individuals with ADHD and those without ADHD (PLMD only). Therefore, there was one group with PLMD and ADHD, and another group of young people with PLMD only.

The parents of the young people from the community control sample completed a detailed sleep habits questionnaire. The questionnaire contained questions regarding ADHD-like activities. Where the parents reported no ADHD or snoring (PLMD only), these young people from the community sample were invited to participate as a control group.

There were n=90 young people identified to have PLMD by the PSG and n=40 of these young people with PLMD also presented with ADHD. The young people with PLMD and ADHD were shown to spend a smaller percentage of the night in REM sleep compared to the group with PLMD only, and the controls. Additionally, the PLMD and ADHD group recorded a higher apnoeic-hypopneic index and spontaneous arousal index throughout the night compared to the PLMD only and control groups, suggesting that the young people with PLMD and ADHD presented with a more severe sleep phenotype compared to the PLMD only group (Crabtree *et al.*, 2003).

There is a scant literature regarding sleep-EEG investigation in young people with ADHD however those studies that are present do show consistent reports of disturbed neurophysiology but do not provide electrophysiological or waveform analyses.

2.4.2 Neurophysiology of ASD

Epileptiform activity is commonly identified by EEG in young people with ASD in the absence of clinical seizures (Chez *et al.*, 2004). Daytime EEG can provide an assessment of neurophysiology during wakefulness however to reliably measure epileptiform activity, the hypnagogic and hypopneic brain activity needs to be accounted for. These two states occur as an individual is falling asleep (hypnagogic) and when an individual is waking up (hypopneic) and therefore transitioning between sleep and wake (Harvey and Barnes, 1996). Seizure-related activity is not isolated to sleep in ASD individuals, yet it shows a preponderance during sleep (Chez *et al.*, 2006). Therefore, 24-hour EEG is required to comprehensively assess brain activity incorporating sleep neurophysiology. Overnight ambulatory EEG discovered abnormalities in brain activity during sleep in young people with ASD (Chez *et al.*, 2004).

Overnight EEG demonstrates abnormalities in slow-wave and delta activity during NREM sleep in young people with ASD and reductions in delta activity compared to a typically developing control group (Rochette *et al.*, 2018). This reduced delta power focussed over the parieto-occipital region and contributed to findings that suggest abnormalities in the thalamocortical system in individuals with ASD. This study was further supported by additional findings in a small (n=13) sample of young male participants with ASD (Lehoux, Carrier and Godbout, 2018). Slow-wave oscillations in this sample were shown to have fewer and smaller differences in the slow-wave density, amplitude and frequency across the cortex when compared to typically developing age-matched controls.

ASD is an overarching diagnosis which includes a broad range of individuals that have variable intellectual functioning. One study of high-functioning individuals with ASD showed reduced beta activity during REM sleep over the temporal-occipital regions compared to typical controls (Daoust *et al.*, 2004). Elevated absolute theta however was observed over the frontal cortex compared to controls suggesting atypical physiology in the frontal cortex in ASD compared to controls and contributes to the literature suggesting atypical thalamocortical mechanisms in ASD.

Accessibility to 24-hour assessments and investigation into atypical sleep physiology in ASD contributes to a better understanding of brain activity abnormalities throughout the 24-hour period showing a persistent problem during wake and sleep that is not isolated to daytime activity and wakefulness.

2.5 Neurophysiology of Sleep and Psychopathology

This section will focus on the neurophysiology of sleep in psychiatric disorders relevant to the neurodevelopmental phenotype of 22q11.2DS.

2.5.1 Neurophysiology of Anxiety Disorders

Widespread sleep disturbance is common in individuals with anxiety disorders. According to the DSM-5 criteria for the diagnosis of generalised anxiety disorder (GAD), tiring easily, more fatigued than usual and difficulty sleeping (insomnia-related) are incorporated into the physical problems assessed (Kocsis, 2013). This demonstrates the inter-relationship of sleep and anxiety and suggests that objective assessment of sleep neurophysiology is of importance to understanding the relationship better.

Sleep studies that include sleep EEG assessments have shown reduced number of awakenings and transitions between stages during the night's sleep, in addition to prolonged REM latency but a shortened duration of REM sleep (Papadimitriou, Kerkhofs, *et al.*, 1988) in individuals with anxiety disorder. Individuals with GAD showed a longer sleep onset latency, taking longer to transition from wake into sleep and a shorted total sleep time and time spent in stage 2 of NREM sleep when compared to control individuals.

Additional studies have suggested that disturbed sleep stage transitions and differing lengths of time in sleep stages compared to typically developing controls is common. A young person sample of individuals with anxiety disorders showed elevated nocturnal awakenings compared to individuals with major depressive disorder, in addition to prolonged sleep onset latency compared with both the MDD and control groups (Forbes *et al.*, 2008; Alfano *et al.*, 2013).

There is consensus in the literature that sleep EEG studies have shown consistency in their objective measurements: prolonged sleep onset latency and differences in the transitions into REM sleep and time spent in REM relative to stage 3 suggesting increased REM sleep which is of higher frequency and reduced time spent in stage 3 where slow-wave oscillations dominate and lower frequency neurophysiology is detected. These variable durations spent in stages of sleep compared to typically developing individuals can be coupled with dysregulation of neurotransmitter concentrations especially serotonin in anxiety disorders with extracellular concentrations of serotonin contributing to changes in slow-wave (stage 3) and REM ratios during a night's sleep (Portas, Bjorvatn and Ursin, 2000).

Anxiety disorders are highly preponderant in 22q11.2DS and one study showed that psychotropic medications in young people with 22q11.2DS were effective in treating anxiety diagnoses with high tolerability (Dori *et al.*, 2017). The presence of medication in young people with 22q11.2DS should be considered when assessing the relationship of anxiety disorders and sleep.

2.5.2 Neurophysiology of Schizophrenia

Deficient sleep spindles (see **section 2.3.3, page 41**) characterised by a reduced number, amplitude and density have been identified in medicated and non-medicated individuals with SCZ (Manoach *et al.*, 2016). Studies have shown similarities in spindle amplitude between the first-degree relatives of individuals with SCZ and healthy controls suggesting spindles are representative of a genetic lesion in SCZ (Manoach *et al.*, 2016; Cosgrave, Wulff and Gehrman, 2018).

Additionally, deficient slow-wave oscillations hypotheses exist. The thalamic reticular nucleus (TRN) (Lewis *et al.*, 2015) relays signals to the thalamus and cortex resulting in the production

of slow-wave oscillations. Disturbance to the TRN in SCZ could contribute to the decrease in delta-power and reduced frequency, amplitude and abundance of slow-wave oscillations (Keshavan *et al.*, 1998).

2.6 22q11.2DS Neurophysiology

There are few studies investigating neurophysiology in 22q11.2DS and those studies including EEG as a technique have focussed on assessments for seizures and epilepsy (Eaton, C.B. et al. *in submission*) and assessment of motor dysfunction (Biria *et al.*, 2018). Despite no report of sleep neurophysiology in 22q11.2DS, these studies are important as they confirm that there is aberrant brain physiology in individuals with 22q11.2DS.

Impairments in source activations and reduced amplitudes of waveforms involved in visual processing (Biria *et al.*, 2018) have been reported as well as spike-wave complexes having been identified in 22q11.2DS. The rate of epilepsies is higher than the general population with one study suggesting 15.2% of the sample experienced epileptic seizures, higher than the prevalence of developmental delay (Kim *et al.*, 2016). There are also different types of epilepsy seen in 22q11.2DS, with one review showing out of n=88 individuals with epilepsy, 44% of patients with epilepsy reported focal epilepsy and 27% generalised epilepsy (Mudigoudar *et al.*, 2017). These potential epileptiform discharges in 22q11.2DS further support the claim of aberrant neurophysiology in 22q11.2DS and further support the investigation of sleep physiology in 22q11.2DS to see whether the wake abnormalities are also detectable during sleep.

Neuroanatomical differences in 22q11.2DS compared to typically developing controls such as a reduced hippocampal volume could be relevant to sleep as sharp-wave ripple activity

originates in the hippocampi (Eschenko *et al.*, 2008). Abnormalities in the volume of the hippocampi and reports of hippocampal mal-rotation in 22q11.2DS could suggest disruption of the translation of short-term stored memories from the limbic system to the neocortex during slow-wave sleep (Andrade *et al.*, 2013).

There are widespread neurological changes in young people with 22q11.2DS including increased cortical surface area in the sensorimotor cortex (Sun *et al.*, 2018), reduced cerebellum size, reduced cerebellar grey matter and white matter in the frontal lobe (Campbell *et al.*, 2006) linking neuroanatomical changes in 22q11.2DS to sleep. Sleep deprivation in addition can effect brain physiology by promoting inflammatory processes which change the brains sensitivity to hormones like insulin and leptin (Suarez, 2008). There is already poor homeostatic regulation of the endocrine system in 22q11.2DS so the additive impact of sleep deprivation could potentially have further impact on brain anatomy.

There is currently no known origin for sleep problems in 22q11.2DS. Beginning to explore the sleep neurophysiology of individuals with 22q11.2DS could help to better understand what is happening at a fundamental neurological level during a night's sleep. To do this, an overnight sleep EEG is required. This thesis aims to describe the first study to systematically assess sleep in this high-risk population.

2.7 Aims of Thesis

The main aim of the thesis is to better understand sleep problems in young people with 22q11.2DS as well as explore the links with their other problems such as within the neurodevelopmental phenotype. These aims are based on the information outlined in the introductions in **Chapters 1** and **2**.

To address this main aim, there were three aims developed with objectives.

1. The first aim was to understand the prevalence, severity, nature and type of sleep problems in young people with 22q11.2DS by conducting comparisons with their unaffected sibling controls. To achieve this, primary carer reports of sleep problems from the semi-structured Child and Adolescent Psychiatric Assessment (CAPA) interview were compared between groups and assimilated into factors which best described the patterns of sleep problems reported in 22q11.2DS.
2. The second aim was to describe the associations between the sleep problems and neurodevelopmental phenotype within young people with 22q11.2DS. The objective was to use the derived patterns of sleep problems from the first aim to further explore associations with the neurodevelopmental phenotype including psychiatric outcomes, neurodevelopmental disorder and cognitive deficits.
3. Aim three worked to better understand the physiology of sleep problems in young people with 22q11.2DS, by conducting comparisons with their unaffected sibling controls. To achieve this, an ambulatory sleep study was developed to assess the neurophysiology of participants using electroencephalography (EEG); cardiorespiratory measures and 24-hour sleep-wake activity using actigraphy watches with a complementing sleep diary. The aimed outcomes to be derived from the measures are: hypnograms and basic measures of sleep stages from the objective EEG analysis (for example percentages of the night spent in each sleep stage); objective nocturnal activity from the actigraphy watches in addition to sleep parameters such as sleep onset latency and sleep timings, analysed using a standardised protocol and

subjective measures of sleep from the validated sleep questionnaires and the sleep diary.

It was predicted that:

1. for aim one, the prevalence and severity of sleep problems in 22q11.2DS would be elevated compared to the unaffected control siblings and the typically developing paediatric population, and that sleep problems would be related to each other, therefore one dominant factor would encompass the different sleep problems. This is based on the evidence for a high preponderance of sleep problems across CNV genetic syndromes, neurodevelopmental disorders and psychiatric disorders (see **section 1.3, pages 8-23**);
2. for aim two, that elevated symptoms of sleep problems would be associated with higher symptoms of the neurodevelopmental phenotype and with cognitive deficit;
3. and for aim 3, differences in the percentage of time spent in sleep stages in 22q11.2DS compared to siblings, with longer periods of REM in 22q11.2DS and shorter NREM Stage 2 and 3 when analysing the EEG was expected. Elevated nocturnal activity was expected to be identified objectively in the 22q11.2DS young people compared to their siblings when analysing the actigraphy data, and sleep parameters to show prolonged sleep timings and increased sleep disturbances throughout the night such as increased wake after sleep onset compared to siblings. The sleep questionnaires were hypothesised to subjectively agree with the sleep problems reported by parents in the CAPA and would show concordance with some objective measures, but namely there would be differences in the parent reports compared to the objective measures, suggesting that multiple methods of sleep assessment are required to

comprehensively assess sleep in the 22q11.2DS population and the typically developing sibling control group. It is important to note, that there is no analysis of individuals sleep architectural markers such as sleep spindles or slow waves. These markers were measured and are available for future analyses and investigation of the underlying sleep physiology in young people with 22q11.2DS.

Chapter 3 General Methodology

The general methodology refers to the samples that have been used throughout this thesis. Therefore, the specific methodologies required for **Chapters 5**, **6** and **7** are not included in this chapter. The samples used throughout the thesis however are all outlined in this chapter for clarity.

The ECHO Study (see **section 3.1** and **Appendices 1** and **2**) and the IMAGINE-ID study (see **section 3.2** and **Appendices 1** and **2**.) are the overarching studies where the young people with 22q11.2DS and the sibling control groups were recruited from to undertake sleep phenotyping and exploring the association between sleep problems and the neurodevelopmental phenotype (see **Chapter 4**, **section 4.2.3**).

Young people from the typically developing population included in the Pilot Sleep Study (see **Chapter 5**, **section 5.4**) were recruited from the general population and colleagues with children at Cardiff University.

The cohort of young people who wore actigraphy watches and completed sleep diaries as part of the Sleep Study (see **Chapter 6**, **pages 162-164**) were recruited from either the ECHO Study (see **section 3.1**) or the IMAGINE-ID Study (see **section 3.2**) as part of a follow-up assessment.

The young people that undertook the polysomnography were individuals that were recruited as part of the same follow-up from the ECHO and IMAGINE-ID studies. There were fewer as this additional part of the sleep study was more invasive and considered problematic for some young people and parents. The cohort of individuals who undertook the polysomnography sleep study are outlined in **Chapter 7**, **pages 217-220**.

The different investigations were all cross-sectional: all analyses were conducted at a single time point. The only exception to this is the small sample longitudinal analysis which is included in **Chapter 4**.

A subsample of adults is included in this thesis to demonstrate the prevalence of sleep problems in a separate adult sample. These individuals were taken from the DEFINE study (see **section 3.3**). The sample of toddlers were recruited through the ECHO Toddler Study (see **page 3.10 and Appendices 1** and **2**.) and were used in this thesis to show presence of sleep problems from a young age. Ultimately, the different sub-samples were used to demonstrate the persistence and maintained prevalence of sleep problems regardless of age of the individual with 22q11.2DS.

For a diagrammatic explanation of how the ECHO Study works in conjunction with other studies mentioned in this thesis, see **Appendices 1** and **2 (pages 327-329**). How the samples were derived from the different studies are also included in the appendices.

3.1 The ECHO Study

The ECHO Study was designed to assess young people with CNVs with elevated risk for psychopathology and intellectual disability, with notable focus on 22q11.2DS. The aim was to understand their lifetime trajectories with regards to their psychological, intellectual and biological development.

3.1.1 Recruitment

Young people with 22q11.2DS were recruited through NHS Medical Genetics clinics in the UK, British 22q11.2DS charities (Max Appeal! 22qCrew and Unique) and social media

advertisements and family networks including '22qAwarenessDays'. Where available, a sibling control closest in age to the child with the deletion was also invited to take part in the ECHO Study. Carriers of the deletion and sibling controls were aged 6 or older and the presence (in carriers) or absence (in sibling controls) of the deletion was confirmed either by a Medical Genetics laboratory providing a medical genetics report, and/or a microarray analysis in the MRC Centre for Neuropsychiatric Genetics and Genomics laboratory at Cardiff University. There was no discrimination of the length of genetic lesion required to participate in the ECHO Study: individuals with both the 1.5 and 3-Mb deletions (see **Chapter 1**) on chromosome 22 were included in the analyses in this thesis.

Consent was obtained prior to participation from carers who consented for all participants. Additional consent was obtained from participants aged ≥16 years with capacity, and assent was obtained from young people aged <16 years and children aged ≥16 years without capacity. The protocols used in the ECHO Study were approved by NHS South East Wales Research Ethics Committee. Additional ethics was required for the sleep and EEG Study and this was in the form of an amendment to the ECHO Study as it required additional measures that had not previously been used (see **Chapters 6** and **7**).

3.2 The IMAGINE-ID Study

The IMAGINE-ID Study is a collaborative project coordinated by researchers from Cardiff University, University College London and the University of Cambridge, as well as the UK rare chromosomal support charity, Unique. The IMAGINE-ID Study focusses on understanding genetics, intellectual disability and the behavioural phenotype, with the aim of understanding the lifetime trajectories and interactions of CNV genetic syndromes. This aligns with the aims

of the ECHO Study; however, the IMAGINE-ID Study focuses on a broader spectrum of CNVs specialising in CNV genetic syndromes with an elevated risk for intellectual disability. The IMAGINE-ID Study includes the recruitment of individuals with 22q11.2DS including young people and adults. Participants are not required to have completed assessments with the ECHO Study, however there are some individuals that have been seen cross-sectionally and followed-up by the IMAGINE-ID Study.

3.2.1 Recruitment

The IMAGINE-ID Study works cooperatively with UK NHS Genetics Clinics where many individuals are initially informed about participation. Cambridge University is responsible for the initial recruitment of the individuals and assimilation of the data. The Cardiff team are responsible for visiting the families and conducting similar assessments regarding psychiatric outcomes and cognition to the ECHO Study. Individuals participating in the IMAGINE-ID Study can also have participated in the ECHO Study or can consent to receiving additional information regarding other research studies including information about the Sleep Study. For further information regarding the overlap of the different studies, please refer to **Appendices 1** and **2**.

3.3 The DEFINE Study

The DEFINE Study aims to integrate basic and clinical neuroscience with animal, cellular and human studies to investigate high-penetrance mutations conferring risk for psychopathology. Of relevance to this thesis, the human aspect of the DEFINE Study assesses individuals over the age of 18 that have a CNV genetic syndrome conferring risk for psychopathology, including 22q11.2DS. Phenotypic and behavioural assessments are conducted which are like those

undertaken by the ECHO Study. Neuroimaging and electrophysiological methods are also included in the DEFINE Study.

A small subsample of individuals from the DEFINE Study (see **Chapter 4**) are included to explore the continued prevalence of sleep problems in individuals with 22q11.2DS into adulthood.

3.4 The Study Samples

This thesis includes multiple samples. These samples have been outlined in flow charts in **Appendices 1** and **2** (**pages 327-329**). For methodology of the different investigations undertaken using the samples see the following chapters: **Chapter 5** for the Pilot Study; **Chapter 6** for the Actigraphy, Sleep Diaries and Sleep Questionnaires analyses and **Chapter 7** for the Polysomnography.

3.5 Psychiatric Assessments

The semi-structured Child and Adolescent Psychiatric Assessment (CAPA) (Angold *et al.*, 1995) was conducted with the primary carer by trained psychologists for the assessment of young people aged 6-18 years old. Sections of the CAPA regarding mood and anxiety disorder were conducted with young people over the age of 12 to gain their perspectives and the psychotic experiences section of the CAPA was conducted with young people from age seven. This was only conducted if the individual was assessed to have capacity to answer and understand the questions. Primary carer and child self-report were both assessed for positive outcomes. The primary carer and the participant were asked to provide responses to the CAPA questions that were relevant to the past three months prior to the assessment date.

All interviews were audio recorded and research DSM-IV diagnoses were obtained. The CAPA assessments were all double-diagnosed with the individual completing the second diagnosis blind to the first assessors' diagnostic opinion. When a particularly difficult case was presented to a researcher, review meetings led by a child and adolescent psychiatrist were held to ensure consistency and accuracy of diagnoses.

The CAPA was used to obtain ADHD, anxiety, oppositional and conduct symptom counts as well as sleep problems. The presence of a symptom was counted if an individual scored one or two on the relevant question. Dichotomising such scores allowed for easier interpretability of the outcomes when summed to generate symptom scores for analyses and therefore, where there were individuals scoring 'two', ultimately these individuals were combined with the individuals scoring 'one' on the same item. On most items, the scoring was arbitrary and referred to a timescale of symptomology which was not explored in this thesis.

3.5.1 Attention-Deficit Hyperactivity Disorder

The overall ADHD symptom score included hyperactivity-impulsivity and inattentive symptoms and therefore, a separate score for hyperactivity-impulsivity and inattention symptoms could be derived. Where there was evidence of ADHD Diagnostic and Statistical Manual for Mental Disorders Fourth Edition (DSM-IV-TR) (Association and Association, 2000; Valo and Tannock, 2010) criteria of presence of symptomology before the age of 7, a research diagnosis could be derived.

3.5.2 Anxiety

The anxiety disorder symptom score included symptoms of generalised anxiety disorder, social phobia, specific phobia, separation anxiety, panic disorder with and without

agoraphobia, agoraphobia and obsessive-compulsive disorder. If any of these research diagnoses were scored for, the individual was given a classification of 'any anxiety disorder'.

3.5.3 Oppositional and Conduct Symptoms

Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD) symptoms were assessed by two subscales: oppositional behaviour and deception for ODD, and acts involving violence and violence against persons for CD. The two symptom scores overlap and literature suggests that many of the symptoms co-occur. As a result, a single research diagnosis indicative of CD and ODD can be given for use in research.

3.6 Sleep Problems

Sleep problems experienced over the past three months were established through the sleep section of the CAPA. There are thirteen questions included in this section (see **Table 1**).

Table 1 Sleep items in the CAPA

Sleep items (CAPA)	***Description***	***Scoring***
Insomnia	Composite insomnia score	0, 1, 2
Initial Insomnia	Trouble initiating sleep	0, 1
Middle Insomnia	Trouble maintaining sleep	0, 1, 2
Early Insomnia	Early morning awakening without being able to return to sleep	0, 1
Medication for Insomnia	Melatonin use	0, 1
Hypersomnia	Excessive daytime sleepiness and extended sleep duration	0, 1, 2
Restless sleep	An inability to sleep comfortably and remain rested through the night	0, 1
Inadequately rested	Lack of restorative and maintained sleep	0, 1
Fatigability	Difficulty in performing to the same standard as previously experienced day-to-day	0, 1
Tiredness	Tiredness for at least half the day and more than usual	0, 1
Nightmares	A distressing event that can be recalled and occurs regularly; the individual will be conscious of their nightmare	0, 1
Night terrors	Distressing, unconscious events without recall or recurrence	0, 1
Sleepwalking	Walking activity whilst remaining asleep	0, 1

These sleep items (see **Table 1**) assess problems rather than clinical diagnoses yet could be indicative of underlying sleep disorders and suggestive of further exploration of the sleep phenotype.

Items were coded “0 = absent” or “1 = present”, except for insomnia, middle insomnia and hypersomnia, which were originally coded on a three-point scale so were dichotomised. Out of the 13 items, 11 were used to obtain an overall sleep problem score. The two items that were excluded were a closed question regarding melatonin use over the past three months and a composite insomnia score, dependent on a score for either initial, middle or early insomnia.

The overall insomnia score was based on the responses from the initial, middle and early questions but the CAPA required the total accumulative time spent awake to reach a threshold of 1 hour or more. Some individuals did not reach the threshold to score for the main insomnia item, however they did report the presence of insomnias illustrated by responses to middle insomnia, where a specific time threshold was not specified. This resulted in positive scores for middle insomnia not always contributing to the overall insomnia outcome. Therefore, despite an individual scoring for middle insomnia set out by the CAPA, this positive score might not contribute to an overall positive score for insomnia. Therefore, it was important to explore the prevalence of insomnia-related problems but exploring the individual insomnias rather than a composite score which could have excluded some individuals who were reporting positive symptoms. The three questions provided a more comprehensive understanding of the types of insomnia experienced by the young people. Each of the 11 items were summed to obtain an overall sleep problem score. The sleep problems symptoms could range from 0-11.

3.7 Social Communication Questionnaire (SCQ)

ASD symptoms were screened for by the Social Communication Questionnaire (SCQ) (Rutter, Bailey and Lord, 2003) completed by the primary carer. The total indicative ASD score comprised subscales: social interaction, communication, and restrictive and repetitive traits. The total score ranged from 0 to 39 with a score of 15 or higher suggestive of putative autism spectrum disorder (ASD) (Berument *et al.*, 1999).

3.8 Developmental Coordination Disorder Questionnaire (DCDQ)

Indicative DCD was established by the Developmental Coordination Disorder Questionnaire (DCDQ) (Wilson *et al.*, 2009) completed by the primary carer. The DCDQ screens for motor coordination impairments with scores ranging from 15 to 75. Lower scores indicated greater coordination problems and discrimination thresholds dependent on age were used (Cunningham *et al.*, 2017).

3.9 Cognitive Assessments

3.9.1 Full-scale IQ (FSIQ), Performance and Verbal IQ (PIQ and VIQ)

Intelligence was assessed by the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) at all waves of assessment as well as in the adults with 22q11.2DS. The selection of subtests used as part of the WASI in the ECHO and IMAGINE-ID studies included two subtests for assessing performance IQ (PIQ) and two subtests for the assessment of verbal IQ (VIQ). PIQ is a measure of nonverbal ability and can be conceptualised as fluid intelligence, whereas VIQ is a measure of verbal ability and considered as acquired crystallised

intelligence. PIQ is assessed by block design and matrix reasoning whereas VIQ is assessed by the vocabulary and similarities subtests.

The four subtests are summed, generating subtest raw scores which are converted to T-scores using the WASI T-score conversion tables. To get the PIQ T-score, the block design and matrix reasoning T-scores are added, and similarly, the vocabulary and similarities T-scores are summed to generate a T-score for the VIQ. Combining the PIQ and the VIQ T-scores results in the generation of the FSIQ T-score. Using this score, a WASI IQ equivalent is generated. The WASI was double-rated to ensure consistency in the ratings (Heaton *et al.*, 1993; Wechsler, 1999).

3.9.2 Wisconsin Card Sorting Test (WCST)

The Wisconsin Card Sorting Test (WCST) (Heaton *et al.*, 1993) was used to assess executive functioning including strategic planning, organised searching, abstract reasoning ability and ability to shift cognitive strategies in response to feedback and contingency. The WCST assesses behaviour directed towards achieving a goal and used to modulate impulsive responding (Kohli and Kaur, 2006).

The WCST consists of four stimulus cards and sixty-four response cards which depict different figures where the shape, colour and form is manipulated. The underlying principle of the WCST was to assess whether the participant was able to determine that a pattern of card-matching changes periodically throughout the assessment. This is demonstrated by the individual changing the method of matching the cards according to the negative or positive response of the assessor. The participant is asked to match each response card to one of the four stimuli cards and they only receive recognition as to whether their positioning of the card is correct or incorrect; the reason is not revealed to the individual and therefore, they remain

unaware to the principle which is enacted until they personally determine it. Ten consecutive correct matches results in the principle of matching changing but the participant remains unaware of this unless they recognise the pattern.

A perseverative error is scored when the incorrect principle is matched by the participant. For example, a perseverative error is scored when the response cards are continually matched to the incorrect stimulus card but the participant has a reason for matching these cards such as matching the form, despite the matching principle being colour. The outcome measure used in **Chapters 4, 6 and 7** is a standard score: a higher standard score, the fewer perseverative errors.

A nonperseverative error is scored however where the response card is placed by a stimulus card without reason and without matching of the colour, shape or form. Nonperseverative total errors is also used as an outcome measure in this thesis in **Chapters 4, 6 and 7**.

3.9.3 Cambridge Neuropsychological Test Automated Battery (CANTAB)

The Cambridge Neuropsychological Test Automated Battery (CANTAB) (Cambridge Cognition Ltd, 2006) is a sensitive and validated cognitive research tool which provides objective measures of cognitive function which has been evidenced to correlate with neural networks (Robbins *et al.*, 1994; Cambridge Cognition, 2019). The CANTAB software was used to assess neurocognitive domains using different tasks. All tasks chosen as part of the ECHO Study and IMAGINE-ID battery had been verified for use in similar populations. Barnett, J.H. et al. (Barnett *et al.*, 2010) showed that the tests that were adopted in the ECHO and IMAGINE-ID studies were sensitive to psychopathology, especially deficits which are present in schizophrenia and therefore, ultra-high-risk populations such as 22q11.2DS are an appropriate model for identifying schizophrenia-related deficits. Gur, R.E. et al. (Gur *et al.*,

2014) used tests that were adopted in the ECHO Study and showed that there is a specific neurocognitive profile in 22q11.2DS young people compared to typically developing young people and age-matched developmental delay groups. Standard scores were used for each of the CANTAB tests other than the MTS total score where no normative score is available.

3.9.3.1 Rapid Visual Processing (RVP)

Visual sustained attention was assessed by Rapid Visual Processing (RVP).

Participants are asked to press a press-pad when they notice a sequence of '3, 5, 7' appear on the tablet screen in front of them. This sequence is hidden within a list of digits from 2-9. There are different levels to the assessment and the '3,5,7' sequence is initially underlined and bold, helping the participant. As each level is completed it becomes less obvious when the sequence is to appear and when to press the button.

The outcome measure used in this thesis is that of the RVP A' prime which is the signal detection measure of the sensitivity to the target sequence (3,5,7). This score ranges from 0 to 1, with 1 the highest.

3.9.3.2 Match to Sample Visual Search (MTS)

Match to Sample Visual Search (MTS) is a measure of visual attention by the number of correct target items distinguished from distractor stimuli. An abstract pattern stimulus is presented in the middle of the screen. Several abstract patterns are then presented in surrounding boxes circling the target stimulus. The participant must then match the target pattern in the middle of the screen with the same pattern on the outer-edge as quickly as possible.

The outcome measure refers to the percentage of correctly discriminated target items: the higher the percentage the better.

3.9.3.3 Five Choice Reaction Time (RTI)

Processing speed was measured by reaction times in milliseconds on the Five-choice reaction time (RTI) task.

The participant holds down the press-pad, only letting go of the press-pad when a yellow spot appears on the screen. Then the participant must respond as quickly as possible and tap the yellow spot displayed on the tablet.

The outcome measure used in this thesis relates to the time it takes to release the press-pad and tap the yellow spot: the quicker the time, the better reaction time.

3.9.3.4 Stockings of Cambridge (SOC)

The spatial planning aspect of executive function is assessed by the Stockings of Cambridge (SOC) task.

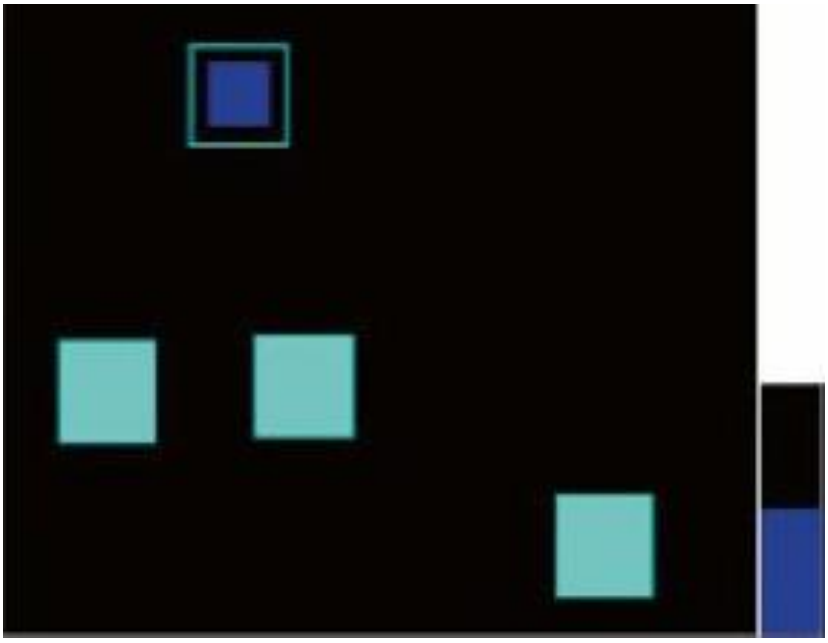
On the screen, there are two scenes where three coloured balls are stacked in stockings suspended from a beam. The participant must use the balls in the lower scene to recreate and copy how the balls are displayed in the upper scene. The balls can only be moved one at a time, and balls at the bottom of the 'stocking' cannot be moved until the balls stacked on top of them are removed and placed elsewhere.

The number of problems that were solved in minimum moves was used as an outcome measure in this thesis. A higher score is better.

3.9.3.5 Spatial Working Memory (SWM)

The Spatial Working Memory (SWM) task is used to assess spatial working memory as an aspect of higher executive function.

Several coloured boxes appear on the screen. The aim for the participant is to locate the darker blue tokens which are hidden in the coloured boxes. Once a dark blue token is found in the coloured box, there will never be another dark blue token under that box.



The number of coloured boxes presented on the screen increases from four to eight as the participant progresses through levels.

Figure 4 An example of the SWM task on CANTAB.

The outcome measure of SWM is 'between-errors'. This is the number of times a participant returns to a box in which they have already found a token. Therefore, the lower the score, the better the performance.

3.10 Subsamples

This thesis includes smaller cohorts of individuals, longitudinal, toddler and adult samples, which are derived from larger studies. These studies and how they are associated with the ECHO Study and the primary cohort of young people with 22q11.2DS is outlined in **Appendices 1** and **2** (**pages 327-329)**.

3.11 Correcting for Multiple Comparisons

Multiple comparisons have been corrected for in the results outlined in **Chapters 4, 6 and 7**, where there is a comprehensive explanation of the technique used to correct for the False Discovery Rate. To correct the analyses for multiple comparisons, we used a Benjamini–Hochberg false discovery rate (FDR) rate of 10% and 5% (Benjamini and Hochberg, 1995). The 10% application is applied in **Chapter 4** whereas the 5% application to the data in **Chapters 6** and **7**. The reason for the different thresholds is that **Chapter 4** was the initial analyses undertaken in young people with 22q11.2DS with regards to sleep problems. Therefore, the intention was to keep lines of enquiry open to explore associations with the neurodevelopmental phenotype. This work has been accepted for publishing in *Psychological Medicine* journal (April 2019).

However, once the initial work had been conducted it was deemed appropriate to align the remaining data with the typical 5% FDR, which is comparable to the 95% CI and p=0.05 in typical analyses. There were many tests completed in **Chapters 6** and **7** and therefore, the smaller FDR seemed to be most appropriate for focussing in on the sleep phenotype in 22q11.2DS.

The Benjamini-Hochberg method ranks the individual p-values from smallest to largest, with the smallest p-value ranking as 1. By comparing each individual p-value to the Benjamini-Hochberg critical value, ((rank/total number of tests) * FDR (i.e., 5%)), if the largest p-value in our analyses before correction is smaller than its critical value, it is considered significant. Any p-values smaller than the largest p-value, that has a p-value smaller than its own critical value, are also interpreted to be significant even if the p-values themselves are not less than their own Benjamini-Hochberg critical value.

For the pre-processing of the EEG data, a standardised early-stage EEG processing pipeline (PREP) (Bigdely-Shamlo *et al.*, 2015) (see **Chapter 7**) was conducted. As part of the pipeline, multiple testing was corrected for.

The results have been corrected for multiple comparisons. The exploratory nature of this work allows for some leniency in the interpretation, however by controlling for multiple testing, the potential for over-interpretation of the findings from the exploratory investigations has been limited.

Chapter 4 – Sleep Problems and the Neurodevelopmental Phenotype in 22q11.2 Deletion Syndrome

4.1 Overview of Chapter

A lack of focus on sleep problems in 22q11.2DS has resulted in scant literature thus far. There is a small pool of literature regarding sleep-related breathing disorders (SRBD), especially obstructive sleep apnoea (OSA). The literature includes case studies and small samples of 22q11.2DS as well as some larger studies of individuals with syndromic craniofacial abnormalities that include 22q11.2DS (Moraleda-Cibrian *et al.*, 2014; Silvestre *et al.*, 2014b). The physical problems of 22q11.2DS such as velopharyngeal insufficiency (VPI), cleft palate and narrow airways increase the risk for OSA and adds to the clinical interest in sleep-related breathing problems in this population (Heike *et al.*, 2007).

Individuals with 22q11.2DS have a broad phenotype (see **Chapter 1**) but how sleep problems feature in this phenotype has yet to be investigated. Sleep problems have been overlooked (see **section 2.2**) and at the current time of writing, there have been no studies exploring the prevalence and nature of sleep problems in this population of young people. It is not known what type of sleep problems are common in 22q11.2DS despite anecdotal parent reports of restlessness, insomnias (particularly initial and early), and daytime tiredness in their children. The topic of Sleep medicine is broad; the literature includes studies on the prevalence of sleep problems in young people and adults in typical populations and there is also expanding literature about sleep problems in neurodevelopmental disorders (Johnson, 1996; Wiggs, 2009; Robinson-Shelton and Malow, 2016). Sleep research in genetic syndromes, however, is

sparse, especially in syndromes where there is a strong biological risk for psychiatric disorders and intellectual disability. The studies reported in the literature tend to focus on Smith Magenis Syndrome and Williams's Syndrome where genetic causes have been attributed to the high preponderance of sleep problems (see **Chapter 2**, **section 2.1.4**) (De Leersnyder *et al.*, 2001; Goldman *et al.*, 2009). An underlying genetic aetiology can help to better understand the sleep and circadian problems seen in specific conditions, but in 22q11.2DS there is currently no known genetic cause to sleep problems.

Once sleep problems in 22q11.2DS have been better understood, relationships between sleep problems and other symptoms can be examined, for example how sleep relates to the neurodevelopmental phenotype. There is variability in the definition of neurodevelopment but in this thesis, the neurodevelopmental phenotype is defined as the psychiatric, neurodevelopmental and neurocognitive domains that are present in 22q11.2DS and outlined in **Chapter 1, section 1.3**, and **Chapter 3, section 3.5-3.9**. The physical phenotype could also play a role in the manifestation of sleep problems especially in individuals with cardiac, respiratory and skeletal problems. Understanding the severity and persistence of sleep problems in 22q11.2DS is clinically important and can contribute toward developing the most appropriate treatments and interventions.

This chapter aims to examine the prevalence and nature of sleep problems in young people with 22q11.2DS and their siblings, cross-sectionally exploring the relationship between sleep problems and the neurodevelopmental phenotype of 22q11.2DS.

To gain further insights into the persistence of sleep problems in 22q11.2DS, this chapter includes the results of longitudinal analysis based on data collected from a follow-up assessment. Additionally, I present sleep problem data collected from the ECHO toddler study

and data on the rates of sleep problems and disorders from adults with 22q11.2DS to gain evidence of the lifetime persistence and clinical importance of sleep problems in 22q11.2DS.

4.2 Part One - Cross-sectional analysis of sleep in young people with 22q11.2DS and sibling controls

4.2.1 Introduction

Sleep is pivotal in healthy brain development, metabolism, cognition and general well-being (Cirelli and Tononi, 2017) and sleep problems from a young age can seriously affect these important mechanisms. Moreover, sleep deprivation and poor sleep quality at any age can elevate risk of neuronal dysfunction (Bonnier, 2008).

There is a high degree of brain plasticity during childhood as changes to neuronal circuitry allow for reorganisation and development of neural pathways (Mundkur, 2005). This period of development however leaves the brain vulnerable to injury and therefore, processes which disturb neuroplasticity such as disturbed sleep, can have a larger impact in children compared to adults (Jan *et al.*, 2010; Bonuck and Grant, 2012). There is increasing evidence that sleep deprivation and inadequate sleep quality is robustly associated with cognitive impairment and poor psychiatric outcomes (Dorris *et al.*, 2008; Krystal, 2012). This indicates that young people may be at higher risk of poor outcomes that may persist into adolescence and early adulthood (Dahl, 2007; Kahn-Greene *et al.*, 2007; Limoges *et al.*, 2013; Rasch and Born, 2013; Krause *et al.*, 2017).

In the typical paediatric population rates of sleep problems range from 25-50% (Owens and Mindell, 2011) (see **Chapter 2**, **section 2.1.2**). The wide range could arise from the use of sleep

measures that have not been systematically evaluated; sleep questionnaires are frequently used as the only parameter of assessment in paediatric sleep studies however the variety of questionnaires include different assessment criteria making comparability of studies more difficult (Spruyt and Gozal, 2011b).

Nonetheless, subjective sleep questionnaires remain the most common tool of assessment and can provide a wealth of data regarding the types of sleep problems experienced, signposting the problem to address. Parasomnias (nightmares, night terrors and sleepwalking) have been identified to be the most prevalent in typical populations. One study showed that 35% of children at the age of one-and-a-half have experienced night terrors; these are one of the more common sleep problems in children under the age of 13 with 56% having experienced night terrors at least once (Moreno, 2015).

Young people with neurodevelopmental disorders (NDD) are at particular risk of sleep problems (see **Chapter 2**, **section 2.1.3**) with an estimated prevalence as high as 86% (Wiggs, 2001; Robinson-Shelton and Malow, 2016). More specifically 40-80% of children with ASD have been reported to have sleep problems, ADHD 25-50%, anxiety disorders 88% and intellectual disability 26-86% (Wiggs, 2001; Staner, 2003; Souders *et al.*, 2009). Furthermore, individuals with NDD who suffer with sleep problems are reported to be at increased risk of impairments in cognitive function and motor coordination (Thapar *et al.*, 2012; Lloyd, MacDonald and Lord, 2013; Ren *et al.*, 2016).

As NDDs are a core feature of 22q11.2DS, impairments in cognitive function are prevalent (see **Chapter 2.9**) regardless of sleep problems. The average IQ is estimated to be ~30 IQ points lower than typically developing children (Niarchou *et al.*, 2014) and deficits in cognitive function including: reaction time, sustained attention, processing speed, spatial working

memory and executive functioning have been documented (Hooper *et al.*, 2013; Niarchou *et al.*, 2014). Additionally, deficits in motor coordination are common in NDDs (Vasserman, Bender and Macallister, 2014) and are highly preponderant in 22q11.2DS with over 80% of young people having been reported to have motor coordination problems (Cunningham *et al.*, 2017). These problems may potentially be linked to some of the widespread brain abnormalities that have been observed in these patients, including increased cortical surface area in the sensorimotor cortex and reduced cerebellum size (Sun *et al.*, 2018).

Sleep in 22q11.2DS remains an understudied area of research despite reports of associations between sleep problems and a range of neurodevelopmental impairments in other populations (Wiggs, 2001; Johnson and Malow, 2008; Robinson-Shelton and Malow, 2016).

4.2.2 Methodology

Participants were part of the ongoing Experiences of CHildren with cOpy number variants (ECHO) study (Niarchou *et al.*, 2014; Chawner *et al.*, 2017; Cunningham *et al.*, 2017). For further information regarding the study see **Chapter 3, section 3.1** and **Appendices 1** and **2**.

The current investigations focussed on 140 children with 22q11.2DS ($\bar{x}$ age = 10.1, range = 6 years 3 month–17 years 1 month, s.d. = 2.45, 45.0% females) and 65 age-matched sibling controls ($\bar{x}$ age = 10.8, range = 6 years 1 month –16 years 7 months, s.d. = 2.26, 43.1% females). Family income information was obtained from primary carer questionnaires and can be found together with information of the age and gender of the participants in **Table 2**.

Table 2 Demographic information on participating families

				%
Family ethnic background				
	European			85.9
	Mixed			4.23
	Non-European			2.11
	Unknown			7.76
Highest maternal education qualification				
	No qualifications			8.57
	Low: O-levels or GCSEs			22.9
	Middle: A-levels/Highers or vocational training			23.6
	High: University degree and/or other higher postgraduate qualification			36.4
	Other			3.52
	Unknown			3.57
Family annual income (£)				
	£≤19,999			20.4
	£20,000-39,000			23.9
	£40,000-59,000			18.3
	£≥60,000			23.9
	Unknown			13.4
Age, years: mean (s.d.) range				***p***
Probands	10.1 (2.46) 6.02-17.1	*Siblings*	10.8 (2.26) 6.29-16.6	**0.04**
Gender, n (%)				
Probands	63/140 F (45%)	*Siblings*	28/65 F (43.1%)	0.796

In the 22q11.2DS cohort, 17 young people were on melatonin for sleep problems; one young person was on antipsychotic medication aripiprazole for psychotic experiences; one was on risperidone for mood disorder and none were on ADHD medication. 10.7% (15/140) of the young people with 22q11.2DS were reported to have experienced seizures during their lifetime or have an epilepsy diagnosis with one person receiving sodium valproate for epilepsy. No unaffected control siblings were medicated for sleep problems, seizure-related problems or epilepsy. Prior to recruitment, primary carers consented for all participants and additional consent was obtained from participants aged ≥16 years with capacity. The protocols used in this study were approved by NHS Wales Research Ethics Committee 1.

4.2.2.1 Assessments

Psychopathology and neurodevelopmental assessment

The semi-structured Child and Adolescent Psychiatric Assessment (CAPA) (Angold *et al.*, 1995) was conducted with the primary carer by trained psychologists. Information regarding the following can be found at the stated locations: psychiatric assessments (**section 3.5**), sleep problems section of the CAPA (**section 3.6**), cognitive assessments (**section 3.9**) and parental questionnaires including the SCQ (**section 3.7**) and DCDQ (**section 3.8**). The DCDQ was introduced later in the ECHO Study accounting for the fewer questionnaires completed compared to the other measures.

The assessments undertaken were completed by different sample sizes as a result of the completeness of tasks and introduction of questionnaires into the wider study. ADHD and anxiety symptoms were available for all n=140 individuals with 22q11.2DS; CD and ODD symptoms for n=134; SCQ total scores for n=128 and the DCDQ score was available for n=97 individuals with 22q11.2DS. All young people had sleep problem scores.

Covariates (age, gender, family socio-economic status and physical outcomes)

Family income information was obtained from questionnaires completed by the primary carer and can be found together with information of the age and gender of the participants in **Table 2** (**page 76**). Physical health problems were also assessed in the parental questionnaire.

4.2.2.2 Aims of the Subjective Cross-Sectional Analyses

The primary aims were:

1. To identify the prevalence of sleep problems in young people with 22q11.2DS compared to their unaffected siblings;
2. To determine patterns of sleep problems in young people with 22q11.2DS;
3. To investigate links between patterns of sleep problems and the neurodevelopmental phenotype.

4.2.2.3 Statistical Analysis

The statistical analyses refer to the aims stated in **section 4.2.2.3**.

For aim 1, chi-squared tests were used to explore differences in the ages, and t-tests were used to investigate the differences in gender between the two groups. Gender was not used as a covariate as there was no difference in proportions between groups (p=0.967) but there were differences in age (p=0.04) (**Table 2, page 76**). Differences in overall sleep problem scores (sum of positively endorsed sleep items) between the two groups were assessed using logistic regression analysis including age as a covariate.

For aim 2, exploratory factor analysis (EFA) (Conway and Huffcutt, 2003) was used to establish the number of factors underlying the structure of the responses to the CAPA sleep section for the children with 22q11.2DS. Statistically, combining items that represent a single underlying

construct is important as it reduces the number of comparisons that would be conducted if each individual item was analysed separately and enhances the robustness of the measured concept. Clinically, it is important to understand whether sleep problems group into particular patterns and have different associations with other outcomes such as psychopathology as this could have implications for interventions.

To conduct the EFA appropriately, pre-processing of the data was required. Initially, two-by-two tables were constructed for each of the different combinations of the sleep variables derived from the CAPA. This was followed by a tetrachoric correlation (Pearson, 1900) which is a suitable type of correlation matrix and analysis for dichotomous variables that assume an underlying normal distribution, with the theory of continuous latent variables to explain variance (Ledesma, Macbeth and Valero-Mora, 2011).

The two-by-two tables suggested that some combinations of variables were highly correlated and there were some tables that showed positive endorsement that equalled zero. This was apparent for parasomnia items and several other items: night terrors and hypersomnia, sleep walking and hypersomnia; night terrors and tiredness; night terrors and fatigability; nightmares and fatigability; and sleep walking and fatigability. As a result, no correlation coefficients could be calculated for these combinations. In addition, there were other two-by-two tables that showed very low cell count and positive endorsement, with many of these correlations including sleep walking as a variable: sleep walking with initial insomnia (r=-0.036); sleep walking with early insomnia (r=-0.002); as well as fatigability with middle insomnia (r=-0.01). The parasomnia items were problematic when combined with the other variables however, they didn't show improved correlations between each other: sleep walking with nightmares (r=0.06, p=0.620); sleep walking with night terrors (r=0.04, p=0.490),

except for nightmares and night terrors (r=0.400, p<0.001). Sleep walking was the only sleep item in the CAPA that was asked dependent on lifetime history suggesting that it might not reliably present the rates of sleep walking in the current cohort. The parasomnia items also did not differ significantly between young people with 22q11.2DS and control siblings (**Table 3**, **page 84),** indicating that they do not represent sleep problems that are specific to young people with the deletion. Therefore, the parasomnia items were removed from the analysis.

Furthermore, of the remaining items, hypersomnia, tiredness and fatigability correlated quite strongly (hypersomnia with tiredness r=0.510; hypersomnia with fatigability r=0.370; tiredness with fatigability r=0.560). Therefore, these variables were combined into a single score capturing tiredness-related sleep problems. Similarly, the items early and middle insomnia also correlated quite strongly (r=0.522, p<0.001) and were combined (into an item capturing mid/early insomnia). By combining these variables, the very low cell counts and positive endorsement were accounted for and the almost zero correlation between middle insomnia and fatigability was corrected. Item combination was as follows: a dichotomous variable was created where a positive score on any of the items translated into a positive score on the combined variable.

This produced a 5-item tetrachoric correlation matrix which was the basis for exploratory factor analysis (EFA) (Conway et al. 2003) to determine the patterns of sleep problems in young people with 22q11.2DS. A tetrachoric correlation matrix is a special type of polychoric correlation which estimates the correlation between two hypothetical parametric continuous latent variables that have been derived from two actual observed ordinal variables (Drasgow, 1986). The tetrachoric correlation is special as the observed variables are dichotomous. The

two-by-two infers Pearson correlation, but with the assumption of bivariate normality (Günther and Höfler, 2006).

The number of factors retained was based on the scree plot of eigenvalues (values ≥0.6) and interpretation of meaningful patterns (Gorsuch, 1983). The factor solution was rotated using a promax method of rotation. A promax rotation is a type of oblique solution used to maximise interpretation. An oblique rotation allows for the factors to be correlated. In this study, it is rationalised that a young person with 22q11.2DS with sleep problems relating to insomnia also has sleep problems relating to tiredness suggesting that the factors should be correlated (Gorsuch, 1983). The oblique rotation of the axes for the distribution of the data accommodates the correlating data better than an orthogonal rotation which keeps the axes at right-angles reducing the interpretability of the data (IBM, 2018). The rationale was based on the literature supporting overlap of insomnia-related problems with restlessness and tiredness (Staner, 2003). Often problems with initiating and maintaining sleep can co-occur with restlessness especially in individuals with an anxiety disorder (Sheehan, Ballenger and Jacobsen, 1980).

The sleep patterns were calculated by summing the binary sleep items that loaded on each factor ("1" = ≥1 positive item; "0" = no positive items) for use in further analysis. Spearman correlation analysis was used to determine the patterns of sleep problems interrelationship.

To explore aim 3, hierarchical logistic regression analyses were used to examine links between the patterns of sleep problems (presence or absence of a pattern) and the children's age (step 1), with the addition of psychiatric disorder symptoms (ADHD, ASD, anxiety, CD and ODD) and DCD, and cognitive performances (IQ, spatial working memory, sustained and visual attention, spatial planning and executive function) each individually (step 2). The patterns of

sleep problems were investigated in relation to potentially confounding factors such as maternal education level and melatonin use. Sensitivity analyses were conducted accordingly. Other potential confounders (e.g., other medication use) were not evaluated because of low sample prevalence.

To correct for multiple comparisons, the Benjamini–Hochberg correction of false discovery rate (FDR) applying a 10% FDR was used (Benjamini and Hochberg, 1995) (see **section 3.11**) . All analyses that survived FDR correction are denoted in tables by an asterisk (*). Statistical analysis was carried out using STATA (version 13.1) (https://www.stata.com/stata-news/news28-4/stata13.1) and R (version 3.5.0) (*R: The R Project for Statistical Computing*, 2018).

4.2.3 Results

In this sample, the individuals with 22q11.2DS were younger than sibling controls (t=2.09, p=0.04) but there was no difference in the gender proportions (p=0.796).

4.2.3.1 Prevalence of Sleep Problems in 22q11.2DS (Aim 1)

There were 60% of young people with 22q11.2DS with at least one sleep problem out of the 11 sleep items outlined in the sleep problems section of the CAPA (see **Table 3**) (22q11.2DS median=1 (range 0-7)). This prevalence was compared to 23.1% of sibling controls (controls median=0 (range 0-5)) (OR=5.00 p=<0.001) (**Figure 5, Table 3**). Analysis of the 11 CAPA items showed that children with 22q11.2DS had higher rates of insomnias, restless and inadequately rested sleep but no differences with sibling controls for hypersomnia, fatigability and parasomnias (**Table 3**).

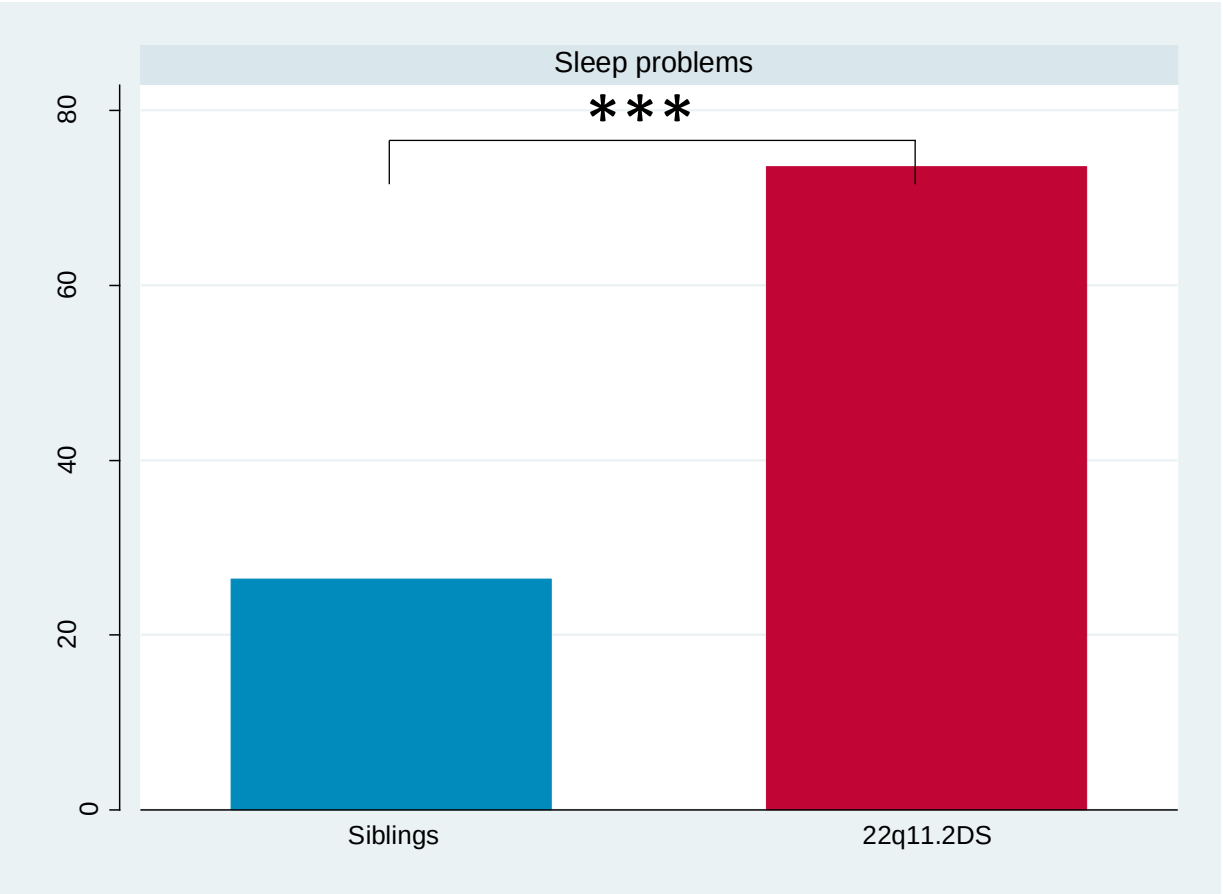


Figure 5 Prevalence of sleep problems in 22q11.2DS and siblings

Table 3 Prevalence of sleep problem items in young people with 22q11.2DS and sibling controls

Sleep items, n (%)	***22q11.2DS***	***Siblings***	***OR***	***95% CI for means/medians differences***	***p***
At least one sleep problem	84/140 (60)	15/65 (23.1)	5.00	2.56-9.76	<0.001
Overall total number of sleep problems	84/140 (60)	15/65 (23.1)	1.86	1.34-2.57	<0.001
	Total, n (%)				
Insomnias					
Initial[b]	32 (22.9)	7 (10.8)	2.46	1.02-5.91	0.045
Middle[b]	23 (16.4)	1 (1.54)	12.6	1.66-95.3	0.014
Early[b]	13 (9.29)	0 (0)	-		-
Hypersomnia[a]	8 (5.71)	1 (1.54)	3.88	0.475-31.7	0.206
Restless sleep[a]	45 (32.1)	4 (6.15)	7.22	2.47-21.1	<0.001
Inadequately rested[a]	27 (19.3)	1 (1.54)	15.3	2.03-115	0.008
Tiredness[a]	15 (10.7)	1 (1.54)	7.68	0.992-59.4	0.051
Fatigability[a]	7 (5.00)	1 (1.54)	3.37	0.406-28.0	0.261
Parasomnias					
Nightmares	16 (11.4)	6 (9.23)	1.27	0.472-3.41	0.637
Night terrors	8 (5.71)	2 (3.08)	1.91	0.384-9.25	0.422
Sleepwalking	11 (7.86)	4 (6.15)	1.30	0.398-4.25	0.664

The sleep items denoted with [a] capture the tiredness-related sleep pattern and those with [b] capture the insomnia-related pattern of sleep problems best.

In the subsample of 22q11.2DS children with a participating sibling control no correlation between the number of sleep problems each sibling experienced was found (r=0.080, p=0.704).

4.2.3.2 Patterns of Sleep Problems in 22q11.2DS (Aim 2)

Table 3 shows the absence of a difference in the rates of parasomnias between 22q11.2DS and sibling controls further adding to the justification of removing the parasomnias from the tetrachoric correlation matrix in the EFA.

The EFA is illustrated in **Figure 6** showing that sleep problems in 22q11.2DS were captured best by two patterns: tiredness-related sleep (see **Table 3** denoted [a]) and insomnia-related (see **Table 3** denoted [b]). The patterns showed a modest correlation (r=0.252, p=0.003) reflecting that the children can have both problems.

54.3% (76/140) of young people with 22q11.2DS scored on at least one sleep pattern. Of these 76 individuals, 44.7% (34/76) scored positive for the tired-related sleep pattern only; 21.1% (16/76) on the insomnia-related sleep pattern only and 34.2% (26/76) on both patterns.

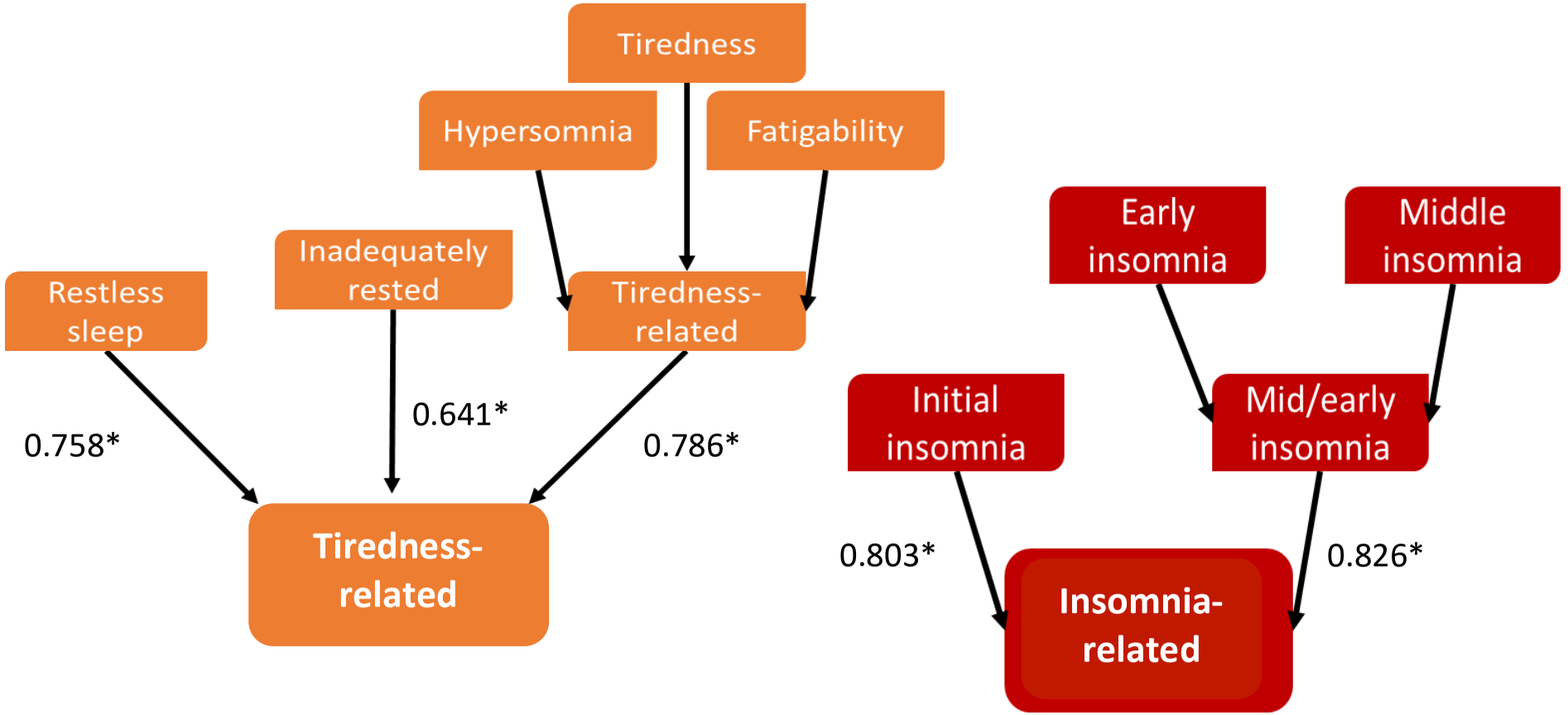


Figure 6 Visualisation of the two-factor outcome from the exploratory factor analysis (EFA).

After exclusion of the parasomnia items (nightmares, night terrors and sleep walking), the hypersomnia, tiredness and fatigability were combined into one item 'tiredness-related' because they correlated strongly. Similarly, early and middle insomnia were combined. EFA indicated that a structure of two patterns, 'tiredness-related sleep' and 'insomnia-related', best described the sleep problems of children with 22q11.2DS.

4.2.3.3 Links between Sleep Patterns and the Neurodevelopmental Phenotype (Aim 3)

Rates of psychiatric disorder in 22q11.2DS are elevated compared to the typical paediatric population (see **Chapter 1, section 1.3**). Comparing rates of neurodevelopmental disorder diagnosis in young people with and without reported sleep problems can potentially provide a better understanding of the pathology of the neurodevelopmental phenotype and the sleep problems.

Young people with 22q11.2DS who scored positive for the tiredness-related sleep pattern compared to those who did not had higher rates of ADHD, anxiety disorder, CD and ODD diagnoses (**Table 4**). No differences were found in the rates of diagnosis in those who scored positive on the insomnia-related sleep pattern compared to those who did not (**Table 4**).

Table 4 Associations between the tiredness-related and insomnia-related sleep patterns and psychiatric disorder in young people with 22q11.2DS

	Tiredness-related Sleep			***Insomnia-related Sleep***		
Psychiatric Disorder	***Positive, n (%)***	***Negative, n (%)***	***p***	***Positive, n (%)***	***Negative , n (%)***	***p***
ADHD diagnosis	33 (55)	21 (26.3)	**0.001**	18 (42.9)	36 (36.7)	0.495
Indicative ASD	25 (43.9)	20 (29.4)	0.094	18 (47.4)	27 (31.0)	0.080
Any anxiety diagnosis	20 (33.3)	15 (18.8)	**0.049**	12 (28.6)	23 (23.5)	0.523
Indicative DCD	38 (88.4)	41 (76)	0.117	24 (88.9)	55 (78.6)	0.241
CD/ODD diagnosis	17 (28.8)	8 (10.5)	**0.007**	11 (28.2)	14 (14.6)	0.065

Sample sizes were different as some CAPA interviews were incomplete: the CD and ODD sections were the final to be completed during the interview and therefore, remained incomplete on some occasions. The DCDQ was introduced later in the overall ECHO Study and the SCQ was part of the questionnaire pack which was sometimes incomplete or not returned by the participating primary carer.

There was a subsample of 39.3% (18/46) of young people with 22q11.2DS who did not meet diagnostic criteria for a neurodevelopmental phenotype, but did score positively for sleep problems.

Investigating the symptom scores of the neurodevelopmental phenotype provided an additional dimension to the findings. The tiredness-related sleep pattern was associated with increased symptoms of ADHD, indicative ASD, anxiety disorder, indicative DCD, oppositional disorder and conduct disorder, as well as a poorer performance on the sustained attention and set shifting ability (perseverative errors on the Wisconsin Card Sorting Task (WCST)) tasks (see **Table 5**). However, the association with sustained attention did not persist when correcting for multiple testing using the Benjamini-Hochberg 10% critical value for FDR.

Table 5 Associations between tiredness-related sleep pattern and psychopathology, IQ and cognitive function

Psychopathology (symptom counts)	***OR***	***S.E.***	***95% CI***	***p***
Attention–deficit hyperactivity disorder	1.16	0.047	1.07-1.25	**<0.001**
Indicative autism-spectrum disorder	1.05	0.027	1.00-1.10	**0.048**[b]
Anxiety disorder	1.10	0.039	1.03-1.19	**0.006**
Developmental coordination disorder[a]	0.968	0.014	0.940-0.996	**0.023**
Conduct disorder	1.41	0.237	1.02-1.96	**0.038**[b]
Oppositional defiant disorder	1.29	0.137	1.05-1.59	**0.015**[b]
IQ	***OR***	***S.E.***	***95% CI***	***p***
Full-scale IQ	0.980	0.0151	0.951-1.01	0.194
Performance IQ	0.984	0.0156	0.953-1.01	0.296
Verbal IQ	0.981	0.0137	0.955-1.01	0.175
Cognitive processing	***OR***	***S.E.***	***95% CI***	***p***
Processing speed (five-choice reaction time)	0.938	0.096	0.769-13.14	0.525
Sustained attention (rapid visual processing)	0.832	0.076	0.696-0.995	**0.044**[*]
Visual attention (match to sample)	0.997	0.026	0.948-1.05	0.900
Spatial planning	0.950	0.192	0.640-1.41	0.800
Spatial working memory	0.909	0.174	0.625-1.32	0.616
Set shifting ability	0.973	0.001	0.954-0.993	**0.008**
Errors on the Wisconsin Card Sorting Task	1.02	0.010	0.998-1.04	0.074

[a]Developmental coordination disorder is reversed scored. [b]Associations did not persist during sensitivity analyses. [*]Correcting for the FDR of 10% meant that these associations did not reach the Benjamini-Hochberg criteria.

Post-hoc analysis of the association between tiredness-related pattern of sleep problems and ADHD symptoms showed inattentive (OR=1.23, p=0.001) and hyperactive-impulsivity symptoms (OR=1.26, p=0.002) were associated with an elevated risk of tiredness-related problems.

The insomnia-related sleep pattern showed associations with increased symptoms of anxiety disorder, indicative DCD and CD as well as impaired performance on the visual attention task (**Table 6**). The association with visual attention did not persist when controlling for multiple testing using the Benjamini-Hochberg 10% critical value for FDR.

Table 6 Associations between insomnia-related sleep pattern and psychopathology, IQ and cognitive function

Psychopathology (symptom counts)	***OR***	***S.E.***	***95% CI***	***p***
Attention–deficit hyperactivity disorder	1.04	0.042	0.961-1.12	0.338
Indicative autism-spectrum disorder	1.04	0.027	0.992-1.10	0.095
Anxiety disorder	1.07	0.036	1.00-1.14	**0.045**
Developmental coordination disorder[a]	0.955	0.017	0.922-0.989	**0.009**
Conduct disorder	1.53	0.278	1.07-2.18	**0.020**
Oppositional defiant disorder	1.22	0.133	0.980-1.51	0.075
IQ	***OR***	***S.E.***	***95% CI***	***p***
Full-scale IQ	0.988	0.017	0.956-1.02	0.467
Performance IQ	0.982	0.018	0.949-1.02	0.315
Verbal IQ	0.992	0.015	0.963-1.02	0.595
Cognitive processing	***OR***	***S.E.***	***95% CI***	***p***
Processing speed (five-choice reaction time)	1.15	0.157	0.881-1.50	0.303
Sustained attention (rapid visual processing)	0.995	0.061	0.882-1.12	0.941
Visual attention (match to sample)	0.945	0.026	0.895-0.998	**0.042**[*]
Spatial planning	1.21	0.277	0.771-1.89	0.410
Spatial working memory	1.09	0.227	0.729-1.64	0.663
Set shifting ability	0.989	0.010	0.970-1.01	0.301
Errors on the Wisconsin Card Sorting Task	1.01	0.010	0.989-1.03	0.381

[a]Developmental coordination disorder is reversed scored. [*]Correcting for the FDR of 10% meant that these associations did not reach the Benjamini-Hochberg criteria.

Comorbid presentation of both sleep patterns with psychiatric symptoms was high. Of the 60 young people who scored on the tiredness-related sleep pattern, 86.7% (52/60) had at least one psychiatric symptom as did 52.4% (22/42) of the young people scoring on the insomnia-related sleep pattern. The most common presentation for the tiredness-related sleep pattern (18.6%) was in combination with symptoms of CD/ODD, anxiety disorder, indicative DCD and ADHD and the most common comorbid presentation for the insomnia-related sleep pattern (35.7%) was in combination with CD/ODD, anxiety and DCD.

Sensitivity analyses

No links between age (tiredness-related pattern, p=0.912; insomnia-related pattern=0.069), gender (p=0.493; p=0.970) nor gross family income (p=0.418; p=0.432) were found with either pattern of sleep problems.

An association between highest maternal education and the tiredness-related sleep pattern (p=0.008) was found. Additionally, associations between melatonin use and both tiredness-related sleep (p=0.003) and insomnia-related (p<0.001) sleep patterns were derived. As a result, sensitivity analyses were conducted by adding 1), highest maternal education and 2), melatonin use to the regression analyses to see if the findings changed and finally 3) the analysis of the links between sleep patterns and anxiety symptoms were rerun whilst removing sleep-related anxiety symptoms from the total anxiety symptom score. These items were: reluctance to sleep alone; sleeps with family member; rises to check on family members; avoidance of sleeping away from family; separation dreams; easy fatigability and restlessness.

The findings showed that higher maternal education was associated with a reduced risk for the child with 22q11.2DS scoring positively on the tiredness-related sleep pattern (p=0.011),

but level of maternal educational attained was not associated with the insomnia-related pattern of sleep problems (p=0.866). A sensitivity analysis was then conducted to see if the associations found with the tiredness-related sleep pattern illustrated in **Table 5** remained when introducing highest maternal education into the regression analyses as a covariate. The associations between tiredness-related sleep and indicative ASD (p=0.101), conduct symptoms (p=0.094) and oppositional symptoms (p=0.087) did not persist when controlling for highest maternal education.

12% (17/140) of the children with 22q11.2DS were taking melatonin at the time of assessment and 94.1% of those (16/17) reported sleep problems. Children taking melatonin had higher levels of sleep problems ($p<0.0001$, $\bar{x}=3.12$ and median=3) compared to those not taking melatonin ($\bar{x}=1.23$ and median =1) with elevated rates of tiredness-related sleep (OR=5.26, p=0.006) and insomnia-related (OR=15.8, $p<0.0001$). 68.8% (11/16) scored on both patterns; 18.8% (3/16) on the tiredness-related sleep pattern and 12.5% (2/16) on the insomnia-related pattern only. Melatonin use was a proxy for sleep problem severity in the sample, and therefore was not included in analyses as a covariate.

On removing the sleep-related anxiety symptoms and repeating regression analyses no changes were found; links with both sleep problem patterns remained.

4.2.4 Discussion

This is the first study to systematically investigate subjective sleep problems in 22q11.2DS. I found high rates of sleep problems affecting around two-thirds of these young people compared to around a quarter of sibling controls. Two patterns of sleep problems were found in 22q11.2DS: tiredness-related sleep and insomnia-related sleep, which occurred at rates of 43% and 30% respectively. Both patterns were associated with elevated symptoms of anxiety

disorder and indicative DCD. The tiredness-related sleep pattern was also associated with increased ADHD symptoms and impaired executive function, and the insomnia-related sleep pattern with conduct disorder symptoms.

4.2.4.1 High Prevalence of Sleep Problems in 22q11.2DS

Young people with 22q11.2DS showed a higher prevalence of sleep problems at 60% than reported for young people in the general population (Owens and Mindell, 2011). The findings add to a scarce literature of sleep problems in young people with genetic syndromes (De Leersnyder *et al.*, 2001; Williams *et al.*, 2006; Trickett *et al.*, 2018). 23% of siblings reported sleep problems which is comparable to typical childhood rates at 25-50% (Owens and Mindell, 2011). Paired analysis of children with 22q11.2DS and their siblings showed no correlation between the number of sleep problems reported. However, data was available from only 63 sibling pairs and these analyses may have been underpowered.

4.2.4.2 Patterns of Sleep Problems in 22q11.2DS

Tiredness-related sleep and insomnia-related were identified as the two patterns of sleep problems in 22q11.2DS suggesting that it may be relevant to distinguish sleep problems dependent on their pattern in further research.

4.2.4.3 Links between Sleep Patterns and the Neurodevelopmental Phenotype in 22q11.2DS

The study showed that ADHD symptoms were linked to increased likelihood of scoring on the tiredness-related sleep pattern and these results are in line with genetically heterogeneous (Lycett *et al.*, 2015) young people with ADHD showing that sleep problems including sleep-related breathing disorders (Chervin *et al.*, 1997; Karen Spruyt and Gozal, 2011) and restless legs syndrome (Chervin *et al.*, 2002) are common in this group.

Previous work showing a higher incidence of inattentive ADHD subtype in 22q11.2DS compared to idiopathic ADHD populations (Niarchou *et al.*, 2018) is supported by the finding that the link between ADHD symptoms and tiredness-related sleep is driven by inattentive ADHD. These results should be interpreted with caution as only three individuals in the 22q11.2DS sample had the hyperactive-impulsive ADHD subtype.

Symptoms of indicative ASD were associated with the tiredness-related sleep pattern complementing previous reports of sleep problems that are common in non-22q11.2 deletion young people with ASD (Reynolds and Malow, 2011; Cohen *et al.*, 2014; Robinson-Shelton and Malow, 2016). These sleep problems are various and of different types including parasomnias, bedtime resistance, insomnia and daytime sleepiness (Johnson and Malow, 2008; Souders *et al.*, 2009). The association found in the study cohort did not persist in sensitivity analyses when accounting for the effects of maternal education; young people with 22q11.2DS of mothers with higher educational attainment were less likely to have tiredness-related sleep, explaining the association with ASD.

Conduct and oppositional defiant disorder symptoms were associated with the tiredness-related sleep pattern (Eastabrook *et al.*, 2003) and conduct disorder symptoms also with the insomnia-related pattern. Children with CD and ODD are reported to have more sleep problems than typically developing children including increased nocturnal awakenings and delayed sleep onset, and conduct problems show particular links with sleep-related breathing disorders and periodic limb movements (Eastabrook *et al.*, 2003). The associations between tiredness-related sleep pattern and symptoms of CD and ODD did not persist when accounting for the effects of maternal education.

Anxiety symptoms were associated with both the tiredness-related and insomnia-related sleep patterns and these links remained after removing sleep-related anxiety symptoms from the total symptom score. Insomnia is a common complaint in anxiety disorders (Vriend and Corkum, 2011) as is fatigue (Chorney *et al.*, 2007) and prior studies have identified links between limb movements during sleep and generalised anxiety (Saletu *et al.*, 2002; Staner, 2003). The findings in this young high-risk population highlight the need for increased awareness of sleep problems (Benca *et al.*, 1997; Staner, 2003; Turnbull, Reid and Morton, 2013).

As previously reported the rate of indicative DCD in 22q11.2DS is very high (80%) (Cunningham *et al.*, 2017) and motor coordination problems are associated with both tiredness-related and insomnia-related sleep patterns. There are few studies reporting sleep problems in DCD with the exception of few reports outlining disturbed sleep patterns (Wiggs, Sparrowhawk and Barnett, 2016) including bedtime resistance and daytime sleepiness (Barnett and Wiggs, 2012) and impaired sleep quality (Wiggs, Montgomery and Stores, 2005). Investigating the mechanism linking sleep and impaired motor coordination in 22q11.2DS can add to the understanding of how sleep problems affect neurological processes.

The tiredness-related sleep pattern was associated with poor executive function and sustained attention. The association with attention did not persist when correcting for multiple comparisons but the link with poor executive function remained. Cognitive flexibility of the frontal cortex is necessary for executive function with studies observing the detrimental impact of sleep deprivation on set-shifting ability using the WCST (Maddox *et al.*, 2009). Anatomical brain abnormalities in 22q11.2DS (Sun *et al.*, 2018) could affect normative

frontal cortical functioning and potentially lead to atypical sleep neurophysiology (Muzur, Pace-Schott and Hobson, 2002).

The insomnia-related sleep pattern was associated with impaired visual attention but this did not remain when correcting for multiple comparisons. The link between insomnia and impaired visual attention has been observed in sleep deprivation studies (Kendall *et al.*, 2006). Cognitive models have shown that insomnia in young adults is associated with poor attention control and negative emotionality (Mitchell, Mogg and Bradley, 2012). Impairments in sustained attention, visual attention and sleep globally are reported in ADHD in young people (H. K. Lee *et al.*, 2014).

Longitudinal studies are required to investigate the relationship of sleep and psychosis as the current sample is too young to look at how sleep problems relate to risk of psychosis and schizophrenia (Ferrarelli *et al.*, 2007; Pritchett *et al.*, 2012; Wulff *et al.*, 2012). Evidence from the Cardiff ECHO Study showed emergence of psychotic experiences in early adolescence in children with 22q11.2DS with an increase in the prevalence of reported psychotic experiences from 4% at the first assessment to 21% at the second time assessment (Chawner *et al.*, 2019). Investigating sleep problems in this emerging cohort may be of interest.

4.2.4.4 Sensitivity Analyses

Highest maternal education associated with reduced Risk for Tiredness-related Sleep Pattern

Higher maternal education level was associated with reduced risk of scoring on the tiredness-related sleep pattern. There is some evidence that parents with higher education attainment may be better informed on practices that can improve the quality of a child's sleep, with specific evidence of long-lasting effects in children with NDDs (Hiscock *et al.*, 2015; Papadopoulos *et al.*, 2015) including ASD (Trickett *et al.*, 2017). There is clear evidence for

higher maternal education to be linked to fewer childhood ODD (Rydell, 2010) and CD symptoms (Etherington *et al.*, 2016).

Melatonin use in 22q11.2DS

Melatonin is commonly prescribed in young people with insomnia-related problems as it mimics endogenous melatonin release, thought to positively influence sleep onset (Ivanenko *et al.*, 2003). Of the individuals in the cohort on melatonin, all but one reported sleep problems. Sleep problems were higher in those on melatonin compared to those who were not hinting that melatonin was prescribed to individuals with the more severe problems. To understand the efficacy of melatonin in young people with 22q11.2DS, a randomised controlled drug trial is required (Appleton *et al.*, 2012). Without systematic investigation of the utility of melatonin, evidence for its appropriate use in these young people is not definitive.

4.2.5 Strengths and Limitations

This is the first study to systematically investigate sleep problems in 22q11.2DS. The sample is large compared to previous studies, including a valuable sibling control group allowing for comparison to typically developing children. Available demographic and medication data allowed us to conduct sensitivity analyses.

In this study the measures used, despite gold-standard research diagnostic psychiatric tools, did not include objective sleep monitoring or the young person's own accounts of sleep problems. Parents might not be able to accurately report night-time activities and behaviours in older children so objective measures such as sleep electroencephalography (EEG) and cardio-respiratory polygraphy would help to further explore sleep problems in 22q11.2DS.

This study was cross-sectional so it is difficult to comment on whether sleep problems preceded or followed the emergence of other associated phenotypes. As a result, it is difficult to infer causal relationships between sleep disorders and other phenotypes. Longitudinal analysis would be required to investigate this further.

The availability of demographics and medication data allowed for us to conduct sensitivity analyses, however as part of the study, there was no data on the rates and seriousness of sleep problems before melatonin use and if melatonin had been taken previously. With the availability of this data, it would be important to identify whether sleep problems have diminished in severity despite their persistence. For example, an individual on melatonin continues to report sleep problems however the melatonin has worked to lessen these problems.

There are endocrine and neurological problems in young people with 22q11.2DS yet this study does not have data reflecting the melatonin profile in 22q11.2DS. Exploring the melatonin profile in individuals with a genetic syndrome could help to identify an endogenous deficiency in 22q11.2DS.

In the literature, there is evidence of obstructive sleep apnoea in 22q11.2DS however these findings were unable to address the high rates of sleep apnoea reported clinically. Further information is also required regarding incidence of physical problems relating to disturbed sleep such as restless legs, pain in limbs during sleep, and periodic limb movements.

The cause/s of sleep problems remains to be elucidated with sleep problems potentially manifesting in a number of ways that could include one or more of the following: unidentified genetic aetiology (S. R. Williams *et al.*, 2012); or behavioural consequences of psychiatric and

cognitive deficits or atypical brain physiology (Flahault *et al.*, 2012; Andrade *et al.*, 2013; Scott *et al.*, 2016; Sun *et al.*, 2018). Imaging studies including use of electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) may provide responses to these questions in the future as imaging will allow for physiological outputs to be assessed during sleep, focussing on objective physiological, anatomical and neurological reasoning.

Similarly, young people with 22q11.2DS who use melatonin report more sleep problems than those not taking melatonin. This needs to be followed up with a longitudinal design to establish whether levels of problems change following the onset of use.

There are a number of different factors which could contribute to the development of sleep problems including physical symptoms seen in 22q11.2DS such as endocrine problems and vascular abnormalities (Sonino and Fava, 1998). There is currently no evidence in this study or current cohort but there have been studies supporting the interaction of sleep problems and cardiovascular disease (O'Driscoll *et al.*, 2009)

4.2.6 Clinical Implications

It is difficult to identify when sleep problems are no longer part of typical development and become long-term disturbances or indicators of underlying problems. Therefore, elevated rates in young people with 22q11.2DS warrants attention. The findings show sleep problems in 22q11.2DS are prevalent and strongly associated with neurodevelopmental problems.

This study shows it is important to distinguish between the two sleep patterns especially when trying to manage neurodevelopmental problems and treat sleep problems. Identifying sleep problems early in 22q11.2DS is vital for intervention and management and there is a need for parents, teachers and clinicians to be aware of the severity of sleep problems and

the possible consequences that untreated sleep problems could have in a population at elevated psychopathological risk.

4.2.7 Future Studies

To further explore sleep in 22q11.2DS, physical problems should be assessed. This includes measuring limb movements during sleep to see whether the restlessness reported in sleep in 22q11.2DS is derivative of periodic limb movement disorders (Provini *et al.*, 2001). Further, a clinical study of sleep disorders in a sleep medicine facility is able to assess cardiac and respiratory sleep-related problems with rigor in a controlled environment (Heike *et al.*, 2007; Kennedy *et al.*, 2014; Moraleda-Cibrian *et al.*, 2014). This physical assessment of sleep problems would complement the evidence this study has found of the prevalence of sleep problems and association with psychiatric and cognitive outcomes.

4.3 Part Two - Description of Sleep Problems in 22q11.2DS through life

4.3.1 Introduction

The cross-sectional findings (**section 4.2.3**) show the incidence of sleep problems at a given time of assessment and helps to identify associated problems, yet the interpretation of development and change in sleep over time is limited.

Anecdotal evidence from parents of young people in the ECHO study suggests that sleep problems occur at birth where issues with getting to sleep and maintaining sleep through the night are observed. Many of the parents have other children without 22q11.2DS and can compare the sleep behaviours, reporting more problematic sleep in comparison to unaffected

siblings. Anecdotal reports from adults with 22q11.2DS suggest that sleep problems continue into adulthood and can be identified as pathological problems resulting in sleep disorder diagnoses.

The importance of investigating change over time and age in individuals with 22q11.2DS is highlighted by studies that have identified increased reports of psychotic experiences between assessment timepoints in young people (Chawner *et al.*, 2019) and elevated reports of mood disorders in late adolescence compared to childhood (Gothelf, Feinstein, *et al.*, 2007). Variability in the prevalence of symptoms of the neurodevelopmental phenotype across age and development could indicate that sleep problems share a similar pattern in 22q11.2DS. However, the occurrence of sleep problems from birth and persistence into adulthood has yet to be investigated.

It is important to identify pathological sleep problems from a young age as they could affect neurological development (Walker and Stickgold, 2006) and could adversely affect attainment of milestones such as physical growth (Brand and Kirov, 2011). Early identification could potentially help to inform interventions that could lessen sleep problems throughout life and potentially have an impact on the neurodevelopmental phenotype in 22q11.2DS.

4.3.2 Methodology

The ECHO Study includes a longitudinal component aiming to assess change in the neurodevelopmental phenotype over time. Assessments by the ECHO Study at wave 2,3, and 4 continue to collect data. Both wave 3 and 4 currently have insufficient cohort sizes for statistical analyses. As a result, these waves are not included in the analyses and this thesis refers to wave 1 (see **section 3.1 and Appendix 2**) and wave 2 (see **section 3.10, Appendix 2**).

Investigation of the neurodevelopmental phenotype across time and ages also included the assessment of toddlers and adults with 22q11.2DS. These two studies were cross-sectional studies exploring the phenotype of 22q11.2DS at different ages rather than a longitudinal assessment. Toddlers with 22q11.2DS and unaffected control siblings were investigated in the preliminary pilot study. The adults with 22q11.2DS were identified and assessed by the ECHO Study working collaboratively with the Intellectual Disability and Mental Health: Assessing the Genomic Impact on Neurodevelopment' (IMAGINE-ID) study (see **Chapter 3, section 3.2**) and the 'Defining Endophenotypes From Integrated Neurosciences (DEFINE)' study (see **Chapter 3, section 3.3**).

4.3.2.1 Recruitment, Demography and Sample

The wave 2 study focussed on 87 children with 22q11.2DS ($\bar{x}$ age =12.6, range = 8 years ~8 months – 17 years ~6 months, s.d. = 2.37, 42.5% females) and 39 age-matched sibling controls ($\bar{x}$ age = 12.8, range = 6 years ~2 months – 17 years ~6 months, s.d. = 1.65, 48.7% females).

The Toddler study focussed on 10 children with 22q11.2DS ($\bar{x}$ age =4.19, range = 2 years ~4 months– 5 years ~7 months, s.d. = 1.12, 70.0% females) and 3 age-matched sibling controls ($\bar{x}$ age = 5.44, range = 2 years ~4 months – 5 years ~10 months, s.d. = 0.584, 66.7% females). The demographic data for this small cohort was not incorporated into analyses.

The adult study focussed on 23 adults with 22q11.2DS ($\bar{x}$ age =37.5, range = 19 years ~6 months– 67 years ~5 months, s.d. = 14.3, 69.6% females. There were no unaffected sibling controls in the adult study. Demographic data was not included in analyses of this cohort. The sample cohort is small (n=23) and as a result, reports of sleep problems were assessed only.

4.3.2.2 Assessments

Wave 2

The wave 2 longitudinal assessments match the cross-sectional methodology (see **section 3.10**) to ensure consistency of the data collected across the waves and for cross-wave comparisons. Sleep problems were assessed by the Child and Adolescent Psychiatric Assessment (CAPA) (see **section 3.6**).

The Toddler Study

The Tayside Children's Sleep Questionnaire (TCSQ) (McGreavey *et al.*, 2005) was completed by the primary carer of the toddler reporting sleep problems. This questionnaire was not consistent with the CAPA sleep problems items: there were different questions, undertaken by different individuals and only a small sample completed such questionnaires.

The Adult Study

The sleep problems for adults were assessed using a validated semi-structured interview for adolescents and adults: Psychiatric Assessment Schedule for Adults with Developmental Disabilities (PAS-ADD) (Moss et al., 1998) (see **Chapter 3, section 3.10**). It is important to note that sleep apnoea is not assessed as part of the PAS-ADD.

The Tayside Children's Sleep Questionnaire and the sleep section of the PAS-ADD were not consistent with the CAPA sleep problems items: there were different questions, undertaken by different individuals and only a small sample completed such questionnaires. This needs to be considered when comparing these findings as part of the discussion.

4.3.2.3 Aims of the Studies

The aim of the wave 2 and longitudinal sleep analyses was:

1. to describe the prevalence of sleep problems at the follow-up assessment.

It was hypothesised that in young people with 22q11.2DS, sleep problems would remain elevated across time and age, whilst sibling controls would experience fewer sleep problems across the same period. As there was no evidence to suggest trajectory of sleep problems in individuals with 22q11.2DS, this was an exploratory analysis of sleep problems across time points.

The aim of exploring sleep in the Toddler study was:

1. To outline the preliminary prevalence of sleep problems in toddlers with 22q11.2DS compared to their unaffected control siblings, illustrating the presence of sleep problems from birth/early age.

Evidence from the cross-sectional study suggested that sleep problems would be elevated in the toddlers with 22q11.2DS. It was hypothesised that elevated reports of sleep problems would also be evident in the Toddler study.

The aim of the investigation of sleep problems in the adult data was:

1. to describe the prevalence of sleep problems and sleep disorders in adults with 22q11.2DS, showing the persistence of problems into older age.

It was hypothesised that the rates of sleep problems in adults would reflect what is seen in adolescents and young people more generally.

4.3.2.4 Statistical Analysis

The prevalence of sleep problems at wave 2 for 22q11.2DS young people and sibling controls was outlined and differences between each of the 11-items from the sleep problem section

of the CAPA (see **Chapter 3**, **section 3.6**) were assessed using chi-squared tests and Fisher's exact tests. The total sleep problem score was compared between groups using Mann-Whitney U tests. Differences in the prevalence of sleep problems between wave 1 and wave 2 for the 22q11.2DS young people and the sibling controls were assessed using a McNemar's test.

Descriptive statistics were used to assess the sleep problem findings from the Toddler Study and the Adult Study. The Adult Study did not include a control sample and therefore no comparisons could be made. Prevalence of sleep problems was determined and a comparison between the toddlers with 22q11.2DS and the sibling controls was determined used a Mann-Whitney U test.

4.3.3 Results

4.3.3.1 Wave 2 and Longitudinal Assessment

The prevalence of sleep problems in young people with 22q11.2DS at the second time point, wave 2, was 57.5% compared to 20.5% of sibling controls (p=0.001). The 11-items of sleep problems were assessed between groups (see **Table 7**) showing elevated rates of initial and middle insomnia, as well as restless sleep in young people with 22q11.2DS compared to siblings.

Table 7 Rates of the 11-items of sleep problems between 22q11.2DS and siblings

Sleep items Wave 2, n (%)	***22q11.2DS***	***Siblings***	***OR***	***95% CI mean/median differences***	***p***
At least one sleep problem	42/75 (56)	7/33 (21.2)	4.73	1.83-12.3	**0.001**
Overall total number of sleep problems	42/75 (56)	7/33 (21.2)	1.79	1.17-2.74	**0.007**
Insomnias					
Initial	21 (28)	2 (6.06)	6.03	1.32-27.5	**0.020**
Middle	16 (21.3)	1 (3.03)	8.68	1.10-68.5	**0.040**
Early	12 (16)	1 (3.03)	6.10	0.759-49.0	0.089
Hypersomnia	2 (2.67)	0	1	/	/
Restless sleep	22 (29.3)	3 (9.09)	4.15	1.15-15.0	**0.030**
Inadequately rested	14 (18.7)	1 (3.03)	7.34	0.924-58.4	0.059
Tiredness	5 (6.67)	1 (3.03)	2.29	0.256-20.4	0.459
Fatigability	3 (4.00)	1 (3.03)	1.33	0.134-13.3	0.806
Parasomnias					
Nightmares	4 (5.33)	2 (6.06)	0.873	0.152-5.02	0.879
Night terrors	3 (4.00)	1 (3.03)	1.33	0.134-13.3	0.806
Sleepwalking	3 (4.00)	1 (3.03)	1.33	0.134-13.3	0.806

Longitudinal Assessment - Wave 1 to Wave 2

The 11-items were then assessed in relation to the prevalence of each item at wave 1 compared to wave 2 (see **Table 8**) showing that there was no overall difference in the total prevalence of sleep problems between the waves of assessment of individuals with 22q11.2DS (p=0.835). Differences in the reported rates of initial insomnia (p=0.02) and early insomnia (p=0.05) however were demonstrated. There were n=10 young people with 22q11.2DS whose sleep problems improved at wave 2. There was an equal amount (n=10) that developed a sleep problem at wave 2 however, resulting in no net change.

Investigating the stability of the wave 1-derived patterns of sleep problems across waves showed that there were no substantial differences: the prevalence remained stable (p=1.00) for both tiredness-related sleep and insomnia-related sleep patterns. Pairwise correlations showed associations between the factors at wave 1 and wave 2: tiredness-related sleep (r=0.450, p<0.001) and insomnia-related (r=0.392, p=0.002). There were no differences in the rates of sleep problems in the sibling controls when comparing reports from wave 1 to wave 2 (p=0.739) (**Table 9**).

Table 8 Comparing the 11-items of sleep problems from wave 1 to wave 2 in young people with 22q11.2DS

Sleep items Wave 1 to Wave 2, n (%)	***Wave 1***	***Wave 2***	χ^2	***p***
Overall sleep problems	84/140 (60)	42/75 (56)	0.04	0.835
Insomnias				
Initial	32 (22.9)	25 (28.7)	5.00	**0.02**
Middle	23 (16.4)	17 (19.5)	0.20	0.655
Early	13 (9.29)	14 (16.1)	3.77	**0.05**
Hypersomnia	8 (5.71)	2 (2.30)	0.00	1.00
Restless sleep	22 (29.3)	45 (32.1)	1.38	0.240
Inadequately rested	14 (18.7)	27 (19.3)	0.17	0.683
Tiredness	15 (10.7)	0.564	0.459	0.564
Fatigability	7 (5.00)	0.480	0.806	0.480
Parasomnias				
Nightmares	16 (11.4)	7 (8.05)	0.53	0.467
Night terrors	8 (5.71)	3 (3.45)	2.78	0.100
Sleepwalking	11 (7.86)	4 (4.60)	1.33	0.248

Differences between the matched n=75 individuals with 22q11.2DS that were in both wave 1 and wave 2 are illustrated in the table.

Table 9 Comparing the 11-items of sleep problems from wave 1 to wave 2 in sibling controls

Sleep items Wave 1 to Wave 2, n (%)	***Wave 1***	***Wave 2***	χ^2	***p***
Overall sleep problems	15/65 (23.1)	7/33 (21.2)	0.11	0.739
Insomnias				
Initial	7 (10.8)	3 (7.69)	0.00	1.00
Middle	1 (1.54)	1 (2.56)	/	/
Early	0 (0)	1 (2.56)	1.00	0.317
Hypersomnia	1 (1.54)	0 (0)	/	/
Restless sleep	4 (6.15)	3 (7.69)	0.00	1.00
Inadequately rested	1 (1.54)	1 (2.56)	1.00	0.317
Tiredness	1 (1.54)	1 (2.56)	1.00	0.317
Fatigability	1 (1.54)	1 (2.56)	1.00	0.317
Parasomnias				
Nightmares	6 (9.23)	3 (7.69)	0.00	1.00
Night terrors	2 (3.08)	1 (2.56)	1.00	0.317
Sleepwalking	4 (6.15)	1 (2.56)	1.00	0.317

Differences between the matched n=33 sibling controls are illustrated in the table.

4.3.3.2 The Toddler Study

The prevalence of sleep problems reported by the primary carer in toddlers with 22q11.2DS was 60% compared to 33.3% of unaffected control siblings (p=0.559). The total sleep problems symptom score showed 22q11.2DS toddlers had a median symptom score of 15.5 (s.d.= ±11.8) and unaffected control siblings had a median score of 2 (s.d.= ±5.20) (p=0.127).

In this current sample, there were no associations seen between the incidence of sleep problems and gender (p=0.266), age (p=0.180) or when including group status as a predictor (p=0.559).

4.3.3.3 The Adult Study

The prevalence of sleep problems reported for adults was 56.5% (13/23). There was a small group of n=3 individuals diagnosed with sleep disorders: slow-wave arousal disorder, early insomnia and restless legs syndrome. One individual was taking the sleep medication Zopiclone for their slow-wave arousal disorder. Two further individuals were considered to have situational initial insomnia with delayed phase sleep onset. This resulted in a total of n=5 individuals either having a clinical diagnosis or a research-assessed diagnosis of a sleep disorder.

In this sample, there were no associations between the incidence of sleep problems and gender (p=0.386) or age (p=0.364).

4.3.4 Discussion

The prevalence of sleep problems in young people with 22q11.2DS was shown to remain high and stable across time-points; 60% at wave 1 to 57.5% at wave 2. Similarly, sleep problems in unaffected control siblings were shown to be relatively stable from 23.1% to 20.5% yet the

proportion reporting sleep problems was consistently lower with fewer symptoms reported than in the young people with 22q11.2DS. These results help to illustrate the seriousness of sleep problems with maintenance of sleep problems over time.

Sleep problems such as parasomnias are considered part of typical development (Siegel, 2005). The preliminary findings suggest that in young people with 22q11.2DS the prevalence of sleep problems does not follow a typical pattern of sleep problems across time. Parasomnias were less prevalent in 22q11.2DS than insomnia-related and tired-related problems, yet the parasomnias were still more abundant than in the siblings. The siblings showed a typical trajectory of decline in parasomnias; fewer were reported over time and as a group, with increasing age.

Group statistics showed that sleep problems decreased from wave 1 to wave 2 however not all individuals followed this trend. There were n=10 young people with 22q11.2DS who reported fewer sleep problems and therefore their sleep problems had improved whereas n=10 others developed sleep problems at the later time point. There was also a small subsample of young people with 22q11.2DS in the cross-sectional analysis that reported sleep problems but did not have a comorbidity (see **section 4.2.4.3**). This variability amongst individuals could suggest that there is no generic solution to treating sleep problems in 22q11.2DS. The problem of sleep is not confined to a particular age or time of development, and personalised treatments and management of sleep problems could be required.

In the Toddler Study, the preliminary results show that the toddlers have a similar incidence of sleep problems to the young people in the cross-sectional and longitudinal studies at 60%. Although the sample of n=13 is very small, the proportion of individuals that report sleep problems is something to note.

These preliminary findings suggest that sleep problems are elevated in individuals with 22q11.2DS from birth and remain a persistent, stable and serious problem across time and development. In toddlers, neurophysiology is different to older individuals (Scholle, Wiater and Scholle, 2011) with different durations spent in stages of sleep for example. At a younger age, development of a bedtime routine is important and behaviour around sleep could be different suggesting that assessing sleep in this vulnerable developmental age might not provide substantial evidence for the high preponderance of sleep problems. However, the similar prevalence of sleep problems in the toddlers compared to the young people should be considered.

The prevalence of sleep problems in the sample of adults with 22q11.2DS was 57%, showing comparative rates with the cross-sectional, longitudinal and toddler prevalence of sleep problems (see **Chapter 4**). This prevalence is shown to be higher than the reported typical rates of sleep problems in adults which range from as little as 10% up to 30% dependent on criteria of sleep problem and diagnosis (Leger *et al.*, 2000; Morin *et al.*, 2006). There were clinical diagnoses in n=3 individuals showing the seriousness of sleep problems in adulthood resulting in clinical diagnosis and medication in one instance. Interestingly, there were no clinical reports of sleep-related breathing disorders in this population despite elevated rates of sleep-related breathing disorders being the only reported sleep problems in 22q11.2DS in the literature. The PAS-ADD did not ask specifically about SRBD and therefore this could have reduced the likelihood of adults reporting this condition. However, a medical report was available for most individuals and this did not indicate the presence of SRBD in the sample in this thesis.

4.3.5 Strengths and Limitations

The longitudinal analyses outlined the stability of the high incidence of sleep problems across waves providing evidence for the consistent issue of sleep across the lifespan and with time. The toddler sample provides preliminary evidence for the high incidence of sleep problems from a young age in 22q11.2DS. These reports are from the primary carer who is the most effective reporter in this young population; there are currently no objective measures of sleep in the toddler sample. The results from the three studies must be interpreted with caution due to the low sample sizes. However, the prevalence of sleep problems is comparable across the ages suggesting evidence of elevated sleep problems throughout life in individuals with 22q11.2DS.

A limitation to the toddler description is that their sleep is often not stable: their neurophysiology during a night's sleep has yet to stabilise (Scholle, Wiater and Scholle, 2011) and their behavioural bedtime routine is inconsistent. The unaffected control sibling sample helps to disentangle whether sleep problems are unique to 22q11.2DS.

There are different sleep questions asked among the samples; the CAPA sleep questions do not map directly onto the Tayside sleep questionnaires and similarly, do not agree with the sleep questions in the PAS-ADD. Furthermore, the PAS-ADD does not address sleep apnoea and as a result, information regarding any sleep-related breathing problems in the adults was not collected.

4.3.6 Clinical Implications

The sleep problem descriptions across the different ages suggests that there are persistent sleep problems throughout life in individuals with 22q11.2DS. Observing sleep problems as

part of these studies can show the prevalence of sleep problems at different ages. However, a longitudinal study into the trajectory of reported sleep problems from birth, through childhood and adolescence, and into adulthood of a group of individuals is needed to understand the sleep phenotype of 22q11.2DS better.

The presence of sleep disorders in the adult cohort suggests that medical intervention and treatment for sleep in 22q11.2DS is important. The earlier the intervention, the higher likelihood there is of a better outcome in later life. Evidence of this needs to be provided in future study.

4.4 Summary

This chapter has shown the high preponderance of sleep problems across different ages in individuals with 22q11.2DS. The systematic assessment of sleep problems in the larger cross-sectional sample of young people with 22q11.2DS showed a prevalence of 60% compared to 23.1% in their unaffected siblings. The rates of sleep problems in individuals with 22q11.2DS was mirrored in the wave 2, toddler and adult sample cohorts studied. There isn't an increase in prevalence shown in these studies but there is persistence of severity and high prevalence from birth into adulthood showing a consistent burden and need for early intervention.

The results from the cross-sectional study showed two dominant patterns of sleep problems: tiredness-related sleep and insomnia-related sleep. Both patterns of sleep problems were associated with the neurodevelopmental phenotype with the tiredness-related sleep pattern associated with higher ADHD, anxiety disorder and indicative DCD symptoms as well as impaired executive function, and the insomnia-related pattern with higher anxiety, indicative DCD and conduct disorder symptoms.

However, there was a subsample of young people with 22q11.2DS that did not have a diagnosable comorbidity but did report sleep problems. This suggests that the presence of the neurodevelopmental phenotype might not explain all the sleep problems observed in 22q11.2DS. However, it is probable that the comparison with diagnoses rather than the symptom scores reduced dimensionality of the analysis with the symptomology providing more reliable and comprehensive results.

This thesis chapter provides evidence supporting the importance of recognising sleep problems in individuals with 22q11.2DS. Treatments targeted to the individual and their additional needs such as neurodevelopmental problems could be trialled and tested. Such therapies could be non-pharmaceutical such as cognitive behavioural therapy (CBT) (Espie *et al.*, 2016), or pharmaceutical as long as a systematic assessment of the efficacy of melatonin was undertaken in these young people.

The longevity of sleep problems is shown by the longitudinal assessment included matched-pairs showing consistency across wave 1 and 2 in the severity of sleep problems. This supported what was found in the cross-sectional samples of the independent toddler and adult groups. Together, these findings provide evidence to support continued sleep problems throughout life for individuals with 22q11.2DS.

This study begins to outline the nature of the sleep problems in 22q11.2DS and the associations that sleep problems have with the neurodevelopmental phenotype. This evidence warrants further study of sleep in 22q11.2DS to understand the underlying neurophysiology of sleep and sleep behaviours to build an evidence base for effective treatment.

Chapter 5 - The Pilot Study and Training

This chapter will outline the rationale behind the pilot study that was conducted prior to the main sleep study. The pilot was conducted to test the feasibility of the proposed protocol including the EEG setup. Therefore, problems could be identified and rectified prior to the development of the 22q11.2DS sleep and brain activity main study.

Moreover, this chapter will comment on the training undertaken to ensure that the researchers could conduct the project suitably. This includes formal training courses as well as volunteering in projects and then on-the-job development and learning.

This chapter provides a brief background to the methods used in the sleep study including electroencephalography and actigraphy. These methods are outlined in detail in **Chapters 6** (actigraphy) and **7** (polysomnography (PSG)).

5.1 Background

In the literature, a pilot study is defined as "A small-scale test of the methods and procedures to be used on a larger scale…" (Samet *et al.*, 2009). The objectives of a pilot study should be outlined from the start ensuring that these work to understand and assess the feasibility of the measures in the study (Leon, Davis and Kraemer, 2011).

The recruitment, protocol and implementation of novel procedures were assessed in the pilot study. As part of this, feedback from the participants was acquired. This could further help to develop and evolve the protocol. The participants were asked to comment on the study protocol and the equipment used. Combining the views of the participants with an objective

assessment of the protocol and equipment especially the method of EEG and its setup, the adherence to the study and equipment could be examined including tolerability.

In summary, a pilot study is a necessary step in exploring novel applications of methodology and protocol in a new patient population.

5.2 Rationale

Currently there are no studies that have objectively assessed brain activity in young people with 22q11.2DS during sleep. The findings from **Chapter 4** demonstrate that there is a high preponderance of sleep problems in young people with 22q11.2DS compared to their typically developing siblings. This investigation is novel in its focus on sleep problems and their association with the neurodevelopmental phenotype in 22q11.2DS. However, the evidence of sleep problems was obtained from a subjective assessment of sleep problems, related to the interviewer by the primary carer. Therefore, the interview itself confined the information that was reported in order to answer the questions, and the subjectivity of the responses does not allow for reliable conclusions to be made regarding the underlying physiology of sleep in young people with 22q11.2DS.

The subjective assessment outlined in **Chapter 4** cannot stand-alone as a comprehensive assessment of sleep problems in young people with 22q11.2DS: the CAPA is not a gold-standard sleep questionnaire and it is limited in the information that it can acquire. To best understand sleep in these young people, it would be preferable to combine the primary carer reports that are subjective perspectives of sleep problems in their child/ren, with objective measures of sleep. As a result, a pilot study was conducted in order to develop an appropriate protocol, which utilises subjective and objective methods of assessing sleep problems in

young people with 22q11.2DS. The pilot study was undertaken in young people that were from the typically developing population to reduce any problems before conducting the protocol with a group of young people with 22q11.2DS and their siblings.

The pilot study allowed for the trialling of the different subjective and objective measures which subsequently shape the themes and results of the following chapters: actigraphy, sleep diaries and sleep questionnaires (**Chapter 6**). These were tested and improved as part of the pilot study to show development in the protocol and appropriateness for the young people with 22q11.2DS. The polysomnography, particularly the technique and equipment used to acquire sleep EEG (**Chapter 7**), was closely examined during the pilot study to ensure the most appropriate equipment was used. This would allow for young people with 22q11.2DS to remain comfortable whilst gathering the most comprehensive dataset.

In total, these foci would contribute to the main aim of the pilot study which was to assess the feasibility of the methodology and protocol that was developed.

5.2.1 In-home Assessment and Logistics

It was proposed that the polysomnography (PSG) should be conducted in the homes of the participants to normalise their experience in the main study. The protocol could have been undertaken in a sleep laboratory, however the disruption to the participants was considered too great. Undertaking the protocol in a constant environment for an individual with 22q11.2DS was important as this removed added disruption to the individuals when moving them into an unfamiliar clinical setting. This is particularly pertinent as levels of anxiety are high which are linked to sleep disturbance (Babson *et al.*, 2013; Swillen and McDonald-McGinn, 2015).

However, to conduct the study in the homes of the participant, the protocol had to be applicable for a home environment with a contained and manageable EEG setup that was ambulatory. Having an ambulatory study resulted in logistical parameters having to be considered such as transportation, ensuring robustness of equipment and the financial impact of staying nearby to the family homes throughout the study. These factors needed to be considered when planning both the pilot and main studies.

5.2.2 Objective Measures of Sleep

To develop the most appropriate protocol for the high-risk 22q11.2DS population, two methods of EEG acquisition were compared in the pilot study: silver-chloride free cup electrode technique to the high-density geodesic EEG net application. In addition to the EEG acquisition, cardiorespiratory polygraphy was used to measure cardiac and respiratory outputs throughout the night's sleep. This technique is routinely used in clinical sleep studies of sleep-related breathing disorders (Ruehland *et al.*, 2011).

Training was needed to learn how to use and interpret electrocardiography, respiratory induction plethysmography (RIP) belt, oxygen saturation, electrooculography and electromyography.

5.2.2.1 Electroencephalography (EEG) – high-density 60-channel EEG net or traditional free electrode?

Two methods of EEG acquisition were trialled during the pilot study to assess brain activity during sleep: the traditional 10-20 EEG, free cup electrode technique which used 21 silver-chloride electrodes glued to the scalp, verses the 60-channel high density EEG geodesic net. The high-density geodesic net acquired brain activity from 60 different electrode locations on

the scalp which could help to accurately map brain activity across the cortex (Pisarenco *et al.*, 2014).

The silver-chloride electrodes are traditionally used to measure brain activity during sleep as well as daytime multiple sleep latency tests (MSLTs) and assessing seizure-related brain activity clinically (Carskadon, 2011). The application of the electrodes however requires the use of collodion, a flammable and highly toxic nitrocellulose preparation that glues the electrodes onto the scalp. Acetone is also used to remove the glue which can also contribute to headaches. These mildly irritant substances would need to be handled and transported carefully as well as stored in secure units in the laboratory prior and after use (Young *et al.*, 1993). The pilot study trialled the use of such substances in the study.

The two techniques required set-up in the evening and either removal in the morning or the following afternoon depending on whether the participant continued wearing the EEG setup for the 24-hour period.

See **section 5.5.2** for the methods used to measure sleep brain activity.

5.2.2.2 Cardiorespiratory Measurements - Breathing and Cardiac

The limited literature regarding sleep and 22q11.2DS refers to the elevated prevalence of obstructive sleep apnoea (Heike *et al.*, 2007). As a result, it was important to monitor breathing during sleep in these individuals. This was completed using a nasal cannula, respiratory inductance plethysmography belts (RIP) (schematic, **Figure 7**) and pulse oximetry. The prominence of cardiac problems in 22q11.2DS suggested

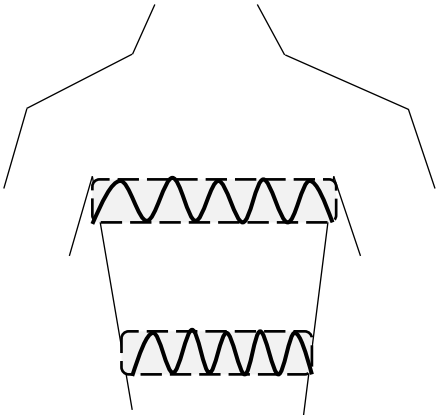


Figure 7 Schematic of the thoracic and the abdominal RIP belts

that it was important to use electrocardiography (ECG) during the night to assess the heart rate of the participant. The respiratory and cardiac measures could be used to control for physiological changes in the EEG that could not be rationalised by changes in brain activity.

The breathing and cardiac sensors were vulnerable to restlessness during the night and this could result in the wires detaching or loosening. The sensors were attached securely before the participant went to sleep.

After consulting with different sleep clinicians and researchers whom had worked with this equipment, it was apparent that there would be difficulties regarding universal adherence to the respiratory measures. The pilot study was a particularly vital time to assess the tolerance of the breathing measures.

See **section 5.5.3** for the methods used to assess cardiac and respiratory measures in the pilot study.

In addition to these measures, there were non-invasive measures that needed to be trialled; actigraphy watches, sleep diaries, and validated sleep-questionnaires.

5.2.2.3 Actigraphy in the Pilot Study

Currently, actigraphy remains unused in a 22q11.2DS population; there is evidence of the use of actigraphy in studies of genetic syndromes such as Angelman syndrome and in neurodevelopmental disorders (NDDs) such as ADHD (Fawkes *et al.*, 2015) and autism spectrum disorders (ASD) (see **Chapter 2**, **section 2.1.4**) (Allen *et al.*, 2013).

The watch was strapped onto the non-dominant wrist of the participant and worn during the day and night for two-weeks. A sleep diary was completed during the same period. Acquiring two-weeks of data allowed a normative sleep-wake pattern and rhythm to be observed for

each individual. It is important to recognise the normal routines and activities of individuals as this helps to identify how a problematic night differs from a routine night's sleep. A single night of EEG could show an abnormality however this could be an isolated event. Actigraphy could be used to confirm this based on abnormalities identified in the sleep-wake patterns and activities.

Sleep parameters such as sleep onset, wake time, nocturnal activity and wake after sleep onset can help to deduce if there is a persistent problem. Actigraphy is used to complement the overnight assessment however the nature of actigraphy, as a non-invasive assessment, enabled individuals that might not want to or be able to complete the overnight assessment to participate.

5.2.3 Parent and Self-Report Measures of Sleep

In paediatric sleep studies, it is more common for subjective measures of sleep to be acquired and parental reports to comment on the sleep behaviours of their child. Sleep questionnaires can be easily completed yielding a large dataset. However, it is recognised in the sleep literature that differences between parental reports and subjective measurements such as actigraphy are present (Meltzer *et al.*, 2012). In the study design, both the objective and subjective sleep measures are included to ensure acquisition of a comprehensive dataset that allows for comparisons between measures also.

Actigraphy is the most common objective measure of sleep with minimal invasiveness and reduced costliness compared to clinical polysomnography. However, actigraphy requires a complementary sleep log or sleep diary to corroborate timings and parents can comment on the behaviours of their child on the sleep diary. Such additional information can help to create a comprehensive understanding of the sleep patterns and behaviours of young people.

5.2.3.1 Sleep Diaries

The sleep diaries were developed based on examples from the National Centre for Mental Health (Lewis, Foster and Jones, 2016) and the University of Bristol (Hellmich *et al.*, 2015). The sleep diaries that were originally trialled were based off the adult examples that were available and therefore, piloting these enabled development and changes to be made to the diaries to ensure appropriateness for the age and developmental level of the young people. The ease of use and adherence to the diary for the two-week period was important, and by assessing the completeness of the sleep diaries, it was apparent as to whether these would be appropriate for the future main study. It was important to note whether participants recorded when the actigraphy watch was removed.

5.2.3.2 Sleep Questionnaires – Parental and Participants

The sleep questionnaires were chosen based on the paediatric sleep literature and a sleep questionnaire reference book (Shahid *et al.*, 2012). The sleep questionnaires were extensively researched and licences were gained for each sleep questionnaire. All questionnaires were validated for the sample age range (see **section 5.4.4.1**).

5.2.4 Sharing the Data

The pilot and main studies were a collaborative effort with a colleague who was investigating seizure-related activity in 22q11.2DS. The EEG method was shared and for adequate data to be acquired for the assessment of seizures, a continuous 24-hour period was needed to capture both daytime and night time activity.

The pilot study showed how a young person could tolerate multiple wires and electrodes during a night's sleep and for the continued day. Assessing the feasibility and tolerability of this was vital in making adaptations to the main study.

5.2.5 Feedback from the Pilot Study

Feedback from the sleep aspects of the protocol helped to develop the delivery and application in the main study. Understanding how individuals without 22q112.DS react to the different sensors informed the approach in the main study.

5.3 Aims and Objectives

The assessment of the feasibility of the protocol and procedures were the main priorities. As a result, the following were the main aims of the pilot study:

1) Assess feasibility of the 24-hour EEG acquisition setup as part of the wider protocol, and the tolerability of the different devices and sensors worn by the child;

2) Monitor the level of disruption to the participants and family from the visit structure (visit in evening, morning and following evening);

3) Deduce whether it was possible to acquire data of reasonable quality when undertaking ambulatory EEG in the participant's home.

To address the aims the following was undertaken:

1) Comparisons between the two methods of EEG acquisition: The Geodesic high-density net and the traditional silver-chloride cup electrodes were made;
2) A dialogue between the researcher and the parents and the young participants; feedback both written, in-person and over email was invited.

5.4 Methodology

The protocol was developed collaboratively. Where there was exaggerated input from an individual, this is emphasised.

5.4.1 Ethics

The ethics were approved by the School of Medicine, Cardiff University. The School of Medicine Research Ethics Committee (SMREC) granted ethical approval for the study. Additionally, SMREC granted two amendments to the pilot study during its delivery. This ensured that the study could be conducted with the intended population and could maximise the measures trialled during the pilot.

The data collected was stored centrally in securely password protected encrypted files, that were accessible by the immediate research team. All data was anonymised. No video or audio recordings during the night period were used during the pilot study despite their intended use in the main study; there was not sufficient time to develop the video recordings.

5.4.2 Recruitment

To recruit individuals for the pilot study, colleagues from across the Division of Psychological Medicine and neighbouring schools were asked if their children were able to partake. The yield of participants from this method was lower than expected and resultantly posters were produced and emails were sent to increase recruitment numbers. All participants had an affiliation with the University either directly or indirectly.

5.4.2.1 Sample Size

The sample consisted of n=12 participants ranging from 6-19 years old ($\bar{x}$ = 12.6 years old, 58.3% females). Geographical location was similar with 75% of the participants located in the

Vale of Glamorgan and the other 25% in Chepstow. The pilot study received ethics for three months and therefore was completed within this time. This resulted in a limited sample for the time period. As the pilot cohort was from the general population, the biological ages were considered to reflect the developmental age.

5.4.3 Information from the Pilot

As previously outlined, the primary aim of the Pilot Study was to assess the feasibility of the methodology and the protocol that had been developed prior to testing. Acquiring feedback from the participants and the primary carers would enable changes to be implemented prior to conducting the protocol in young people with 22q11.2DS (**Table 10**).

Table 10 Information we wanted to acquire from the Pilot Study

What is to be decided from the Pilot Study?	***How the information was acquired?***
What method of EEG acquisition is best with regards to data acquisition?	To assess the density of information acquired: can the net provide more information than the traditional electrodes?
What equipment is most comfortable for the participants?	Discuss the comfort levels with the participants to gauge a perception of the comfort level
Is the proposed protocol and the timings feasible?	Discuss and note the perceptions of the primary carers and the participants with regards to time spent at house and participating in the study
How did the set-up timings differ between the EEG methodologies?	Note how long set-up was for the different methods of EEG acquisition and compare

5.4.4 Sleep Assessments

5.4.4.1 Sleep Questionnaires

Many sleep studies in the literature have a design built around the objective assessment of sleep problems resulting in polysomnography, or actigraphy often worn for a minimum of seven nights. Objective measures are often supported by subjective measures such as sleep questionnaires.

Questionnaires can be used without restraints on cost as they can be widely distributed to families. Wearing an EEG net over a single night is an invasive process which some young people, especially those with neurodevelopmental disorders, might find uncomfortable. Having a non-invasive measure of the study that can still yield a large wealth of data allows those individuals to still participate and contribute to the understanding of sleep. It isn't only that individuals with NDD might not be able to tolerate the study, the study is completely voluntary and as a result, some individuals might not want to participate in the overnight aspect but are receptive to completing questionnaires. Whether the questionnaires are used to support the sleep EEG and actigraphy data or stand alone to assess behaviours of sleep problems in young people, each component added a new dimension.

I chose age-validated sleep questionnaires to cover the age span of the participants intended to be in the main study. These sleep questionnaires were trialled in the pilot study to see how easily they were to complete. The younger participants were not required to complete the sleep questionnaires whereas older participants completed a sleepiness questionnaire with the primary carers providing the remaining information.

Logistically, the best time for the questionnaires to be completed by the parents and the participants was trialled in the protocol; sending the questionnaires prior to the study visit

and taking them along for completion during the study visit were both trialled. Remaining flexible with the times and providing pre-paid envelopes for returning the questionnaires, along with the sleep diaries and actigraphy watches, proved to be a reliable method of receiving the questionnaires back. The different sleep questionnaires are outlined.

Cleveland Adolescent Sleep Questionnaire

The Cleveland Adolescent Sleep Questionnaire (CASQ) (Spilsbury *et al.*, 2007) is a validated sleep questionnaire for 11-17-year olds that includes sixteen questions aiming to identify daytime sleepiness in adolescents. The degree of sleepiness and of alertness in an individual is assessed on a 1-5 Likert-scale response. The CASQ is scored based on the higher the total score, the greater the implied sleepiness. There is no normalised scale. Some of the questions in the CASQ are reversed-coded (a smaller number on the Likert-scale equates to a 'stronger' response in some questions whereas in other, a higher number equates to a 'stronger' response) and care was taken when coding these questions. Where there were difficulties, primary carers could help the participant in the understanding of the reversed question. In the pilot study, the CASQ was completed by n=6 young people.

Children's Morningness-Eveningness Scale

The Children's Morningness-Eveningness Scale (CMES) is a validated questionnaire for 12-18-year olds (Shahid *et al.*, 2011) and was completed by the participant to obtain a subjective opinion regarding their morningness/eveningness (M/E). The CMES consists of ten multiple choice items based on a 1-5 Likert-scale including questions regarding perceived preferred time to complete exams, to do exercise and preferences regarding wake and bedtimes if they were able to choose these times themselves. The CMES was completed by n=6 young people.

Children's Chronotype Questionnaire and the Munich Chronotype Questionnaire

The Munich Chronotype Questionnaire (MCTQ) (Roenneberg and Merrow, 2016) is a self-rated scale assessing individual phase of entrainment on scheduled days and free-days. It was designed to work in ages 6 to over 60 years old, but a younger version was adapted for the assessment of younger children to be completed by their primary carer called the Children's Chronotype Questionnaire (CCTQ).

The MCTQ estimates the chronotype (CT) on an individual. Chronotype or CT is the behavioural manifestation of underlying circadian rhythms and develops as an individual's propensity to want to sleep at a particular time during a 24-hour period. The CT is derived using the midpoint between sleep onset and offset on work-free days, called the mid-sleep on free days (MSF) variable. The MSF quantifies the average mid-point of the sleep duration across free days. However, if a particular night were to be atypical, for example if an individual slept longer on the weekends to compensate for any 'sleep debt' they had accumulated during the scheduled days of the week, this would need to be accounted for. Therefore, the MSF can be corrected for the 'excess' sleep resulting in the MSFsc variable. MSFsc represents a measure of CT.

Many variables can be derived from the MCTQ: sleep latency (how long is needed to fall asleep (mins)); time in bed (TIB, get-up time-bedtime); sleep onset (SO, sleep latency + lights-off); sleep duration (SD, wake-up time - sleep onset (SDf (free) or SDw (work))); average weekly sleep duration ((SDweek, SDw x number of 'work' days + SDf x number of 'free' days/7)); sleep inertia (SI, time fully alert-wake-up time) and midsleep point defined as (MSF, sleep onset (SO) + sleep period)/2)).

The Children's Chronotype Questionnaire (CCTQ) is an adaptation of the MCTQ. The CCTQ was devised for parents to answer open-ended questions regarding sleep/wake parameters for their child's scheduled and free days. The parameters assessed and variables derived are like the MCTQ: bedtime, lights off time, sleep latency, wake-up time, get-up time and time taken to be fully alert. Including the scheduled and free day assessments shows whether sleep/wake patterns are directly influenced by activities such as school on scheduled days and on the free days these restrictions aren't present and sleep/wake patterns could differ.

There are three different parental report measures of children's chronotype in the CCTQ: the midsleep point on free days (MSF), the Morningness/Eveningness (M/E) scale score and the Chronotype (CT). These measures are comparable to the MCTQ derived variables and chronotype measures. This allows for the entire sample to have a chronotype as the MCTQ and CCTQ together are inclusive of the age-range of the sample.

There were n=6 young people who completed the MCTQ and n=6 who completed the CCTQ. These numbers of completion were based on the age distribution of the sample.

Paediatric Sleep Questionnaire and Sleep-related Breathing Disorder Scale

The Paediatric Sleep Questionnaire (PSQ) is an extensive questionnaire completed by primary carers of young people aged 2-18 years old. The PSQ includes general information including demographic information; night time parameters including snoring, breathing, restlessness, growing pains and number of nocturnal awakenings; daytime section regarding how refreshed the child is, complaints of sleepiness and sleep paralysis; health which includes growth rate, tonsillectomy and breathing conditions; stimulant use regarding caffeine and Ritalin; medical information about medication, psychiatric symptoms and sleep diagnoses, as well as clinic attendance and an ADHD behavioural section. Overall, these sections can be

divided into subscales which assess the total scores for behavioural problems, sleepiness, and a total score for snoring-related outcomes. In addition, there is a periodic limb movement index (PLMI).

Within the PSQ there is an additional stand-alone scale called the Sleep-related Breathing Disorder Scale (SRBD) which is a 22-item scale including variables taken from the main PSQ questionnaire such as sleepiness, hard to wake, headaches and snoring frequency. The SRBD scale has a total sleep-breathing disorder score outcome that can be added to a five-point ADHD scale to get an overall SRBD score. The ADHD scale is a 5-point Likert scale regarding how well the outcomes match the participant: doesn't listen, difficulty organising, easily distracted, fidgets, on the go and interrupts.

There were n=12 parental completions of the PSQ and SRBD. An option of 'don't know' was available to parents and this could be scored as such and included in calculating subscales.

Sleep Disturbance Scale for Children

The Sleep Disturbance Scale for Children (SDSC) is a 27-item questionnaire rated on a five-point Likert-type scale based on how well the question describes the individual. The parents completed the questionnaire on behalf of their child. The instrument is used to categorise sleep disorders in children and gives an overall score for the instrument matched to a t-score. There are five subdomains: disorders of initiating and maintaining sleep (DIMS), sleep breathing disorder (SBD), disorders of arousal (DA), and sleep-wake transition disorder (SWTD), disorders of excessive somnolence (DOES) and sleep hyperhidrosis. The scale is validated for use on 2-18-year olds, with n=12 parental reports completed.

5.4.4.2 Actigraphy and Sleep Diaries

The actigraphy watches were purchased from Philips Respironics (Philips, 2018) and calibration was completed by volunteers to ensure accurate data acquisition. The actigraphy protocol for the pilot study was developed in line with the standardised procedure of actigraphy acquisition outlined (Meltzer *et al.*, 2012). Further information regarding the finalised actigraphy protocol is in the experimental section for the main sleep study (**Chapter 6**).

The watches were used in the pilot study to determine whether they were tolerable and whether the participants were likely to adhere to wearing the watches for the total two weeks' period. The watches have been extensively used in published research both in younger populations and in adults suggesting that these watches were reliable in their data acquisition (see **Chapter 6**).

The watches were designed to be used in research conditions which in the circumstance of this study, would eventually be in the homes of young people who experienced developmental and behavioural difficulties. The watches were chosen for the validity, robustness and water resistance to mitigate damage to the watches. Minimal damage was inevitable as these watches were intended to be used as part of the pilot study, main study and future studies.

The type of actigraphy watch that was used was not the same for all ages. Phillips had a smaller, more appropriately sized watch for young children to wear which looked similar to a wristband. This was especially important in this study sample with younger children, both of physical age and of developmental age, so a larger watch with a digital watch face and date

on it might not be as appropriate. The pilot study assessed the effectiveness in measuring sleep/wake activity across two weeks across the two different types of watches.

In conjunction with wearing the watch for two weeks, the participants were asked to complete a sleep diary for the same two-week period as wearing the actigraphy watch either on their own or with a primary carer ensuring accuracy. The primary carer completed the sleep diary if the child was considered not able to.

The first sleep diary was a basic assessment of timings: time in bed, lights off time, sleep time, wake time and out of bed time in addition to sections for commenting on removal of the watch, whether medication had been taken and whether they had napped during the day. The sleep diary evolved over the pilot study and in the preliminary stages of the main sleep study (see **section 5.5.4** and **Chapter 6**) generating a more comprehensive diary.

5.4.4.3 Polysomnography

Sleep EEG

Dr Ullrich Bartsch, a Lilly sponsored Postdoctoral Fellow, supported all technical aspects of EEG acquisition during the study including sponsoring the acquisition setup.

Training

A three-day polysomnography course organised by the University of Edinburgh and Dr Lizzie Hill from NHS Lothian provided guidance and training on how to practically acquire EEG. This course included application of polysomnography sensors with sessions on breathing apparatus and the interpretation of breathing pressures and data from nasal cannula, RIP belts, pulse oximetry and thermistors. A small number (n=5) of sleep EEGs were available to practice on. A trained somnologist was available to assist in the interpretation and provide

help with scoring. Experience of sleep scoring was gained, however this was complemented with by continuing to practice scoring of EEG traces downloaded from open sourced databases online (Grandchamp, Braboszcz and Delorme, 2014). Additionally, an open-source EEG database from Boston, USA was made available from collaborators of Dr Ullrich Bartsch for practising sleep scoring. Ten sleep EEGs were available to practice scoring and to become accustomed to working with sleep EEGs.

At the time of developing the in-home EEG setup, the University of Bristol Clinical Research Imaging Centre (CRIC) housed a longitudinal population-based sleep study (Hellmich *et al.*, 2015). This was based on a 'recall-by-genotype' study protocol of male participants aged 25 who were members of the Avon Longitudinal Study of Parents and Children (ALSPAC) study. By volunteering to help run the study overnight, I was provided with an opportunity to develop a better understanding of EEG setup in addition to contributing to interpreting EEGs acquired in the study. There were ten sleep EEG traces that were available to practice scoring on. Once I had scored these EEGs, a post-doc re-assessed these EEG traces and then a trained somnologist re-scored a 10% sample of the EEGs to ensure concordance. The somnologist used an agreement of 80% (average) when scoring the EEGs.

To increase exposure to EEG data, I attended six sessions with an electrophysiologist at the University Hospital Wales, Heath. I was able to ask questions regarding the electrophysiological traces. This did not include sleep EEG however it did provide an opportunity to learn how to interpret traces and to spot inconsistencies. Each week provided a new EEG sample that had been recorded as part of the epilepsy clinic.

The pilot study compared two methods of EEG acquisition. For the traditional silver-chloride cup electrode setup, the amplifier used for the acquisition of the EEG signals could be used to

acquire the cardiorespiratory signals also. This was an Embla Titanium amplifier (Natus, 2018b) resulting in the full PSG assessment to be acquired on one device. However, with regards to the high-density 60-channel Geodesic net from EGI (Philips Company, 2018), this method of EEG acquisition required an EBNeuro BE Plus LTM 64 amplifier and the Galileo acquisition software (EBNeuro, 2018). Therefore, this meant that two amplifiers were required for the full PSG assessment as there were no additional inputs in the EBNeuro amplifier. The signals were synchronised post-acquisition before analysis.

Electrooculography (EOG) and Electromyography (EMG)

When undertaking EEG setup, regardless of technique, the minimal PSG application is the electrooculography (EOG) and electromyography (EMG) electrodes to the face. These free adhesive electrodes are required for the interpretation of the sleep EEG.

Eye movements were measured by one electrode positioned 1 cm lateral to the outer canthus of the left and right eyes and then 1 cm up from the right eye, and 1 cm down from the left eye. Eye movements such as saccades and rapid and slow eye movements could be detected dependent on the polarisation of the eye. This was especially important in determining sleep stages as eye movements in REM sleep are biological markers of the stage. The impedance of the EOG electrodes was under the 10kΩ for the best quality signal to be measured.

In addition, free adhesive electrodes were placed under the chin to the left and right of the midline of the neck. These were used to assess muscle tone and atonia during REM sleep. The impedance levels of the EMG electrodes were under 10kΩ for the best quality signal to be measured.

Once the EOG and EMG electrodes were applied to the participants face, the EEG electrodes or the EEG net were glued to the scalp/placed over the head.

10-20 International Electrode Placement System

The head of the participant was measured before application: the circumference; left pre-auricular to right pre-auricular distance and the nasion to inion measurements were recorded. Marks were made where 21 electrodes could be applied to the scalp using a porcelain pencil. This was easily removable by an antiseptic wipe.

For the standard sleep study, there were 12 electrodes that were applied to the scalp: F3, F4, Fpz, Fz, C3, C4, Cz, Pz, O1, O2 and M1 and M2 (the left and right mastoid bones behind the ears) (see **Figure 8**).

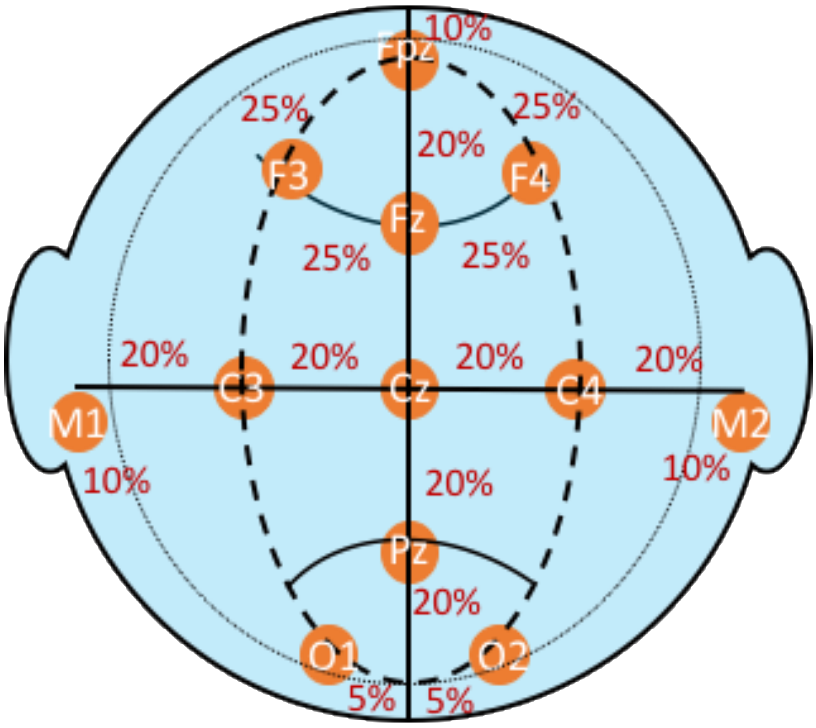


Figure 8 10-20 International electrode placement

The areas marked were swabbed with abrasive NuPrep gel (*Nuprep Skin Prep Gel | For Use in Cardiac Rehabilitation Monitoring*, 2018) which works to clean and moisten the skin, reducing the impedance so that a higher quality signal can be detected. Once the positions were dried, the silver-chloride cup electrodes were glued to the scalp with collodion glue, ensuring that any hair was separated to ensure best contact with the skin. Once dry, Signa gel (*SignaGel Electrode Gel: Parker Laboratories, Inc.*, 2018) an electro-conductive gel, was injected into the small hold in the centre of the silver-chloride electrodes. The electro-conductive gel works to increase the signal quality detected globally across the cortex and conducted across the scalp which is detected by the 21 electrodes.

Geodesic Net

The primary difference of the two methods was that the net included 60 active recording electrodes which is like an EEG cap available from other manufacturers.

The net application required the circumference, left pre-auricular to right pre-auricular, nasion to inion and the central position known as 'Cz' to be measured. The technique did not require collodion glue but NuPrep was used to clean the head and exfoliate the area removing with an alcohol wipe. A curved plastic syringe was filled with Signa gel and was used to move hair away from the area to expose a larger area of the scalp for the electrode to be in contact with. Each of the electrodes was housed in a plastic pedestal where the electro-conductive gel was injected into from the curved syringe.

Electrode impedances were checked using the impedance check tool in Galileo. The tool displayed electrode locations and impedance values which can then be individually checked and improved. An impedance of 30KΩ was aimed for and once this was achieved across the net, the signal quality was tested and calibrated.

Bio-calibration

Once the electrodes were prepared using either technique, the signals had to be calibrated to ensure detection of the correct physiology. The protocol included a bio-calibration of the electrodes that was the same for both techniques.

Different instructions were given to the participants such as 'blink', 'eyes closed', 'eye open' and 'grind teeth.' Once the participant had reacted to the command, a marker was added to the computer physiological trace displayed on the Galileo programme to mark where the activity had occurred. This was relevant for analysis when assessing the signal quality during different activities.

Bio-calibration was also completed for the EOG and EMG on a separate programme, REMLogic (Natus, 2018a) which displayed the cardiorespiratory signals. Similarly, the commands were noted for future analyses when assessing changes in physiology based on different activities.

The cardiorespiratory and EEG bio-calibrations remained separate during the acquisition but would be combined post-acquisition for analyses.

Cardiorespiratory Polygraphy

The cardiorespiratory polygraphy protocol was developed in collaboration with Dr Claire Durant from CRIC, University of Bristol and Dr Jose Thomas, Respiratory and Sleep Consultant, Nevill Hall Hospital, Abergavenny. Dr Jose Thomas was available to help with the Pilot Study, however did not continue to assist throughout the study nor the main study.

Working closely with Nevill Hall Hospital ensured that the correct measures were included in the study ensuring that the cardiorespiratory events would be detected accurately through

the night and could be used in further analysis. The cardiorespiratory measures were also important to identify whether there were any undetected clinical sleep problems relating to breathing or changes in the heart rate.

The pilot study incorporated the use the following respiratory measures: nasal cannula to measure the breathing pressures and derive the breathing rate; respiratory inductance plethysmography (RIP) belts, one around the thorax and one around the abdomen which expand and contract based on breathing and a pulse oximeter which measures the oxygen saturation of the haemoglobin in the blood worn on a finger of the non-dominant hand.

The cardiac measures were the electrocardiography (ECG) which was assessed by two adhesive electrodes connected by ECG wires to the amplifier that in turn was connected to the EEG net. There were an additional two adhesive electrodes that were connected by ECG wires to the cardiorespiratory Embla titanium amplifier. Pairs of electrodes were placed under the participants left and right clavicle; two adhesive electrodes on each side. The impedances of the ECG electrodes were assessed to be under the 10kΩ and were checked on both the Galileo (EBNeuro, 2018) and REMLogic (Natus, 2018a) programmes to ensure the signal was detectable and interpretable.

5.5 Outcomes and Discussion

Outcomes from the pilot study included the optimal protocol and the appropriate method of EEG acquisition. There were no quantitative analyses undertaken. For instance, the sleep EEG was not analysed from the pilot study in a strategic and systematic manner: it was assessed for quality of the signal and the duration of continuous assessment as well as assessing the wireless technologies. There were however results from the sleep questionnaires that have

been included but the remainder of this section will refer to the results obtained from the pilot study generally regarding feasibility, tolerability and the development of the protocol.

5.5.1 Recruitment

Individuals in the pilot study were recruited through the Division of Psychological Medicine and Clinical Neurosciences (the university Division that the researchers belonged to) and Cardiff University. Colleagues were asked whether their children, if the correct age, could volunteer for the pilot study. As a result of the nature of how the participants were recruited, neurological, psychiatric and psychological conditions were not controlled for. The parents and/or participants were encouraged to disclose problems to the researchers if they wished to. There was no evidence to suggest that any of the individuals had a neurodevelopmental problem. However, we recognise that because the participants were the children of individuals working at Cardiff University, there could be resistance to disclose problems to our research team.

5.5.2 Polysomnography - Sleep EEG, and Daytime Recording

Coordinating an evening, night and following day for the acquisition of data proved to be difficult. The following protocol was trialled:

- Visit in the evening to undertake EEG setup
- Return the following morning to remove EEG/to re-gel to lower the impedances
- If continuing with the daytime assessment, return in the evening.

Therefore, the total visit could last for 24-hours including the night sleep. Otherwise, the evening protocol should have lasted around three hours, followed by the following morning taking up to two hours.

One night seemed to be acceptable by the parents, but there were limitations in the acquisition of daytime EEG. Some of the assessments however were conducted during the summer holidays which allowed for more flexibility in arrangements.

There were only n=3 participants that wore the EEG net for one night and up to 12-hours of the following day. These individuals wore the net for an average of 20.7 hours. When recruiting the young people, confirmation was required as to whether they were free the following day. All individuals excluding one confirmed that they were free however the small number of individuals completing the entire study suggested that the participants no longer wanted to complete the study after sleeping in the equipment.

As a result, during the EEG setup and throughout EEG acquisition in the main study a suggested incentive could be provided to encourage continuous wearing of the geodesic net for the 24-hour period. Communication to the parents and the participants was important ensuring that the most comprehensive set of data could be acquired.

There were n=2 participant who did not complete the overnight sleep EEG. Reasons for this were that one participant did not want to wear the net after attempting, and the second participant was unable to complete the overnight aspect of the study on the arranged night however their sibling was able to. Both individuals did however complete the other parameters such as the actigraphy watch and the sleep diary and completed the sleep questionnaires.

For those individuals who asked for the net to be removed after the night's sleep, they were asked to hypothetically say whether they would have been able to continue wearing the EEG net for an extended period during the day.

Table 11 outlines the feedback received from the participants based on their experience. **Table 12** shows the outcomes of what was intended to be learnt from the Pilot Study (outlined in **Table 10**, **section 5.4.3**).

Table 11 Findings from the Pilot Study

Participant	*Age (years)*	*Set-up time (mins)*	*Hours EEG*	*Why removed?*	*Wear during day?*	*Sleep feedback*
PS-01	10.4	-	22	End of study	Yes	6/10 comfort – okay to sleep in
PS-02	8.18	-	0	Didn't want to wear after trying net on	-	Did not wear EEG
PS-03	6.16	-	15	Wanted to remove after 1 hour of daytime recording	No	Fine to sleep in.
PS-04	11.0	-	-	-	-	Did not wear EEG
PS-05	11.7	115	11	Had badminton practice at 9 am	No (would wear if opportunity to)	Net dug into the back of head during sleep
PS-06	8.8	-	13	Didn't want to wear during the day	No	Uncomfortable to wear
PS-07	13.5	-	18	Wore for portion of daytime	Yes	Uncomfortable to wear
PS-08	17.7	55	13	Didn't want to wear during the day	No	Uncomfortable to wear
PS-09	14.2	50	15	Didn't want to wear during the day	No	Uncomfortable to wear
PS-10*	17.2	173	15	Didn't want to wear during the day	No	Good sleeping position; okay to wear
PS-11*	15.4	125	20	Had to leave at 11:00	Yes	Free electrodes were quite comfortable
PS-12	17.8	95	20	Didn't want to wear during the day	No	Net uncomfortable to sleep in

Asterisks (*) refer to those individuals which wore the silver-chloride free electrodes over the Geodesic net

Table 12 Outcomes from the Pilot Study

What is to be decided from the Pilot Study?	***Outcome***
What method of EEG acquisition is best with regards to data acquisition?	The Geodesic EEG net
What equipment is most comfortable for the participants?	The traditional electrodes
Is the proposed protocol and are the timings feasible?	Protocol fine and timings, when using the Geodesic EEG net, were fine for the evening.
How did the set-up timings differ between the EEG methodologies?	Time it took to apply the traditional electrodes in 10-10 placement was too long.

One particular outcome that was shared by participants was the mild discomfort of the EEG net during the night's sleep. The free electrode method was shown to be more comfortable however, the preparation was substantially longer and involved the use of irritant chemicals that required safe storage and transportation. The sleep study was coupled with the seizure-related study that required a high density of electrodes suggesting that the high-density EEG net was a more appropriate technique.

The EEG net would yield a higher quality of data for the sleep EEG and covered a wider area of brain activity for assessment. However, the EEG net also introduced potential discomfort and an abnormal sleep night. The discomfort was something that would be particularly intrusive for the individuals sleep so efforts to minimise this, by suggesting the use of soft toys and blankets under the occipital electrodes at the back of the cranium, was important to include in the main sleep study protocol.

5.5.3 Polysomnography - Cardiorespiratory Polygraphy

All participants wore the RIP belts during the night's sleep who completed the overnight sleep EEG. There were n=10 individuals who wore the pulse oximeter and n=10 wore the ECG electrodes.

The nasal cannula was problematic as it was the most invasive sensor that was inserted slightly in the nostrils of the participant to accurately measure breathing. Two of the pilot participants wore the nasal cannula; both reported discomforts.

5.5.4 Actigraphy, Sleep Diaries and Sleep Questionnaires

The subjective measures in the pilot study were easily adhered to with all the n=12 participants wearing the watch for the two-week period other than when they were taking a bath, doing contact sport or swimming. There was one individual who worked as a lifeguard so had to remove the watch whilst on duty two times a week and another participant who went on a surfing holiday during the two-week period.

All participants completed the sleep diary in conjunction with the actigraphy watch, and the necessary sleep questionnaires that were then sent back with the watch.

5.6 Strengths and Limitations

The pilot study aimed to assess the feasibility of the current protocol to allow for the development of an optimal setup for the EEG and for the overall sleep study protocol with young people with 22q11.2DS.

It is important to note that there was a lack of reliability in the training that was provided regarding sleep EEG scoring. This was not utilised in the Pilot Study due to the inconsistency

of the software to acquire and store the data reliably. Once acquired, the data could not be exported and in some situations, data was lost. This was rectified before the main study. Nonetheless, the training provided for sleep scoring was insufficient to derive clinical conclusions from any further sleep EEG scoring that is commented on later in this thesis. I am not a qualified somnologist nor am I a certified sleep physiologist. The training I received was provided from such professionals however, the training was from numerous sources and from different individuals and therefore, introduces some unreliability in the reporting of the sleep EEG scoring.

The feasibility of the intended measures was assessed resulting in mixed success. The small sample size suggested that results could not be extrapolated. However, they suggested the strengths of the protocol and areas that required modifying before the main EEG study.

The recruitment will be easier in the main EEG study as the participants are current recruited members of the ECHO Study (see **Chapter 3**). Of the people which expressed interest in the pilot study n=3 withdrew their interest and n=2 didn't partake in the EEG measure. There was a 66.6% success rate in the recruitment and execution of the sleep EEG.

The pilot study helped to develop the protocol and helped to devise a step-by-step protocol for each of the experimenters working on the project as well as for the parents and participants so that they were able to know exactly what was going on and ask as many questions as possible. Making sure that the families were completely aware of what was happening and what the study was for needed to be reinforced so that the main study protocol involved the development of pre-study information packs, during the study information and post-study information and evaluation to ensure that all participants received the same information and guidance throughout.

The sleep measures showed some problems in the pilot study with regards to comfort and adherence. Having developed an EEG setup and for that to not be undertaken as intended, added to personal development of the researchers. Resilience and adaptability of the researchers ensured that each study was approached with openness for flexibility. Maintained communication with the participant and their family was vital to ensuring trust and participation in the study. If a participant was uncomfortable then in some instances, it would be deemed appropriate to conclude the study. Having the ability to objectively judge this ensured that the researchers, parents and participants remained comfortable and satisfied. Some measures were considered more invasive such as the nasal cannula. If the nasal cannula could not or would not be worn, this did not detract from the wealth of other monitoring systems and data analysis would be adapted accordingly.

The pilot study was conducted with typical young people of which the majority were related to an academic at Cardiff University so biasing the sample slightly with regards to IQ and socioeconomic status. This homogenous sample might not be reflected in the main sleep study sample. It would be important to remember that each different family, each participant and different environment must be tailored to ensure that the family is as comfortable as possible and the participant at ease.

The actigraphy watches were a proven success and were shown to be tolerated by a range of ages. The sleep diaries were also completed by all, but from the pilot feedback suggested that the lengthiness of the sleep diaries should be reduced and that sleep diaries for different ages should be developed. For example, adding an element of fun to the task for younger children, and in order to gain a direct response to a question (i.e. have you consumed caffeine today), include more closed questions. The sleep dairies were developed to be gender-neutral and

age-appropriate for example, including colouring for young children. There was an additional area for the participant to provide comments on how they felt after waking and how they perceived their quality of sleep. An example of the various sleep diaries that were included in the main sleep study can be found in **Appendix 4, pages 334-344**.

The sleep questionnaires were answered and returned with the only complaint from parents of older children suggesting that they were unaware of their child's sleeping behaviours and patterns because of their independence. As a result, these primary carers were unable to provide responses to some questions. Parents and carers of older children from the typically developing population anecdotally might not have as much involvement with the sleep behaviours and activities of their children compared to the parents and carers of young people with 22q11.2DS. This is anecdotal and based on evidence provided verbally from parents.

The primary strength of the pilot study was that it could gather a comprehensive and broad picture of sleep behaviours and physiology from the participants. This was promising for the main sleep study in identifying sleep phenotypes of young people with 22q11.2DS.

5.7 Future Work

The future work was the main EEG sleep study which was developed from what was learnt from the pilot study. These findings were incorporated into an ethical application for the main sleep EEG study.

Most importantly, the pilot study motivated the development of step-by-step protocol, a clinical research framework (CRF) for the experimenters and the parents for the main sleep study that would include a list of equipment to ensure that everything was packed and taken

with us on every study, tick boxes for different procedures from measuring the head to turning the EEG amplifier on and there were also sections for further comments relating to the participant. This would ensure that any individual with a working knowledge of the sleep EEG study would be able to follow the protocol step-by-step without trouble.

Incorporating these measures, updating the sleep diaries and finalising the EEG net protocol was necessary for the ethics of the main sleep study and to ensure that the main sleep study was a great success.

5.8 Summary

The pilot study was pivotal in the development of the main sleep study and without it, a succinct and reliable protocol would not have been delivered during the main sleep study. The feasibility and the tolerability of the measures throughout the night was assessed and showed varying acceptance which potentially related to age whereby older young people tolerate more of the measures throughout the night. This is an important outcome that needs to be taken forward into the sleep study. The intricacies of the protocol were apparent during the pilot. As a result, a clinical research framework (CRF) was developed that could be used explaining step-by-step the protocol for the experimenters as well as the participants and parents.

From the pilot, it was apparent that the high-density EEG net was a fast and efficient technique for acquiring high quality datasets and could be system could be set up with greater ease for use in young people. The pilot also suggested that further practice with the EEG net was required to shorten the duration of the protocol before the main sleep study, quickening the process and lessening the disruption to the families and their homes. The EEG net was

considered uncomfortable so maximising comfort during the night would be important to allow the participant to sleep as typically as possible.

The actigraphy yielded high adherence with watches worn for the two weeks and only removed when suggested. The sleep diary was completed by all, yet the information provided was less comprehensive in some than others. Developing the sleep diaries to make them easier to complete and requiring fewer written comments would be vital in the main study.

The main study could now incorporate these changes, improving efficiency and the quality of data obtained. The pilot study ensured development of a better organised and succinct protocol, yet further practice and protocol development would be required before the main study and in the earlier stages of the main study.

Chapter 6 – Actigraphy, Sleep Diaries and Sleep Questionnaires in 22q11.2 Deletion Syndrome

6.1 Overview of Chapter

Parent-reports have anecdotally suggested that sleep problems are preponderant in young people with 22q11.2DS. These subjective views of their children's sleep can incorporate unintentional bias. Therefore, parent-reports should be followed-up and sleep problems in young people should be objectively assessed. Parental perspectives can contribute to the development of a comprehensive assessment of sleep including both subjective and objective assessments.

Movement patterns can be monitored using accelerometers (Kavanagh and Menz, 2008) that can exist within wearable devices such as actigraphs. Actigraphy watches can be worn by an individual to assess their movement and provide an objective measure of activity during wakefulness but also during sleep. This equipment can measure nocturnal activity in young people and help to support the evidence of sleep problems from parents. Nonetheless, the perspective from the individual themselves is important in understanding the sleep behaviours including whether an individual prefers sleeping in later or waking earlier compared to others. These perspectives can be derived from the actigraphy measures but validation from self-reports can help to substantiate the evidence. Difficulties can occur where younger children are unable to verbalise their views on their sleep or where learning disability prevents a child from having the capacity to report. Parental-reports are important

for these individuals, acting as a replacement to self-report to compare with actigraphy measures.

This chapter is divided into two sections. The first section will comment on the use of actigraphy in the main sleep study assessing the nocturnal activity of young people with 22q11.2DS and unaffected sibling controls. The actigraphy-derived sleep parameters and sleep diary data are used to investigate differences between the individuals with 22q11.2DS and siblings, in addition to exploring the relationship between nocturnal activity in individuals with 22q11.2DS and the neurodevelopmental phenotype.

The second section of this chapter will outline and describe the different sleep questionnaires that were used in the main sleep study. These validated sleep questionnaires are described and used to cross-validate the sleep problems section of the CAPA (**Chapters 3** and **4**). Validation of the sleep questionnaires, parent and self-report, alongside parental-report of sleep problems from the CAPA can help to identify the appropriateness of the CAPA in the assessment of sleep problems in young people with and without neurodevelopmental problems.

6.2 An Introduction to Actigraphy

6.2.1 What is Actigraphy?

The practice of actigraphy was defined in 2007 by the American Academy of Sleep Medicine (AASM), the world authority in sleep medicine, as '*…is indicated for delineating sleep patterns, and to document treatment responses in normal infants and children (in whom traditional sleep monitoring by polysomnography can be difficult to perform and/or interpret), and in special paediatric populations*' (Iber, 2007).

Actigraphy can provide an alternate assessment to polysomnography techniques which are clinically required for diagnosis of certain sleep disorders. Actigraphy records the frequency and degree of movement of an individual over time with an actigraphy watch worn on either the wrist or ankle, or as a band around the waist. The position of the watch on either the wrist or ankle allows for the recording of triaxial movements (in axes X, Y and Z) in space. Despite the increasing use of actigraphy, there is currently no single definitive and replicable protocol or method used to analyse the data. This results in inconsistencies in the protocols used and therefore, there are differences in the parameters assessed and algorithms used. There are a number of different watches that have undergone different validation processes resulting in added irregularities in the variables that can be derived from actigraphy assessments. One review of the literature showed that of the papers they systematically reviewed, 72 did not report the protocol for their actigraphy data scoring or interpretation resulting in nonreplicable results (Meltzer et al., 2012).

Clinically, an actigraphy assessment can last between one and two weeks to acquire a normative profile of the sleep-wake patterns. This time period aims to include examples of weekend and weekday nights of sleep. This is important when analysing the data as there are often differences in sleep-wake patterns during weekdays, defined as 'scheduled days', and the weekends which tend to have fewer restrictions. It is widely acknowledged that a period of seven days (Ulate-Campos, Tsuboyama and Loddenkemper, 2017) including at least one weekend with the remaining five days as 'scheduled' days of work or study is adequate in the analysis of sleep-wake patterns and nocturnal activity.

Actigraphy can generate estimates of similar sleep parameters to those derived from PSG. These include sleep onset latency (SOL), total sleep time (TST), time in bed (TIB), wake after

sleep onset (WASO) and sleep efficiency (SE). Actigraphy watches present an adequate alternative to acquire more objective measures of sleep activity but cannot replace the clinical gold-standard assessment of PSG which can obtain measures of neurophysiology and breathing. Actigraphy can be used across all demographics however should be supported by PSG to comprehensively assess sleep.

6.2.2 Actigraphy in the Typical Paediatric Population

There is a limited number of actigraphy studies in the typical paediatric population and the validity of actigraphy in young people is debated. Evidence from one study suggested that actigraphy underestimated TST and SE and did not accurately report SOL when compared to PSG assessment (Meltzer et al., 2012). Assessing young people's nocturnal activity can be difficult as normative sleep patterns are not often acquired until later childhood with physiology changing until the age of 10 (Berry et al., 2012).

Bedtime behaviours and nocturnal activity in very young infants can be assessed by different types of accelerometer: a baby-mat with an integrated accelerometer can detect movement of babies during the night period for example (Lambiase et al., 2014). These techniques could be used to map the development of nocturnal activity over different ages.

In young children it can be difficult to undertake clinical studies, especially studies which require the participant to stay away from home for at least one night of sleep. Actigraphy can be a helpful tool by contributing to the understanding of a child's problems, whilst causing minimal disruption.

6.2.3 Actigraphy and the Neurodevelopmental Phenotype

6.2.3.1 Actigraphy in Neurodevelopmental Disorders

In individuals with neurodevelopmental problems, objectively assessing sleep can be difficult as a result of behavioural, communicative or intellectual impairments (Thackeray and Richdale, 2002). Parental-reports of sleep problems are commonly used to assess sleep in these young people however this is a subjective measure contributing bias to the assessment. A combined assessment can yield the most data and help to identify inaccuracies.

Attention-Deficit Hyperactivity Disorder

Actigraphy watches in individuals with neurodevelopmental disorders can be used to assess motor or activity-related phenotypes such as hyperactivity in ADHD. Small studies of children with intellectual disability can provide objective insights into the sleep phenotype (Goldman, Richdale, et al., 2012). Actigraphy is more suitable for these children where invasive techniques could cause substantial distress; actigraphy is generally tolerated by young people with intellectual disability (ID) (Goldman, Richdale, et al., 2012; Ashworth et al., 2013). However, some individuals find it difficult to wear a band around the wrist or the ankle (Fawkes et al., 2015; Veatch et al., 2016).

Actigraphy studies in young people with ADHD have shown higher levels of average activity during sleep, but a similar sleep duration and no substantiate alterations in the sleep-wake pattern compared to typical young people (De Crescenzo et al., 2014). Another study investigated the role of actigraphy in identifying ADHD subtypes however, this was not nocturnal activity and did not show any differences between the subtypes based on daytime activity either (Dane, Schachar and Tannock, 2000). The use of actigraphy in neurodevelopmental populations has helped to explore the validity of parental reports,

especially in young people who may have problems communicating. One study found that parents of children with ADHD reported a substantially higher number of sleep problems compared to the parents of typical children. This was confirmed by actigraphy assessments suggesting that the parents had reported these problems accurately apart from the longer sleep duration, which was not objectively identified, and bedtime resistance (Corkum et al., 2001).

Autism Spectrum Disorders

The problem of bedtime resistance is a common complaint of parents of children with autism spectrum disorders (ASD). Objective measures including actigraphy have been used to confirm parent-reports of sleep problems (Wiggs and Stores, 2004; Goldman, Richdale, et al., 2012; Malow et al., 2016) in studies of young people with ASD. However, there is contrasting evidence that suggests some parental responses to sleep questions that form part of behavioural assessments can overestimate problems in their children when compared to actigraphy (Humphreys et al., 2014).

Elevated levels of sleep problems have been reported by parents across NDDs. Potentially there could be elevated reports through parents being hyper-aware of problems in young people with existing conditions including ASD and resultantly, this could influence the report of sleep problems in these individuals (Couturier *et al.*, 2005). However, there is a larger literature which continues to report sleep problems in this cohort of young people. Therefore, the evidence suggests there is a high preponderance of sleep problems in young people with ASD. One study demonstrated that parents reported higher numbers of sleep problems in their child with ASD compared to a control cohort, but reported a comparable sleep duration (Schreck and Mulick, 2000). Behavioural interventions in young people with ASD have

changed parental perception of sleep problems suggesting improved sleep but actigraphy measures did not demonstrate the same marked differences (Johnson et al., 2013).

Objective measures can be used to complement the subjective parent reports (Moore *et al.*, 2017). The best way to capture the sleep phenotype in young people with NDDs is a combined approach. This is particularly pertinent in these young people because of the complexity of their phenotype. Parent-reported sleep diaries can capture the behaviour of children and comment on the impact of the child's poor sleep quality on the rest of the family. Actigraphy can provide a more objective measure of the actual changes in the child's sleep itself. Actigraphy detects movement and activity and therefore, can provide information regarding restfulness and fragmentation across a night. In many cases, it would be difficult for parents to report such activities during the night (Moore *et al.*, 2017).

6.2.3.2 Actigraphy in Psychiatric Disorders

In young people with psychiatric disorders, actigraphy allows the individual to remain in their own environment, reducing disruption. It allows for the objective assessment of sleep-wake patterns that have been reported in the sleep literature (Pritchett et al., 2012) without requiring the individual to stay overnight in a sleep clinic. There is a limited literature regarding actigraphy assessments in young people with anxiety disorders and schizophrenia with most studies focussing on adult samples, especially in schizophrenia.

Anxiety Disorders

Young people have been assessed with actigraphy in combination with parent-report sleep questionnaires to measure sleep in anxiety disorders. The objective measure showed in one study that anxiety was not associated with average sleep variables including sleep onset latency, sleep duration and daytime sleepiness however an association between elevated

anxiety and night-to-night variability of the sleep-wake patterns was shown (Fletcher et al., 2018).

Schizophrenia

Actigraphy has been used to assess individuals with SCZ as it provides an objective, non-invasive technique, minimising the disruption to the individual. Prolonged latency to sleep onset, delayed awakening times and increased nocturnal awakenings across six-weeks of actigraphy have been illustrated by one study (Wulff et al., 2012). Reduced daytime activity relative to nocturnal is shown in SCZ with frequent reports of daytime naps supporting a dysfunctional 24-hour sleep-wake cycle (Wichniak et al., 2011).

6.2.4 Actigraphy in Copy Number Variant Research

Young people with CNVs often have many comorbidities which complicate the assessment of physiology, especially where young people have an intellectual disability. Comprehensively examining the sleep phenotype can be completed by a combination of different assessments. An individual might not be able to undertake one aspect of a study such as a PSG, but other methods used might be more appropriate for them, such as wearing an actigraphy watch. This flexible approach ensures the maximal yield of data.

6.2.4.1 Angelman Syndrome

Individuals with Angelman Syndrome (AS) show elevated rates of ID with delayed development, severe speech impairment and problems with motor movements. These problems could be detrimental in assessing sleep problems subjectively from the participant so the use of objective measures can play a significant role in understanding the sleep phenotype of these individuals. One study used actigraphy in combination with PSG and sleep questionnaires to assess sleep parameters in young people with AS showing major problems

such as delayed sleep onset latency, fragmented sleep and a shorted sleep duration compared to typical controls (Goldman, Bichell, et al., 2012).

6.2.4.2 Prader-Willi Syndrome

Individuals with Prader-Willi Syndrome (PWS) have been reported to have more WASO but shorter SOL. This demonstrates that young people with PWS take a shorter time to fall asleep but spend more of the night in wake periods compared to typical populations (Gibbs, Wiltshire and Elder, 2013). Young people with PWS have a high incidence of hyperphagia in which obesity is a main component of the phenotype (Heymsfield et al., 2014). This puts these young people at a higher risk for the development of sleep-related breathing disorders such as OSA which can lead to fragmented and disrupted sleep, adding to the sleep phenotype in PWS.

6.2.4.3 Williams Syndrome

Sleep problems have been reported in up to 63% of young people with Williams Syndrome, suggesting that the sleep phenotype plays a significant role in the prognosis of the syndrome. Actigraphy has shown extended SOL and work in adolescents and young adults has replicated these extended SOL coupled with poorer sleep efficiency, more fragmented sleep and increased nocturnal activity (Goldman et al., 2009). Intellectual disability is common and similarly to 22q11.2DS, there can be craniofacial dysmorphisms and cardiac problems, as well as reports of ADHD in around half of individuals (Mason et al., 2011).

6.3 Rationale

There are no studies that have explored sleep in 22q11.2DS and to date, the study in **Chapter 4** is the first study of its kind to outline the prevalence, nature and associations of sleep

problems in 22q11.2DS. This study formed the foundations of investigating sleep in 22q11.2DS using actigraphy, sleep diaries and sleep questionnaires. The physiology of sleep has yet to be defined in 22q11.2DS. This chapter provides the first evidence of objective study of sleep problems in 22q11.2DS. **Chapter 4** outlined that young people with 22q11.2DS had higher parent-reported sleep problems compared to parent-reports of their unaffected siblings. In the young people with 22q11.2DS, two distinct patterns of sleep problems were identified: tiredness-related sleep and insomnia-related sleep. These patterns of sleep problems were associated with the neurodevelopmental phenotype suggesting impairment was associated with experiencing more sleep problems. Additionally, there were individuals with 22q11.2DS using melatonin at the time of interview for their sleep problems. Evidence suggested that most of these young people continued to report sleep problems whilst on the melatonin. From these initial findings, further investigation into the nature and the aetiology of sleep problems in young people with 22q11.2DS is required.

In the sleep literature, there are a large number of studies where subjective questionnaires have been used to assess sleep problems. It is recognised however that to be able to assess sleep problems comprehensively, a combined approach of using subjective and objective measure of sleep is required. Questionnaire data can provide an essential foundation for the evidence of sleep problems. Building on these findings with objective sleep studies of actigraphy or PSG (Markovich, Gendron and Corkum, 2014) can help to validate the findings and develop the understanding of the overall picture of sleep problems. Actigraphy watches and sleep diaries are non-invasive objective assessments of sleep in young children that are important to use in young children who experience problems that could affect whether they

could undertake an overnight sleep EEG assessment. The actigraphy watches provide a simple method of acquiring a large dataset of nocturnal activity.

The evidence from the NDD, psychiatric and paediatric literature suggests that actigraphy is an effective tool in monitoring nocturnal activity in young people. Incorporating actigraphy watches positions this study at the forefront of understanding the sleep phenotype better in 22q11.2DS.

6.3.1 Aims of the Actigraphy Study and Sleep Diaries

The primary aims of the actigraphy and sleep diaries were:

1. to acquire a large dataset of objective activity in young people with 22q11.2DS compared to their siblings;
2. to assess whether there are differences in sleep parameters and nocturnal activity of young people with 22q11.2DS compared to their siblings;
3. to determine whether the sleep parameters and nocturnal activity in young people with 22q11.2DS are associated with the psychopathology and cognitive outcomes that were identified previously in **Chapter 4** i.e. the outcomes that were associated with the patterns of sleep problems.

To address aim 1, a relatively large dataset of individuals with 22q11.2DS and siblings were collected.

Aim 2 was addressed by extracting sleep parameters from the actigraphy using the automatic sleep scoring algorithm. Unpaired two-tailed t-tests were used to explore the differences between the young people with 22q11.2DS and the sibling control group (see **Tables 14** and **15**). Paired t-tests were used to explore the differences between the 22q11.2DS and their

paired, related sibling in further analyses. Findings were corrected for multiple testing and the initial analyses did not account for covariates however in **section 6.5.3**, **pages 178-180**, covariation using age, gender and highest maternal education level is explored.

Aim 3, which involved exploring the relation between the actigraphy-derived sleep parameters and the neurodevelopmental phenotype in 22q11.2DS, was investigated through hierarchal linear regressions. Covariates such as age, gender and highest maternal education level were included. There were only a few families that completed psychopathology and cognitive assessments on the same day as the sleep study. Therefore, when exploring these associations, the age difference between the date of assessments and date of actigraphy was accounted for as a covariate. Melatonin was also investigated as a covariate of the regression analyses.

6.4 Methodology

In **Chapter 5**, the pilot study protocol outlines the difference aspects of the sleep study. This includes the use of actigraphy and the sleep diaries. The actigraphy protocol was developed during and after the pilot study to develop its consistency and replicability within research groups in the MRC Centre at Cardiff University.

6.4.1 Recruitment, Sample and Demography

Participants were part of the ongoing Experiences of CHildren with cOpy number variants (ECHO) study (Niarchou et al., 2014; Chawner et al., 2017; Cunningham et al., 2017). Recruitment for the ECHO Study can be found in **Chapters 3** and **4** and in **Appendices 1** and **2**.

During recruitment, the aim was to invite the families to complete the entire sleep study including PSG, actigraphy and the sleep diary, and sleep questionnaires. As previously mentioned, the sleep study was combined with a seizure-related study and therefore when families were recruited, they were asked if they would be happy for their child/children to wear the EEG net for a 24-hour period inclusive of a single night's sleep. The extent of the study proved to dissuade some families from taking part therefore as recruitment progressed, the study was broken down into sections and the families were able to consent into the different aspects of the study resulting in different overall sample sizes of the different aspects. See **Appendix 2** for the different sample sizes.

The actigraphy watches were worn by a larger sample than those who completed the PSG. The study asked that the watch was worn for a two-week period, however during those two weeks whenever the watch was removed, it was noted in the sleep diary. There were n=22 young people with 22q11.2DS and n=10 sibling controls who did not wear the watch for all 14 nights. There is evidence in the literature that n=7 nights of complete actigraph is sufficient for interpretation (Allik, Larsson and Smedje, 2006; Goldman et al., 2009) if the period includes weekdays and weekends. This parameter resulted in n=1 young person with 22q11.2DS and n=1 sibling control excluded from the interpretation of the actigraphy.

In addition to the actigraphy watch, the protocol included a sleep diary. Some of the participants however did not return their sleep diaries, or they were returned incomplete. In these cases, where there were no or insufficient sleep timings on the sleep diary, sleep timings that had been automatically determined by the actigraphy analysis could not be compared to the sleep diary sleep timings. In n=3 young people with 22q11.2DS and n=2 sibling controls, there was enough automatically scored actigraphy data that could be analysed, however

there were no subjective sleep diary timings to compare the timings to. There were n=22 young people with 22q11.2DS and n=7 sibling controls that had at least one night of sleep timings incomplete in the sleep diary over the two-week period. Despite the absence of a complete sleep diary, if the actigraphy data was sufficient (>7 nights including a weekend) and the quality of the data was good, these findings were still included in analyses.

After processing and quality control of the actigraphy data, the sample size of analysable data included n=49 young people with 22q11.2DS ($\bar{x}$ age=14.5, range = 6 years, ~8 months – 19 years, ~8 months, s.d.=3.45, 52% females) (98% of the original sample) and n=27 unaffected sibling controls ($\bar{x}$ age=13.2, range=7 years, ~6 months – 19 years, ~1 month, s.d.=3.55, 52% females) (97% of the original sample). Not all individuals wore the actigraphy watch for the total 14 nights. The sleep diaries were not returned by all individuals. For some individuals however, the sleep diaries were completed more frequently than the watch was worn, resulting in n=44 sleep diaries returned for the young people with 22q11.2DS ($\bar{x}$ age=14.6, range=7 years, ~9 months – 19 years, ~7 months, s.d.=3.47, 61% females), but n=28 sleep diaries for the unaffected sibling controls ($\bar{x}$ age=13.2, range=7 years, ~6 months – 19 years, ~1 month, s.d.=3.40, 46% females). Therefore, there was n=1 more sleep diary completed than the analysable actigraphy data for the siblings. The demography of the final sample of 22q11.2DS and sibling controls is outlined in **Table 13**.

Table 13 Demographics of the sleep actigraphy and sleep diary sample

			%
Family ethnic background			
	Mixed		4.26
	British		89.4
	Other		6.39
Highest maternal education qualification			
	No qualifications		2.13
	Low: O-levels or GCSEs		2.13
	Middle: A-levels/Highers or vocational training		29.8
	High: University degree and/or other higher postgraduate qualification		38.3
	Other		27.7
	Unknown		0.00
Family annual income (£)			
	£≤19,999		14.0
	£20,000-39,000		32.0
	£40,000-59,000		24.0
	£≥60,000		30.0
	Unknown		0.00
Age, years: mean (s.d.)			
Probands	14.5 (3.45)	***Siblings***	13.2 (3.55)
Gender, n (%)			
Probands	26 F (52)	***Siblings***	15 F (51.7)

In the 22q11.2DS cohort, 30/49 (61.2%) of young people were on medication and n=8/30 (26.7%) of these young people were taking melatonin. There were n=3 sibling controls who were on medication; one sibling took clonal modulate, salbutamol and multivitamin, for asthma and general health; one sibling took Pizotifen for migraines and another took codeine phosphate for surgery-related limb pain.

Prior to recruitment, primary carers consented for all participants to partake in the main ECHO Study and consented to further contact regarding other studies. Additional consent was obtained. For further information regarding consent in the ECHO Study, refer to **Appendices 1** and **2**.

6.4.2 Assessments – Actigraphy and Sleep Diaries

Actigraphy data was scored using Actiware software version 6.0.9 (Respironics, 2018). The medium threshold sensitivity value (TSV) (40 counts per epoch) is the default setting for this program and therefore was used as the primary threshold in these analyses (Meltzer *et al.*, 2012).

The sleep diaries were created for the pilot study and modified so that they could be understood better and for ease of use in the main study. Three versions of the sleep diaries were developed: parent, adolescent and child that could be distributed to the families appropriately. Examples of the sleep diaries can be found in **Appendix 4**, **pages 334-344.**

6.4.2.1 Actigraphy Protocol

The actigraphy was measured by either of two watches: The Actiwatch 2 and Actiwatch Spectrum Plus (Mini Mitter Division of Philips Respironics Ltd.). The Actiwatch 2 is a light-weight strap weighing 16 grams and 43mm x 23mm x 10mm in size. Where available, this

watch was given to younger participants or where the participant had a strong preference for it. The Actiwatch 2 had a typical battery life of 30 days and included solid state piezo-electric accelerometer with a 32Hz sampling rate. The light sensor had a wavelength range of 400-900nm and was waterproof at 1m for 30 mins. The Actiwatch Spectrum Plus was a heavier watch with a full digital display. It weighed 31 grams and is 48mm x 37mm x 15mm and was often given to older participants or those who preferred replacing a watch display that they removed during the study. This watch had a standard battery life of 60 days and included a microelectromechanical system (MEMS) type of accelerometer that works on change in capacitance, but with the same 32Hz sampling rate. The light sensor wavelength range is slightly shorter in the longer wavelengths, 400-700nm, but was also waterproof.

Actiwatch data was scored using Actiware software version 6.0.9 (Respironics, 2018) using the automatic scoring algorithm that has been validated on numerous populations including young people (Meltzer *et al.*, 2012). The activity across the two-week period was measured in 30-second epochs with each epoch of activity determined based on comparison to the other epoch counts. For example, the activity in the epochs immediately before and after the said epoch under analysis were considered and each epoch was weighted dependent on the standardised threshold sensitivity value (TSV or activity count). Any activity above 40 TSV would be regarded as wake and below would be sleep.

Actiware software produced preprogramed outcome measures including bedtime, get up time, time in bed (TIB), total sleep time (TST), sleep onset latency (SOL) (how long it takes to get to sleep), sleep efficiency (SE) (TST/TIB*100), wake after sleep onset (WASO) (the accumulated time spent awake during the night after sleep onset) and number of awakenings during the night based on the 40 TSV. It is important to note that for the sleep onset latency,

the Actiware software calculates a proxy onset latency based on the estimated time (in minutes) that a subject takes to fall asleep. This is based on automated calculations calculated from parameters of 'rest' and 'sleep' periods. As an example, the Actiware software takes the start time of the 'Rest' interval and the 'Sleep' interval start time to then calculate the onset. This is what was used in this study however it is recognised that sleep latency calculations require information regarding 'bedtime'. This is often derived from the sleep diaries as that can help to distinguish the differences between immobile wakefulness or 'rest' as the actigraphy is measuring movements. An example where sleep diaries have been used to correct for the sleep latency timings can be found in a 2012 paper by Gringras et al (Gringras *et al.*, 2012).

Further to these variables the exported raw data made more variables available: sleep duration (SD), total activity during sleep, average activity during sleep, average activity for each epoch, standardised activity during sleep and maximum activity during sleep. Each of the outcomes were measured in minutes.

Actiwatches were either sent out to participants who did not complete the overnight sleep-EEG aspect of the study or they were handed out on the night of the sleep-EEG. The participants wore the watches on their non-dominant wrist and were asked to keep this on for a continuous two-week period other than bathing or playing high impact, contact sports such as rugby. A sleep diary was provided alongside this.

6.4.2.2 Sleep Diaries

Sleep diaries were provided to each participant to complete in conjunction with wearing their actigraphy watch for two weeks. A primary carer copy of the sleep diary was produced and given to them if their children were under the age of 12 or if the child was considered to not

have capacity to complete the diary themselves. All children over the age of 12 were offered the opportunity to complete their own sleep diary with or without the assistance of the primary carer. A simplified version was offered to younger children where they were able to colour areas of a table showing how long they had slept for each night.

The sleep diaries included open ended questions such as what time did you get into bed, what time did you turn the lights off, what time did you fall asleep, what time did you wake and what up time did you get out of bed. In addition to these questions, the participants were asked to note whether they had napped during the day, exercised, drunk caffeine-containing drinks or alcohol and what medication they had taken that day. The sleep diary data was entered into a datasheet and was used to create the custom intervals on the actigraphs as mentioned previously.

Examples of the different sleep diaries which were used in the main sleep study are available in **Appendix 4**. It is important to note that the sleep diaries have not been used in the results section of this chapter. The sleep diaries cannot be analysed as a result of the lack of validation and reliability data across the different types of sleep diaries. For example, some of the sleep diaries were completed by parents and others by the participants. Unfortunately, there is not a consistent report regarding who completed the sleep diaries across the two weeks and therefore by combining all of the sleep diaries, findings would be unreliable. Future use of sleep diaries in this manner will require a consistent approach to recording who completed the sleep diary each night and therefore, reliability and validation data would also need to be calculated. The sleep diaries did form part of the protocol and have therefore been included in the methodology. However, there will be no further reference to the sleep diaries in the results.

6.4.3 Statistical Analysis

The actigraphy data was analysed in the Philips Respironics Actiware Version 6.0.9 (Respironics, 2018). Individuals that completed seven or more nights of total actigraphy are included in the analyses: the other individuals did not have sufficient data to compare with the larger dataset.

Statistical analysis was carried out using Microsoft Excel Microsoft Office Professional Plus (2016), STATA (version 13.1) (https://www.stata.com/stata-news/news28-4/stata13.1) and R (version 3.5.0) (R: The R Project for Statistical Computing, 2018).

Unpaired two-tailed t-tests were used to explore the differences in the sleep parameters between the young people with 22q11.2DS and siblings. Paired t-tests were used to explore the differences between the 22q11.2DS and their paired, related sibling in further analyses. The initial analyses did not account for covariates however in **section 6.5.3**, **pages 178-180**, covariation using age, gender and highest maternal education level is explored.

Hierarchal linear regressions were used to explore the relationship between the actigraphy-derived sleep parameters and the neurodevelopmental phenotype in 22q11.2DS. Covariates such as age, gender and highest maternal education level were included. There were only a few families that completed psychopathology and cognitive assessments on the same day as the sleep study. Therefore, when exploring these associations, the age difference between the date of assessments and date of actigraphy was accounted for as a covariate. Melatonin was also investigated as a covariate of the regression analyses.

To correct the analyses for multiple comparisons, we used a Benjamini–Hochberg false discovery rate (FDR) rate of 5% (Benjamini and Hochberg, 1995). The Benjamini-Hochberg

method ranks the individual p-values from smallest to largest, with the smallest p-value ranking as 1. By comparing each individual p-value to the Benjamini-Hochberg critical value ((rank/total number of tests) * FDR (i.e., 5%)), if the largest p-value in our analyses before correction is smaller than its critical value, it is considered significant. Any p-values smaller than the largest p-value are also interpreted to be significant.

6.5 Results

The sleep parameters were derived from the Actiware software. The nocturnal activity and sleep parameters were generated using the standardised algorithm in the software. All of the parameters are averages taken across the total time the actigraphy was recorded for (average nights = 13.1 nights for the siblings and n = 12.7 nights for the 22q11.2DS) and the activity during sleep is measured in units relating to the threshold sensitivity value (TSV) based on the standardised algorithm: 40 units and above are considered wake, and below 40 considered immobile restfulness or sleep.

6.5.1 22q11.2DS young people compared to sibling control group: objective assessment

Tables 14 and **15** show comparisons between young people with 22q11.2DS and siblings not controlling for covariates. This table shows comparisons between the two groups only using two-tailed unpaired t-test. The tables illustrate that young people with 22q11.2DS had longer time in bed, total sleep time and a higher sleep efficiency than the sibling group. However, no notable differences in the nocturnal activity across the actigraphy period were identified between the young people with 22q11.2DS and the sibling group not controlling for covariates. The absence of group differences in activity was not hypothesised.

6.5.2 Relatedness between 22q11.2DS young people and their sibling control groups

There were sibling pairs in the sleep study and this needed to be explored to ensure that relatedness did not affect the findings. Therefore, **Tables 16** and **17** show direct paired t-test comparisons between the related couples (not controlling for covariates). The findings reflect the same findings from the overall group analyses: there was a longer time in bed, total sleep time and higher sleep efficiency for the young people with 22q11.2DS compared to the siblings. There were no differences identified between the young people with 22q11.2DS and their siblings with regards to their nocturnal activity. This also agrees with the findings from the larger cohort analysis.

In these analyses, where there were two siblings available for analyses, which occurred in two instances, the sibling closest in age was paired with the young person with 22q11.2DS for the analyses.

Table 14 Comparing the automatic, objective, actigraphy-measured sleep parameters between groups

	22q11.2DS	***Siblings***				
Automatic Actigraphy Scoring	***Average, (s.d.)***	***Average, (s.d.)***	***t***	***p***	***95%CI (combined)***	***n***
Sleep Onset (hours)	22:24:16 (±01:12:23)	22:49:27 (±01:17:57)	1.40	0.166	.928-.951	76
Get Up Time (hours)	08:29:00 (±01:21:56)	08:20:42 (±01:11:27)	-0.473	0.638	.339-.364	76
Time in Bed (hours)	10:05:26 (±00:54:04)	09:31:15 (±00:51:55)	-2.65	**0.001***	.403-.421	76
Total Sleep Time (hours)	09:07:53 (±00:51:10)	08:02:25 (±00:48:33)	-6.28	**<0.0001***	.360-.380	76
Sleep Onset Latency (hours)	00:28:19 (±00:24:29)	00:22:52 (±00:16:28)	-1.02	0.312	.0149-.0219	76
Sleep Efficiency (%)	92.4% (±4.92%)	84.48% (±4.09%)	-7.03	**<0.0001***	88.3-91.0	76
Wake After Sleep Onset (hours)	00:48:05 (±00:13:08)	00:44:27 (±00:12:27)	-1.15	0.252	.0305-0.346	76

Table 15 Comparing the automatic, objective, actigraphy-measured nocturnal activity between groups

	22q11.2DS	***Siblings***				
Activity measures (nocturnal average)	***Average, (s.d.)***	***Average, (s.d.)***	***t***	***p***	***95%CI (combined)***	***n***
Total Activity	6768.7 (±2443.0)	5970.1 (±1808.1)	-1.47	0.146	5977.7-7013.3	76
Average activity per epoch	5.93 (±1.78)	5.75 (±1.86)	-0.412	0.682	5.46-6.28	76
Average activity per minute	11.9 (±3.56)	11.5 (±3.73)	-0.412	0.682	10.9-12.6	76
Standard activity	28.2 (±6.80)	27.1 (±6.37)	-0.68	0.500	26.3-29.3	76
Maximum activity	386.1 (±99.1)	362.9 (±76.3)	-1.04	0.301	357.1-399.2	76

Table 16 Paired comparison between the young people with 22q11.2DS and their siblings for the automatic, objective, actigraphy-measured sleep parameters between groups

Paired sibling analysis	**22q11.2DS**	**Siblings**				
Automatic Actigraphy Scoring (paired)	***Average, (s.d.)***	***Average, (s.d.)***	***t***	***p***	***95%CI (combined)***	***n***
Sleep Onset (hours)	22:18:41 (±01:16:03)	22:51:23 (±01:13:16)	-2.05	**0.05**	-.0458-.000362	44
Get Up Time (hours)	08:28:02 (±01:29:11)	08:15:45 (±01:05:02)	0.936	0.360	-.0104-.0275	44
Time in Bed (hours)	10:09:20 (00:59:33)	09:24:21 (00:52:04)	2.57	**0.0177***	.00599-.0565	44
Total Sleep Time (hours)	09:23:43 (±00:46:56)	07:59:09 (±00:59:33)	5.88	**<0.0001***	.0379-.0795	44
Sleep Onset Latency (hours)	00:29:07 (±00:27:35)	00:20:26 (±00:15:10)	1.23	0.231	-.00414-.0162	44
Sleep Efficiency (%)	92.71% (±4.99%)	84.92% (±3.64%)	5.96	**<0.0001***	.0508-.105	44
Wake After Sleep Onset (hours)	00:46:03 (±00:14:48)	00:43:41 (±00:12:50)	0.83	0.416	-.00278-.00576	44

Table 17 Paired comparison between the young people with 22q11.2DS and their siblings for the automatic objectively actigraphy-measured nocturnal activity between groups

Paired sibling analysis	***22q11.2DS***	***Siblings***				
Activity measures (nocturnal average)	***Average, (s.d.)***	***Average, (s.d.)***	***t***	***p***	***95%CI (combined)***	***n***
Total Activity	6426.9 (±2442.3)	5903.5 (±1900.8)	1.13	0.271	-438.7-1485	44
Average activity per epoch	5.55 (±1.69)	5.65 (±1.91)	-0.273	0.788	-.855-.657	44
Average activity per minute	11.1 (±3.37)	11.3 (±3.81)	-0.271	0.789	-1.71-1.32	44
Standard activity	27.3 (±5.65)	26.9 (±6.63)	0.287	0.777	-2.20-2.91	44
Maximum activity	386.2 (±93.2)	264.7 (±80.4)	0.990	0.334	-23.7-66.7	44

6.5.3 Investigating covariation

Covariates were not accounted for in **section 6.5.1** and **6.5.2** as the associations between groups was explored first. However, it is important to account for covariation to better understand the relationships between the young people with 22q11.2DS and the siblings in relation to the sleep parameters.

Before controlling for covariation, it was important to explore the total cohort and examine whether covariates of age, gender and highest maternal education were more generally associated with the sleep parameters. This meant that the covariates were explored using linear regression analyses without including the group status and therefore not distinguishing

between the young people with 22q11.2DS and the siblings. The following relationships were identified. In relation to the sleep parameters derived from the objective automatic actigraphy sleep scoring, later sleep onset was identified in older individuals (B=.00938, p<0.0001), later get up time was identified in older individuals (B=.00931, p<0.0001), sleep onset latency was shown to be longer in older individuals (B=.00246, p<0.0001) and sleep efficiency was shown to be lower in older individuals (B=-.000139, p<0.0001). It is important to note that the effect sizes are relatively small and therefore associations would need to be explored further and corrected for multiple comparisons.

When investigating the objective actigraphy nocturnal activity measures, only the maximum nocturnal activity count was associated with age showing a reduced maximum activity count in older individuals (B= -6.58, p=0.031).

However, there were differences in nocturnal activity derived from the objective actigraphy data when controlling for gender. The average nocturnal activity count was higher in male individuals (B= -.867, p=0.035) compared to female. Similarly, the average nocturnal activity measure per minute was higher in male individuals (B= -1.73, p=0.035) compared to females also.

Highest maternal education was added as a covariate to determine whether the sleep parameters were associated with the covariate that was associated with the pattern of tiredness-related problems in **Chapter 4**. No associations were found other than with the sleep efficiency measured by the actigraphy in the young people with 22q11.2DS showing an increase in sleep efficiency with better maternal educational attainment (B=.0250, p=0.003).

These findings helped to identify where particular associations could exist. It was important to explore the covariates in linear regression models taking into account group status. This would then allow for better interpretation of the findings between the young people with 22q11.2DS and siblings, and explore whether the presence of age, gender and highest maternal education could account for the differences. Therefore, only the associations which showed significant differences after correcting for multiple testing were further explored for covariation. It is important to note that when adding the covariates, the sample size of the model reduces resulting in small cohorts being explored. This needs to be considered when reviewing the findings and basing decisions off this work. Exploring covariation is important however it would need to be repeated and replicated in a larger cohort sample to better understand robust associations.

Covariates age, gender and highest maternal education were added to the associations noted by an asterisk in **Tables 14-17**. Changes to the associations between the young people with 22q11.2DS and siblings are commented on. The difference in the time in bed derived from the actigraphy data between the young people with 22q11.2DS and siblings was attenuated when adding highest maternal education as a covariate (differences between the groups was accounted for B=0.0220, p=0.052, by adding highest maternal education.) It is important to note that the cohort size was reduced to n=47. The effect and relationship between the paired 22q11.2DS young people and their siblings in the time in bed derived from the actigraphy data (reflecting the prior results) was attenuated when highest maternal education was added as a covariate (B=0.0186, p=0.153). All other associations that had been corrected for multiple testing and highlighted in **Tables 14-17** remained when controlling for age, gender and highest maternal education.

6.5.4 Actigraphy and the Neurodevelopmental Phenotype

The next step in the analysis was to examine whether there were differences in the actigraphy sleep parameters based on the symptomology of psychiatric problems and cognitive impairment in the 22q11.2DS young people only. Symptom scores for the sibling group were not sufficiently high to explore further.

These analyses were based on the significant findings in **Chapter 4** analyses that explored the patterns of sleep problems and comorbidities in 22q11.2DS. The patterns of sleep problems however could not accurately be applied to these current analyses as they were derived from a single time-point. The actigraphy could be compared to the psychopathology and cognitive data that was closest in time to the sleep data acquisition: some participants completed the sleep study on the same day as psychopathology and cognitive tests for wave 1 of the ECHO Study for example whereas others completed wave 2 of the ECHO Study three months prior to the sleep study acquisition.

The time difference between the psychopathology and cognitive assessments and the actigraphy assessment was accounted for when investigating the associations including the differences in ages as a covariate in the analyses (mean time difference = ~10 months, s.d. = ±13 months). Associations identified were tested with covariates to determine whether the relationship was robust or could be explained by another predictor.

There were no associations between the more objective-actigraphy derived sleep parameters and the neurodevelopmental phenotype in young people with 22q11.2DS. There were no associations found for the nocturnal activity in young people with 22q11.2DS compared to the neurodevelopmental phenotype and this finding was particularly surprising.

6.6 Discussion

This is the first study to explore sleep in young people with 22q11.2DS and in addition, to include a comparison sibling group in these sleep analyses. This study included the use of an objective assessment of sleep using actigraphy watches. Several different variables were derived and investigated in the young people with 22q11.2DS and their siblings. Surprisingly, there were no differences in the nocturnal activity between the 22q11.2DS and siblings however differences in the sleep parameters derived from the objective actigraphy data were found between the groups.

6.6.1 Differences in sleep parameters between groups in actigraphy-derived sleep parameters

From the actigraphy-derived sleep parameters, differences between the 22q11.2DS young people and their siblings were identified in total time in bed, total sleep time and sleep efficiency. The young people with 22q11.2DS slept for longer, remained in bed for longer and had a higher sleep efficiency than the sibling control group.

There is conflicting evidence in the literature with one study suggesting that young people with neurodevelopmental disorders such as ADHD, when compared to typically developing controls, on average spend longer time in bed and have a longer total sleep time during weekdays and at the weekends across a seven night assessment (Corkum et al., 2001). However, it is widely reported than young people with ADHD and ASD have shorter sleep times when compared to typically developing controls and longer sleep onset latencies (van der Heijden *et al.*, 2018) in other studies. The differences between young people with ADHD and ASD have been shown to be negligible. More severe ADHD symptoms have been shown

to be associated with shorter sleep duration when compared to young people with milder ADHD symptoms. However, ADHD symptoms in general have been repeatedly reported to associate with shorter sleep duration (Hysing *et al.*, 2016). There is consistent report of sleep variability among young people with NDDs notably variability in sleep timings suggesting poorer sleep quality and quantity with common reports of insomnia and bedtime resistance (Hodge *et al.*, 2014).

The young people with 22q11.2DS despite having an elevated preponderance of neurodevelopmental problems compared to their siblings have demonstrated contrasting findings to the majority of the literature regarding NDDs and sleep. This work needs to be replicated with a larger sample of young people with 22q11.2DS in addition to using a different control sample such as an ADHD cohort that has been genotyped to exclude the presence of 22q11.2DS. Having a comparative NDD control group could help to identify the differences between the young people with 22q11.2DS and the non-22q11.2DS NDD populations if these differences are robust.

Young people with 22q11.2DS do present with a number of problems which include physical problems. There are potential reasons for why an individual with 22q11.2DS might demonstrate differences in their sleep to other NDDs. Speculation could suggest that young people with 22q11.2DS might be confined to bed once in it as a result of their physical problems such as musculoskeletal problems which might require wearing a back brace at night. The comorbidities, especially affective disorders in 22q11.2DS, could also play a bigger role in influencing the sleep phenotype in 22q11.2DS than the NDDs. This is highly speculative but something which should be explored in the future.

6.6.1.1 Paired sibling analyses showed similar findings

It was important to identify individuals who were related in these analyses. Therefore, undertaking paired analyses showed differences in the same parameters in the unpaired analyses between the 22q11.2DS and their paired sibling.

Controlling for highest maternal educational attainment did explain the difference in the time in bed derived from the actigraphy data between the paired siblings, attenuating the finding. However, no other findings were account for in the paired analyses when adding covariates. In the literature however age has been shown to account for variation in sleep timings with younger individuals showing longer total sleep times (Hirshkowitz *et al.*, 2015) and adolescents having a preference for later sleep onset times than younger children, with a later chronotype marking adolescence (Roenneberg *et al.*, 2004). However, age did not account for differences in these analyses. Behaviours at bedtime can influence sleep onset (Jones and Ball, 2014) and potential daytime tiredness resilience a contributing factor. Sleep restriction studies in young typically developing children compared to individuals with neurodevelopmental disorders have shown evidence of daytime tiredness and impaired attention and poorer attainment of cognitive tasks, however the typically developing children showed more resilience and fewer general errors, with no individuals reaching a clinical inattention threshold (Gruber et al., 2011).

These analyses do not account for a number of different parameters however including preferences and chronotype. Potentially, differences in the propensity for the young people to want to sleep at different times could influence findings. Twin-studies which have explored chronotype and diurnal preference have shown a stronger genetic link between monozygotic twins regarding their preferences than dizygotic twins. Nonetheless, there was evidence of

heritability but does suggest some convergence in siblings (Watson, Buchwald and Harden, 2013). Therefore, these findings are interesting, however should be considered with caution as currently it is not known what factors are driving these sibling differences.

6.6.2 No associations between actigraphy-derived sleep measures and psychopathology

The parent-reported psychopathology did not show an association with any of the objective actigraphy-derived sleep parameters. This was surprising as it doesn't agree with the literature surrounding psychopathology and objectively measured sleep problems. This was particularly interesting when taking into consideration the findings from **Chapter** 4 which showed the prevalence of sleep problems and associated neurodevelopmental phenotype. Sleep patterns and bedtime variability have been shown to be relevant to anxiety symptoms in early adolescence (McMakin and Alfano, 2015) however the absence of findings from the actigraphy suggest that it is not objectively supported. Understanding more about the bedtime behaviours and sleep patterns could help to better understand this finding.

6.7 Strengths and Limitations

Strengths include the use of the verified standard actigraphy analysis protocol that was conducted on this data. This algorithm and software have been used in many papers within the sleep medicine literature with verification on samples of young people. Furthermore, the combined use of the objective and subjective assessments of sleep allows more a comprehensive understanding of the sleep problems in 22q11.2DS and begins to explore the most appropriate method of sleep assessment in these young people.

The actigraphy watches were worn for two weeks however the watches could be worn for longer to provide further data for verification of sleep patterns. Furthermore, the most appropriate time for the participants to undertake the study was at the weekends and during the summer holidays. Therefore, it is important to note that the sleep timings would have fluctuated depending on whether they were assessed on free days or the school/work days. Unfortunately, it was not feasible to undertake the sleep study in the same period of the year among individuals. For example, some young people would have been at school for the two weeks whereas others were on summer holidays.

The sleep diaries were not used in the analyses for this thesis. This was not the original intention however on reviewing the data that was acquired, it wasn't possible to provide reliable and valid comparisons with the objectively measured actigraphy data. This was as a result of not having a consistent record of who was completing the sleep diary across the two-week period. This was a mistake in the design of the protocol and in the future, this needs to be rigorously recorded especially where there are differences in the average IQ of individuals such as young people with 22q11.2DS compared to their siblings.

There could also be an issue of reporter error as there has to be an assumption that the sleep diaries were reported accurately however some may have been completed retrospectively and therefore could introduce some errors into the outcomes. As a result, these findings need to be considered with caution before further objective sleep assessments are conducted.

6.8 Introduction to Sleep Questionnaires

Sleep questionnaires are widely used in the paediatric literature to obtain a parental report of child sleep behaviours. Having a questionnaire response from a parent can provide

information regarding the perceived phenotype of the individual. However, it cannot be used as the only assessment of sleep, especially when addressing sleep behaviours of adolescents. Sleep questionnaires are a subjective measure which take a snapshot of sleep-related behaviours of an individual. It is important to verify these measures with more objective measures. Nonetheless, validated sleep questionnaires have been used extensively in the paediatric literature to acquire a large yield of information about sleep in young people. Sometimes sleep questionnaires are the only measures that can be conducted reliably in children of a very young age or in a population with a high prevalence of learning disability.

The second part of this chapter aims to outline why sleep questionnaires were used in the study and how they can be used to validate and support other measures to create a comprehensive assessment of sleep in 22q11.2DS.

6.8.1 Sleep Questionnaires in the Paediatric Literature

There are an extensive number of published and unpublished sleep questionnaires that are used in the paediatric sleep literature. Sleep problems should be assessed by validated sleep questionnaires that have been tested in the target population however there are a number of instruments that remain non-standardised (Spruyt and Gozal, 2011a).

Sleep questionnaires are used initially to obtain a general assessment of the sleep problems, behaviours and potential phenotype. They are used to provide a screen of the individuals sleep and therefore inform further study and potentially intervention. Sleep questionnaires should not be used clinically as the only assessment tool (Spruyt and Gozal, 2011b). However, the validated questionnaires provide a reliable tool that can be distributed easily and at a reduced cost with the benefit of acquiring a large yield of data.

Validation of sleep questionnaires should be conducted against more objective measures of sleep such as polysomnography (PSG). However, undertaking a PSG in young people can be difficult and therefore, could contribute to a reason why there is limited validation. For example, the Children's Sleep Habits Questionnaire is one of the most commonly used children sleep questionnaires that assessed sleep quality and quantity. Validation of the questionnaire was conducted in 2014 and the study demonstrated incongruence between the questionnaire and the PSG and actigraphy-derived sleep measures (Markovich, Gendron and Corkum, 2014). This study demonstrated that sleep questionnaires in the paediatric literature should be interpreted cautiously and that further verification of sleep problems in young people should be conducted.

6.8.2 Sleep Questionnaires in Neurodevelopmental Populations

Sleep questionnaires are predominantly validated in typical control samples of young people. Therefore, it can be difficult to obtain a sleep questionnaire that is appropriate for use in young people at elevated risk for learning disability. Parental-reports are used as a replacement for these young people as they are considered to provide a more reliable assessment of their child's sleep behaviours. However, more objective measures have shown discrepancies in parent reports (Esbensen et al., 2018). Therefore, in young people with capacity, self-reports of sleep problems can provide a reliable and representative perspective of sleep habits (Richdale and Baglin, 2013).

There are sleep questionnaires that were developed based on a typical population that have been adapted and validated for the assessment of sleep in young people with NDDs. The Paediatric Sleep Questionnaire (Chervin et al., 2000) is a 22-item symptom assessment of sleep problems in young people. However, it includes an assessment of behaviour and

specifically includes a 6-item scale that assesses attention and hyperactivity in reference to ADHD symptoms (Chervin et al., 2002). This questionnaire can be used in NDDs and can help to identify whether sleep problems and symptoms of NDD are overlapping and comorbid.

Problems were using subjective sleep questionnaires include validation in the appropriate populations but using sleep questionnaires in large datasets can yield vast amounts of data as demonstrated by the assessment of sleep in the UK Biobank where in one sample sleep problems provided as a self-report were assessed in nearly 500,000 participants (Boakye *et al.*, 2018).

6.9 Rationale

This thesis started with assessing sleep subjectively by the Child and Adolescent Psychiatric Assessment. The gold standard psychiatric assessment includes the sleep problems section which was used to explore the prevalence and nature of sleep problems in 22q11.2DS and siblings in **Chapter 4**. This assessment however was a semi-structured interview with the primary carer but did not account for the perspective of the individual. This analysis helped to form the basic questions that led to the further development of the sleep study.

The sleep questionnaires outlined in **Chapter 5** have been used to collect data from the largest subset of individuals. The questionnaires were short for the young people and often included closed responses on Likert-scales which involved little writing so that they were appropriate for most developmental ages. The questionnaires did not involve the participant having to wear any equipment or for the study team to visit the home of the family as the questionnaires were sent out before any of the home visits for all individuals. The parents also received sleep questionnaires which has added to the wealth of data now available for

the assessment of parental reports compared to the individuals report of sleep problems, allowing for further investigation of the state of sleep problems in 22q11.2DS and also in the typical young person population which can be acquired from the sibling data.

The sleep questionnaires importantly have allowed validation of the CAPA sleep problems section showing that the evidence from the CAPA is validated by tested sleep questionnaires used in the target paediatric population.

6.9.1 Aims of the Sleep Questionnaires

The aims of the sleep questionnaires were:

1. to acquire a large dataset of information from the primary carers and the individuals themselves (young people with 22q11.2DS and siblings);
2. to validate the sleep section of the Child and Adolescent Psychiatric Assessment (CAPA) with the sleep questionnaires to determine the reliability of sleep problems (see **Chapters 3** and **4**);
3. to begin to compare the subjective measures to the objective measures of sleep in the 22q11.2DS and siblings.

6.10 Methods

Participants were part of the ongoing Experiences of CHildren with cOpy number variants (ECHO) study (Niarchou et al., 2014; Chawner et al., 2017; Cunningham et al., 2017). Recruitment for the ECHO Study can be found in **Chapter 3** and information regarding the recruitment process for the sleep study can be found in **section 6.4.1**.

6.10.1 Assessments – Sleep Questionnaires

The sleep questionnaires outlined in **Chapter 5**, as part of the Pilot Study, were used in the main study. It was confirmed that these questionnaires could accurately provide information regarding sleep behaviours and patterns in the young person population. However, there was a small gap in the information acquired. In individuals under the age of 11, there was no assessment of the daytime sleepiness as the Cleveland Adolescent Sleepiness Questionnaire (CASQ) could not be used in these younger children as it had not been validated. This resulted in the main study including a Teacher's Daytime Sleepiness Questionnaire (TDSQ) to capture the daytime sleepiness activities of the younger children. By asking the teachers who spend school days with the child to assess their daytime sleep activity, sleepiness throughout a school day was assessed.

6.10.1.1 Teacher's Daytime Sleepiness Questionnaire (TDSQ)

The remaining participants that were younger than 11 years old did not report their sleepiness. Primary carers provided consent regarding contact with their child/ren's teacher. If the primary carer consented, an address was provided and subsequently the Teacher's Daytime Sleepiness Questionnaire (TDSQ) was sent out with a pre-paid envelope for the teacher to complete and return. The TDSQ asks teachers about their perceived sleepiness of the young person in their class considering physiological responses such as yawning and behavioural responses such as does the young person complain about sleepiness/being tired, the child's discipline and their hyperactivity. The TDSQ includes 10-items measured on a 1-3 Likert-scale response. There were n=18 teachers who successfully completed and returned the TDSQ. There were n=3 teachers who did not return the TDSQ.

6.10.2 Statistical Analyses

Pairwise correlations were conducted to explore the relationship between the CAPA-derived sleep problems and the total scores derived from the sleep questionnaires. Linear regression analyses were conducted to determine the relationships between correlating continuous scores and ordinal logistic regressions were used to investigate categorical scores. Statistical analysis was carried out using STATA (version 13.1) (https://www.stata.com/stata-news/news28-4/stata13.1) and R (version 3.5.0) (R: The R Project for Statistical Computing, 2018).

6.11 Results

The questionnaires were completed by different samples sizes. Some of the questionnaires were not returned by the families. The numbers completed are outlined in **Table 18**.

Table 18 Number of sleep questionnaires completed and returned in the sleep study

Sleep questionnaire	***Sent to***	***Returned incomplete***	***Returned completed***
Cleveland Adolescent Sleep Questionnaire (CASQ) completed by ≥12-year olds	69	0	69
Children's Morningness-Eveningness Scale (CMES) completed by ≥12-year olds	59	2	54
Children's Chronotype Questionnaire (CCTQ) completed by primary carer of child <11 years old	28	1	27
Munich Chronotype Questionnaire (MCTQ) completed by ≥12-year olds	58	7	49
Paediatric Sleep Questionnaire (PSQ) completed by primary carer	89	4	84
Sleep-related Breathing Disorder Scale (SBDS) completed by primary carer	89	4	84
SDSC completed by primary carer	89	2	86
TDSQ completed by teacher	22	1	18

6.11.1 Cross-validation

The total scores were derived from each of the sleep questionnaires and where appropriate the scores were correlated with the parent-reported sleep problems from the CAPA to demonstrate cross-validation of the CAPA as a sensitive tool for sleep problems (see **Chapters 3** and **4**).

The CASQ did not correlate with any of the other subjective measures and showed poor effect sizes. There was no association between the CASQ total and the CAPA sleep problems ($p=0.893$). The CAPA sleep problems did show an association with the other parental reports focussing on disorders of initiating and maintaining sleep (DIMS) ($p\leq0.001$), sleep-wake transition disorders (SWTD) ($p=0.001$), disorders of arousal (DA) ($p=0.0088$) and disorders of excessive sleepiness (DOES) ($p=0.0007$) (see **Table 19**). After controlling for multiple testing, all associations remained. The disorders of initiating and maintaining sleep can be defined as insomnia-related which is pertinent for the two patterns of sleep problems derived from the

CAPA sleep problems: tiredness-related and insomnia-related. The CASQ was a participant report showing that parent-report did not correlate with the young person report.

Table 19 Pairwise correlations between the CASQ participant-completed questionnaire and the parent reports of sleepiness

QA total score	***CASQ***	***PSQ – sleepiness scale***	***SDSC – DIMS***	***SDSC – DA***	***SDSC – SWTD***	***SDSC - DOES***	***CAPA Sleep Problems***
Cleveland Adolescent Sleep Questionnaire (CASQ) completed by ≥12-year olds	1						
Paediatric Sleep Questionnaire (PSQ) Sleepiness scale completed by primary carer	0.171	1					
Sleep Disturbance Scale for Children, Disorders of Initiating and Maintaining Sleep subscale completed by primary carer	0.0336	**0.417**	1				
Sleep Disturbance Scale for Children, Disorders of Arousal subscale completed by primary carer	0.127	0.0898	**0.181**	1			
Sleep Disturbance Scale for Children, Sleep-Wake Transition Disorders Subscale completed by primary carer	0.140	**0.235**	**0.487**	**0.372**	1		
Sleep Disturbance Scale for Children, Disorders of Excessive Sleepiness completed by primary carer	0.163	**0.738**	**0.542**	0.149	**0.425**	1	
Child and Adolescent Psychiatric Assessment Sleep Problems	0.0165	**0.257**	**0.578**	**0.278**	**0.341**	**0.353**	1

Significant associations are highlighted in **bold**

The CMES score is categorical and therefore an ordinal logistic regression showed associations with the SDSC DIMS (OR=0.844, p=0.028) and the DOES scores (OR=0.786, p=0.032) (see **Figure 9**). Controlling for multiple testing, the association between the CMES score and SDSC DIMS remained. These results show that an elevated score for DIMS and DOES is associated with a reduced risk for morningness chronotype preference. This indicates that individuals that have an eveningness preference associate with worse scores for DIMS and DOES.

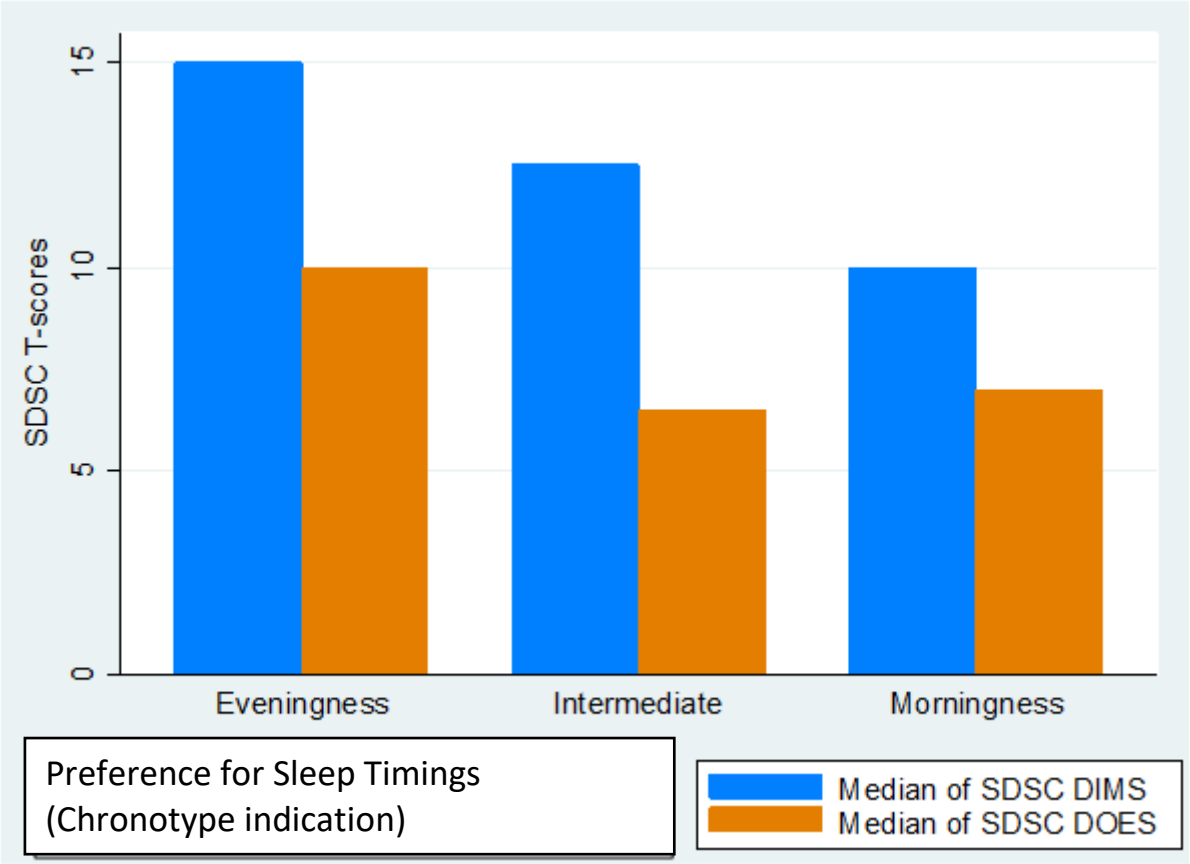


Figure 9 Lower scores for SDSC were shown to associate with the morningness preference in young people with 22q11.2DS on the CMES

The TDSQ did correlate with parental measures of sleepiness including the CAPA sleep problems in the total group (p=0.018) however this was driven by siblings as no association was seen for individuals with 22q11.2DS (p=0.195).

6.11.2 Comparing Actigraphy measures with Self-Report

The subjective, participant-reported measures derived from the sleep questionnaires were compared to the objective actigraphy measures. Elevated scores on the CASQ, indicative of more sleepiness, were associated with shorter total sleep time in young people with 22q11.2DS (B=-0.393, p=0.0133). Lower scores on the CMES, suggestive of a preference for an 'eveningness' chronotype, showed associations with later get up time (B=-0.501, p=0.0035) (see **Figure 10**) and later sleep onset time (B=-0.369, p=0.0377 in young people with 22q11.2DS. Controlling for multiple testing resulted in only the association between the lower CMES total score and later get up time surviving. For the siblings, similarly an association between lower total CMES score and later get up time was seen (B=-0.585, p=0.0172) however this association did not survive correction for multiple testing.

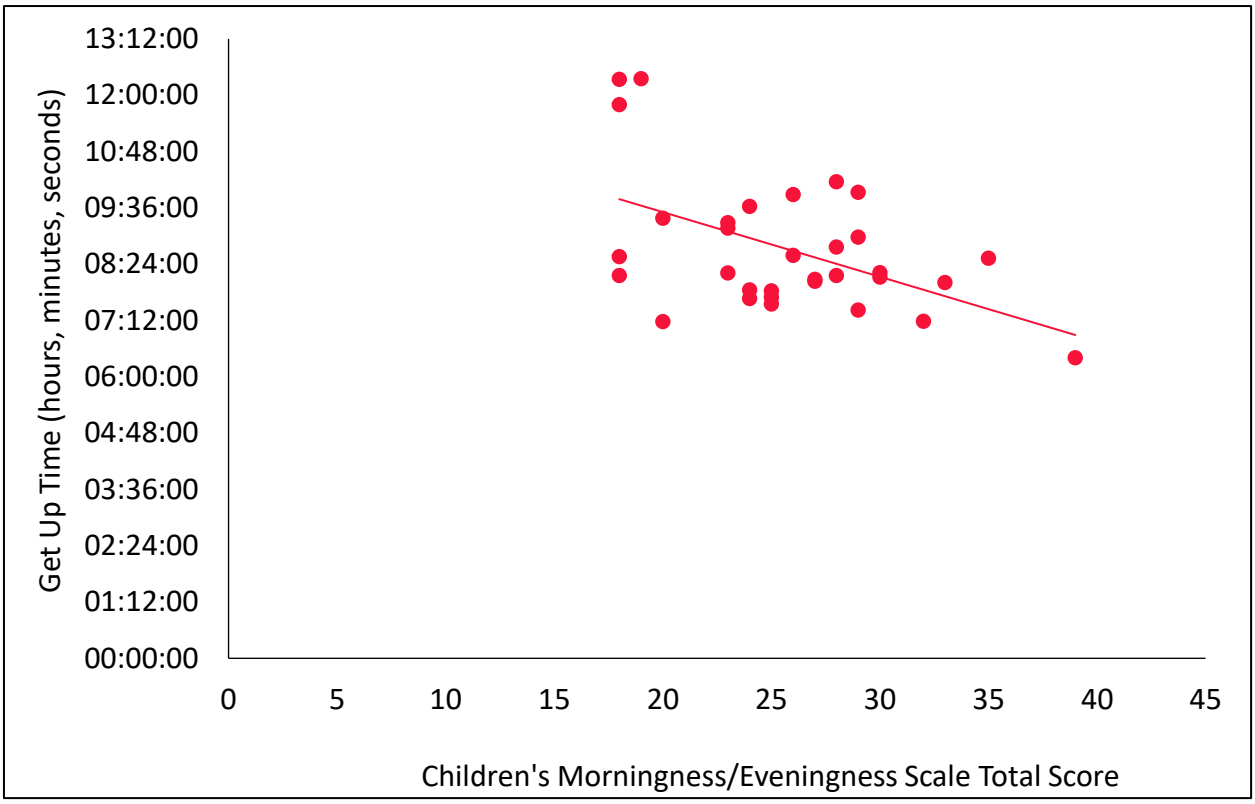


Figure 10 Correlation between the objective actigraphy Get Up Timings, and the subjective 22q11.2DS young person, participant-reported CMES total score: the only associations to survive correction for multiple comparisons

6.12 Discussion

This the first time, at time of writing, that validated sleep questionnaires have been used in a young person population with 22q11.2DS. The sleep questionnaires were used to assess the validity of the CAPA sleep problems and the parent reports generally. In addition, a brief description relating sleep problems identified by the sleep questionnaires is included, however further analysis is required.

The Paediatric Sleep Questionnaire (PSQ) and the Sleep Disturbance Scale for Children (SDSC) subscale scores of parental-reports of sleep problems showed strong, positive correlations. However, the CASQ participant self-report sleep questionnaire did not show concordance with the CAPA sleep problems or any of the parent-reported sleep problems. Furthermore, for the younger participants, a sleep questionnaire was completed by their teachers. The outcome score from the Teacher Daytime Sleepiness Questionnaire (TDSQ) did not show concordance with the parental report sleep problems for the young people with 22q11.2DS, but it did show correlation with the parent report of the unaffected siblings' sleep problems.

The SDSC Disorders of Initiating and Maintaining Sleep (DIMS) and Disorders of Excessive Sleepiness (DOES) subscale scores showed an association with more problems and reduced risk for a morningness chronotype preference. This suggested that parent-reports of DIMS and DOES were associated with individuals reporting that they had a preference for an 'eveningness' chronotype or 'an owl' chronotype.

6.12.1 Cross-validity of the CAPA Sleep Problems Section with Parent-reports but Discordance between Self-report and Parental-reports

The parent-reported PSQ and SDSC validated the parent-reported CAPA sleep problems, showing a correlation between the two reports for the young people with 22q11.2DS and their unaffected siblings. This suggests that the CAPA Sleep Problems section could reliably identify similar sleep problems to validated sleep questionnaires used in the paediatric sleep literature. However, the CASQ showed a lack of concurrence with the CAPA sleep problems, suggesting that parent-reports and self-reports generated diverging scores. This potentially indicates that the parent reports are not the most reliable assessments of young people's sleep problems. The CASQ was completed by young people over the age of 12 and therefore suggests that the older children and adolescents report differently to their parents. The findings support one study examining parent and adolescent reports of sleep problems in a population sample (not in 22q11.2DS). Parental reports accurately identified a third of the sleep problems that adolescents reported (Fatima et al., 2016). Moreover, another study assessing parent-reported sleep problems in their children compared to the adolescent reports showed that the parents were more sensitive to reporting sleep problems in their child who had a physical problem compared to the parents of the adolescents that did not have a physical problems (Pirinen et al., 2010). These parent-reports did not reflect the adolescent reports which showed that sleep problems did not vary dependent on a physical problem.

Furthermore, the findings showed that the teachers of the younger children did not identify similar sleep problems to the parents in those young people with 22q11.2DS however they did accurately report siblings' sleep problems. This potentially suggests that the parents are

more aware of potential sleep problems in their child with 22q11.2DS and therefore, they could over-report sleep problems. The teachers however anecdotally, have a class of young people that they can compare and are of potentially varying developmental ability. This could allow for teachers to forge reliably informed judgements of sleep problems.

6.12.2 Preference for Heightened Evening Activity in Self-reports relates to Parent-reports of Sleep Problems

The Children's Morningness Eveningness Scale (CMES) was completed by young people over the age of 11 to acquire an understanding of their preference for their most active time of the day: would the young person prefer to be awake later into the night, or wake early in the morning for example. The self-reported preferences were shown to associate with elevated parent-reported problems in initiating and maintaining sleep and with parent-perceived excessive tiredness. The findings that adolescents and older children preferred going to bed later, and felt personally more active into the evening, was potentially misinterpreted by the parent as a problem relating to their sleep. For example, that their child had prolonged onset of sleep and difficulties with initiating sleep. This potential finding could support the absence of correlation between the parent and self-reported outcomes discussed in **section 6.11.1** also. A study including sleep questionnaires completed by parents and adolescents, in addition to actigraphy in the adolescents demonstrated that parent's had an idealised perspective of their child's sleep with inconsistencies in bedtimes on weekdays and weekends suggesting that they were earlier than the adolescent report and also the parent suggested later wake times on weekends compared to the adolescents (Short et al., 2013). Actigraphy parameters suggested that parents reported longer total sleep times than the more objective measures and the adolescent reports.

6.12.3 Uniting the Objective and Subjective Assessments of Sleep

Comparing the participant-reported sleep problems and the objective actigraphy worn by the participants themselves showed interesting findings. After surviving multiple testing, lower CMES total scores were shown to associate with later get up times. The CMES assesses the preferences of a young person (over the age of 12) regarding sleep timings. The participants can comment on the preferred timing for sleep onset, and get up times and times of the day that they consider themselves to be most active. The fact that the objective measure in the adolescents corroborated the subjective report of their preference getting out of bed later, shows that the two methods of assessment can complement each other. In these analyses however, this was the only finding that survived multiple comparisons in young people with 22q11.2DS and there were no findings relating the participant-reported sleep problems and the objective actigraphy measures in the siblings. There is mixed success in actigraphy corroboration of subjective reports of sleep problems however , with one study showing one significant associations between reported night time awakenings with actigraphy (Markovich, Gendron and Corkum, 2014).

Therefore, this could suggest that it is important that objective actigraphy and subjective sleep questionnaires are used in a complementary manner to assess the sleep phenotype as sleep diaries can provide a cost-effective assessment of sleep times with actigraphy providing added information regarding nocturnal events (Werner *et al.*, 2008). For the younger individuals, parent reports can provide an accurate and sensitive representation of the sleep problems in certain cases, especially in young children with NDDs (Wiggs and Stores, 2004). However, how the objective actigraphy measures relate to the parent-reported sleep problems in older children and adolescents suggests that parent-report=ed sleep problems

might not be representative of adolescent reports of sleep problems. The findings relating the objective actigraphy measures and the participant-reported subjective sleep problems, suggests that older individuals can provide reliable and replicable information about their sleep in some instances particular sleep parameters relating sleeping and waking preferences.

The sleep questionnaire outcomes are more robust when the more objective actigraphy data can support outcomes. Nonetheless, together and in addition to the data outlined in **Chapter 4** and the next chapter, **Chapter 7**, these measures contribute to a comprehensive assessment of sleep in a young person population that has yet to be investigated.

6.13 Strengths and Limitations

This is the only study to have used actigraphy in young people with 22q11.2DS and the sample size is relatively large for a high-risk young person population. This is a novel assessment in these young people and the invaluable sibling control group allows for comparisons to typically developing children.

The actigraphy and sleep questionnaire allowed young people who were not interested in completing an overnight assessment to contribute to the data acquisition in a non-invasive way. The participants were able to remove their actigraphy watch, complete their sleep diaries (yet this was not rigorously recorded and should have been) and sleep questionnaires with freedom and an understanding between the parent, child and researcher increasing the yield of data, with most young people keeping the watch on over the period of two weeks.

A limitation to the actigraphy was that watches had to be sent out to participants and this relied on the families to return the watches in good time for the data to be removed from the watches. Unfortunately, there were instances where the watches were not returned and

therefore data was lost. If actigraphy was to be used in the future, devising a better way of returning the watches would be advisable.

The actigraphy watches were placed on the non-dominant wrists of the young people. Studies have used actigraphy watches on the dominant wrist, ankles and have used bed mats containing accelerometers to assess night time activity. For the younger individuals, another device such as a mat might be more appropriate and cause less discomfort as the watches were sometimes bulky and larger than the wrists of the smaller children. Furthermore, new and innovative technologies that can measure multiple different physiological measures are not readily available. The assessment only focussed on the acquisition of movement however future work could consider a technology with several different physiological measures of the young people.

The neurodevelopmental phenotype was assessed on a different day to the actigraphy assessments in many of the individuals. The age differences were accounted for however it would be interesting to conduct cognitive and psychiatric assessments throughout a period of actigraphy or in relation to an objective-measure of sleep.

Actigraphy has been shown to accurately estimate TST in the literature however the sensitivity of actigraphy to detect immobile wakefulness, such as lying in bed awake has been questioned. This is important to the findings as the sample included young people and adolescents that biologically would have a predisposition to 'lying-in' during the weekends or during a free day (Sadeh and Acebo, 2002; Sadeh, 2011). This could cause some inaccuracies in the TST and therefore the SE. This is one of the reasons the sleep diary is completed in concordance with the actigraphy to attempt to remove inconsistencies between the two measures.

Statistical comparisons were corrected for multiple comparisons and therefore, the findings can provide the foundations to build upon to investigate sleep problems further in young people with 22q11.2DS.

6.14 Clinical Implications

These findings have suggested that parent-reports might not potentially be the most reliable assessment of sleep in young people with 22q11.2DS. This indicates that the perspective of the individual, particularly in adolescents, should be obtained with regards to their sleep. Having a parent-report, self-report and a more objective measure such as actigraphy, will allow for a more reliable assessment of the sleep phenotype in 22q11.2DS. This can help to examine whether there are underlying sleep problems or if there are potential genetic and individual variations in sleep parameters such as bedtimes which might present as sleep problems to a parent. This information can help to develop better sleep interventions including sleep hygiene and sleep education which can involve the individual, teachers and the family.

6.15 Future Work

The sleep questionnaires should be used to validate the more objective measures of sleep in young people in 22q11.2DS. This would be the first validation of sleep questionnaires in this population and would unite the different sleep assessment measures to create a comprehensive, valid sleep assessment that can be replicated in other groups with 22q11.2DS.

There are many physiological changes during sleep and nocturnal activity is only one assessment. Heart rate, oxygen saturation and hormone levels vary over the night time period and sophisticated innovative technologies have been developed which could give an insight into other physiological measures. This could be conducted through smart technologies to identify stress or skin conductance through the night. Furthermore, stress hormones fluctuate overnight and circadian profiles of cortisol and melatonin are inherent to sleep-wake cycles. Therefore, melatonin and cortisol samples could be acquired through the night and daytime to determine circadian profiles in 22q11.2DS.

6.16 Summary

This chapter has shown that there are actigraphy-derived differences in sleep parameters between young people with 22q11.2DS and their unaffected sibling controls. There are potential relationships between the sleep problems and the neurodevelopmental phenotype in the young people with 22q11.2DS also. This chapter has also helped to identify the validity of the CAPA sleep section supported by parental-reports of sleep problems, but it has further indicated that parent reports might not be the most reliable perceptions of sleep problems in older adolescents whether young people with 22q11.2DS or siblings. Associations with the neurodevelopmental phenotype suggest that more work is required to determine whether actigraphy can be used to identify particularly symptoms in young people with 22q11.2DS from monitoring their sleep.

Chapter 7 – Polysomnography in 22q11.2 Deletion Syndrome

7.1 Overview of the Chapter

Sleep problems in 22q11.2DS have been reported anecdotally by parents who suggest that sleep problems are disruptive to the young person and their family. To be able to investigate sleep problems clinically and comprehensively explore the sleep phenotype in the young people with 22q11.2DS, a gold-standard sleep study is defined as a 'polysomnography' (PSG). A PSG is conducted after a referral from a general practitioner to assess sleep architecture and physiology in an individual suspected to have sleep problems such as sleep breathing disorders (SBD) or parasomnias. There are several measurements conducted in a PSG: sleep EEG, EOG, EMG, ECG and respiratory measures. The equipment and measures used in this study are outlined in **Chapter 5**.

The pilot study showed that PSG could be used in the homes of young people to assess their sleep. The high-density Geodesic net was adopted as the best technique for acquiring neurophysiology data in addition to assessing cardiorespiratory measures. These methods of assessment provide a comprehensive measurement of the different physiological changes throughout the sleep period. This maximises the possibility for interpretation of sleep physiology in young people with 22q11.2DS and the sibling control group.

7.1.1 Recognising Waveforms of Sleep

To characterise the neurophysiology of sleep in young people with 22q11.2DS and compare this to unaffected sibling controls, normative brain physiology during sleep needs to be understood. Typical sleep physiology is outlined in **Chapter 2** and studies including the

acquisition of sleep EEG, in the typically developing population and within the neurodevelopmental phenotype, are reviewed. However, to be able to assess and analyse the data acquired from a sleep EEG, markers need to be identified in the EEG trace. This allows for staging of sleep and further analysis. The different types of waveforms and architecture of sleep is discussed in **Chapter 2**. The AASM guidelines (Berry *et al.*, 2016) used to score sleep EEG are dependent on these waveforms.

The physiology of the brain changes throughout the night with stages of sleep transitioning in a 90-minute cycle. Changes in neurophysiology and categorisation of sleep stages was initially developed by Allan Rechtshaffen and Anthony Kales (Rechtschaffen and Kales, 1968) who produced the first staging criteria in 1968. Scoring criteria for sleep physiology have been developed into a systematic assessment that is re-evaluated each year to ensure consensus within the sleep research community. As a result, guidelines are uniformly adopted by sleep researchers to score and assess PSG physiology. The American Academy of Sleep Medicine (AASM) unified these guidelines and it is important to note that 'The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications, Version 2.3' (Berry *et al.*, 2016) was used to acquire and interpret the data (see **section 7.3.2**) and analyse data outputs outlined in **section 7.3.3**.

Across a lifetime, sleep neurophysiology changes with neonates often spending around 50% of their time in NREM and 50% in REM sleep, with 50-minute sleep cycles (see **section 2.3**). Physiology stabilises in the first year of life showing increasing similarities in activity and cycle-length with adolescents and adults. In adults, on average 18-25% of a night' sleep is spent in REM with a full cycle around 90-minutes.

The following section will briefly highlight the fundamental aspects of each sleep stage that are needed for the interpretation of the results in this chapter. It is important to note in this thesis that the physiological markers in the EEG are not analysed. Instead, the sleep physiology is assessed more generally and can provide the foundations to further exploring sleep physiology in the future.

7.1.1.1 Wake

Figure 11 shows a characteristic wake EEG signal: the wake brain exhibits fast and low desynchronised voltage activity. There is characteristic alpha activity (8-14Hz) that localises to the occipital cortex and is present in quietened and relaxed wakefulness (Blume, 2006). When eyes are shut, alpha dominates and can continue accompanied by slow eye movements when transitioning into sleep and stage 1.

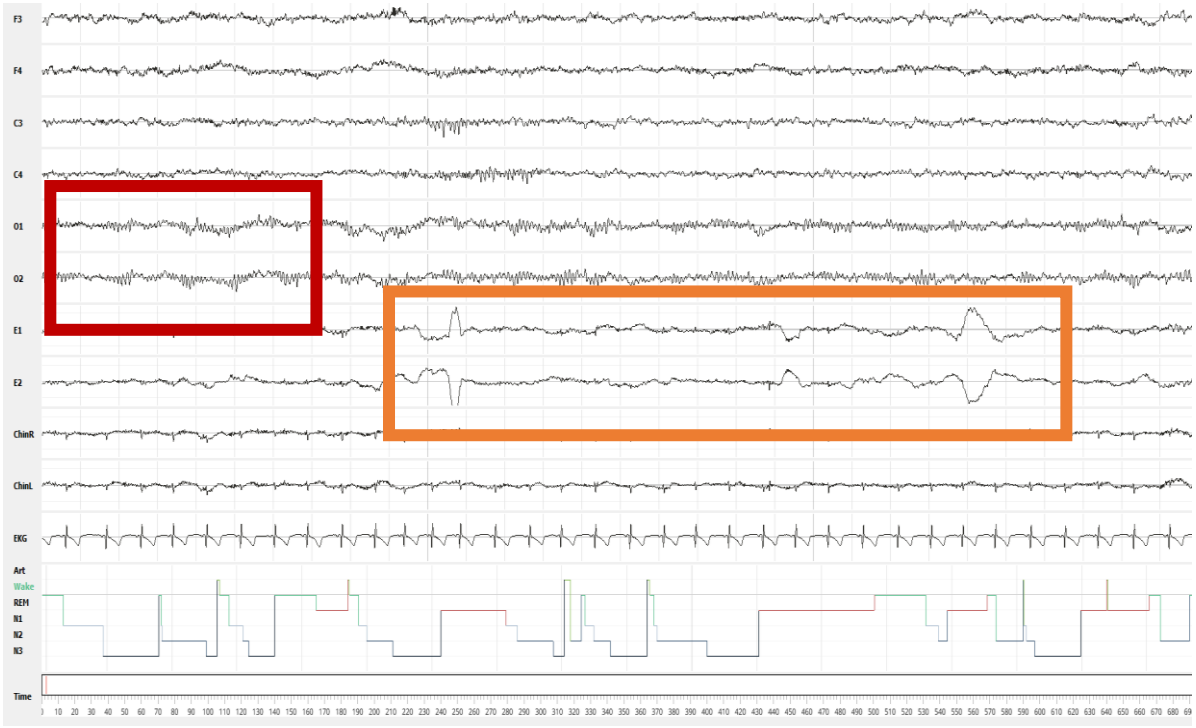


Figure 11 A 30-second epoch illustrating wakefulness. The red box shows alpha activity whereas the orange box shows slow eye movements. These are 30-second epochs from my own acquired data.

Identifying wake is important in an EEG trace as nocturnal awakenings can occur through the night. Periods of wakefulness can be scored if there is 10 seconds of normal EEG followed by an arousal into 15 seconds of continuous alpha activity. An arousal is an abrupt shift in the EEG frequency in sleep stages NREM Stage 1, 2 or 3 or REM sleep. The frequency change must be greater than 16 Hz and includes alpha and theta (4-8 Hz) frequencies but not sleep spindles. The shift must last for a minimum of three seconds and 10 seconds of stable sleep frequencies must precede the shift. In REM specifically, an arousal can only be scored if there is a concurrent change in the EMG showing that the muscle tone has increased for a minimum of 1 second (Berry *et al.*, 2012).

Brain activity is variable amongst individuals, but most brain activity will present like a standardised model of each stage of sleep. However, there are some individuals (around 10% of people) who do not generate alpha rhythm and as a result it is much harder to identify the restful wakefulness and transition into stage 1. As a result, other waveforms and characteristics need to be identified to recognise stage 1 such as theta activity, vertex sharp waves and slow eye movements (Berry *et al.*, 2016).

7.1.1.2 NREM - Stage 1

NREM Stage 1 is scored by observing low voltage activity, with dominant low-amplitude mixed frequency (LAMF) waveforms (highlighted by the orange box in **Figure 12**) some slow eye movements and a transition from alpha activity into theta frequencies (4-8 Hz) (Biswal *et al.*, 2010).

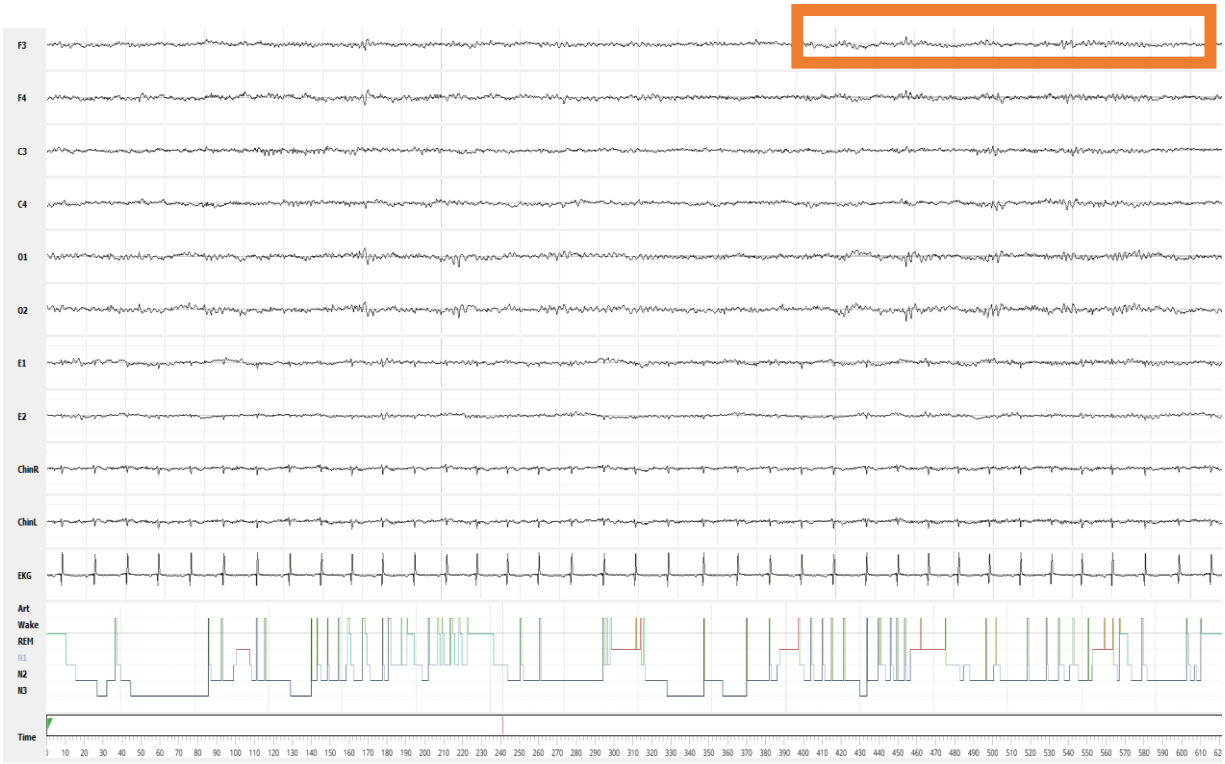


Figure 12 A 30-second epoch showing stage 1 sleep. There is evidence of low-amplitude mixed frequency waveforms throughout. These are 30-second epochs from my own acquired data.

7.1.1.3 NREM - Stage 2

Stage 2 of NREM sleep can be characterised by the dominance of theta waveforms (4-8 Hz) and cortical signatures of patterned network activity called 'K-complexes' and 'sleep spindles' (see **Chapter 2** for definitions). **Figure 13** demonstrates the architecture that needs to be detected to score a 30-second epoch of sleep as stage 2: the star denotes the peak of a K-complex (contributing to the frequency band ranges 0.5-0.9 Hz and 1-4 Hz) and arrows show sleep spindles (11-16 Hz, most commonly 12-14 Hz) in stage 2 of the sleep EEG.

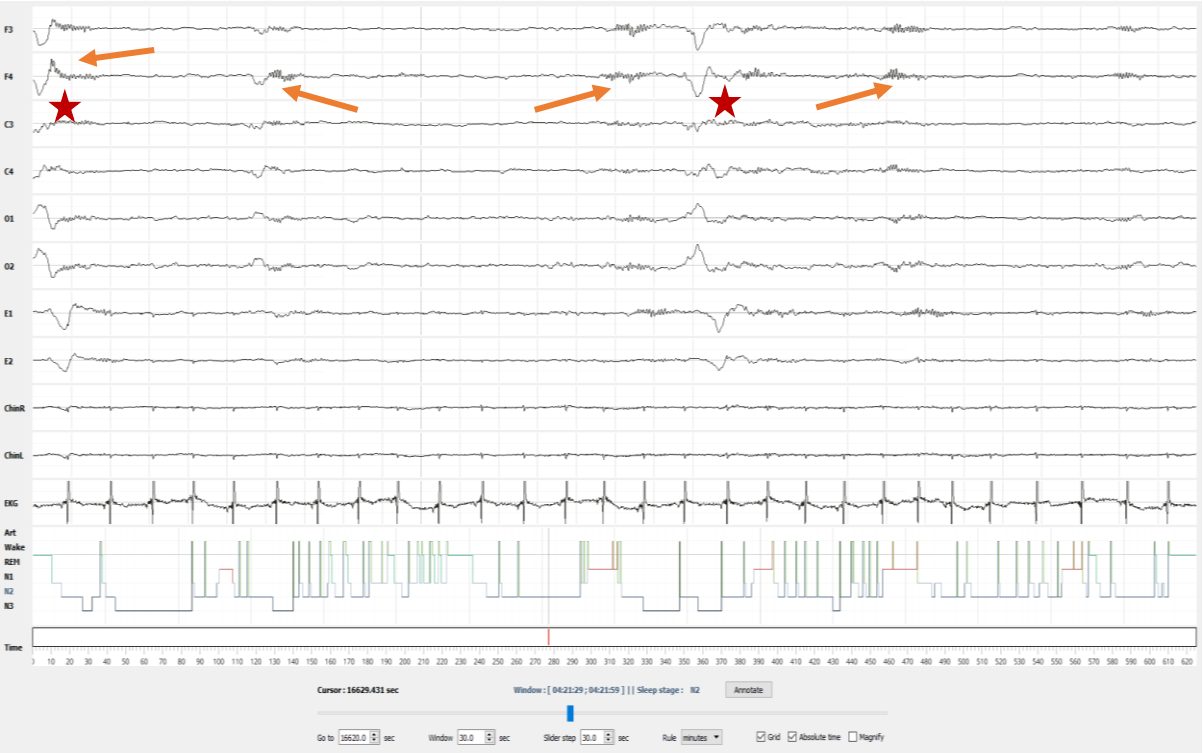


Figure 13 A 30-second epoch showing stage 2 sleep EEG. The orange arrows show examples of polyphasic spindling (sleep spindles) and the red stars show examples of K-complexes which are shown here to be coupled with the spindles. These are 30-second epochs from my own acquired data.

K-complexes that are connected to an arousal effects the interpretation of the sleep stage. If an arousal is scored in the first 15 seconds of stage 2, and no other arousal occurs during the next 30 seconds, the next epoch will be scored as stage 1. The occurrence of the next K-complex that is not associated with an arousal allows for NREM Stage 2 to be scored again. K-complexes and sleep spindles are important physiological features which have been and continue to be explored in conjunction with psychiatric disorders (see **section 2.4** and **2.5**).

7.1.1.4 NREM - Stage 3

Stage 3 is characterised by slow-wave activity (SWA) which is inclusive of slow-waves (0.5-2 Hz) and delta activity (1-4 Hz). Evidence of K-complexes in NREM Stage 3 has suggested that a K-complex could be an isolated slow-wave or down-state (Cash *et al.*, 2009). This is due to their frequency band range contributing to both slow-wave and delta frequencies. The

presence of K-complexes is considered an indicator of a cortico-thalamic processing mode (Cash *et al.*, 2009).

In NREM Stage 3, the general physiological activity observed can be defined as slow-wave activity (SWA) which encapsulates the 0.5-4 Hz frequency band (outlined by the orange box in **Figure 14**) SWA can be measured locally at the frontal regions of the brain by EEG and is generally modulated in the neocortex (Achermann *et al.*, 1993). Slow-wave oscillations derive primarily from the cortex however delta waveforms are generated deeper in the mid-brain from the thalamus, showing that SWA does not originate from one isolated brain region (Esser, Hill and Tononi, 2007).

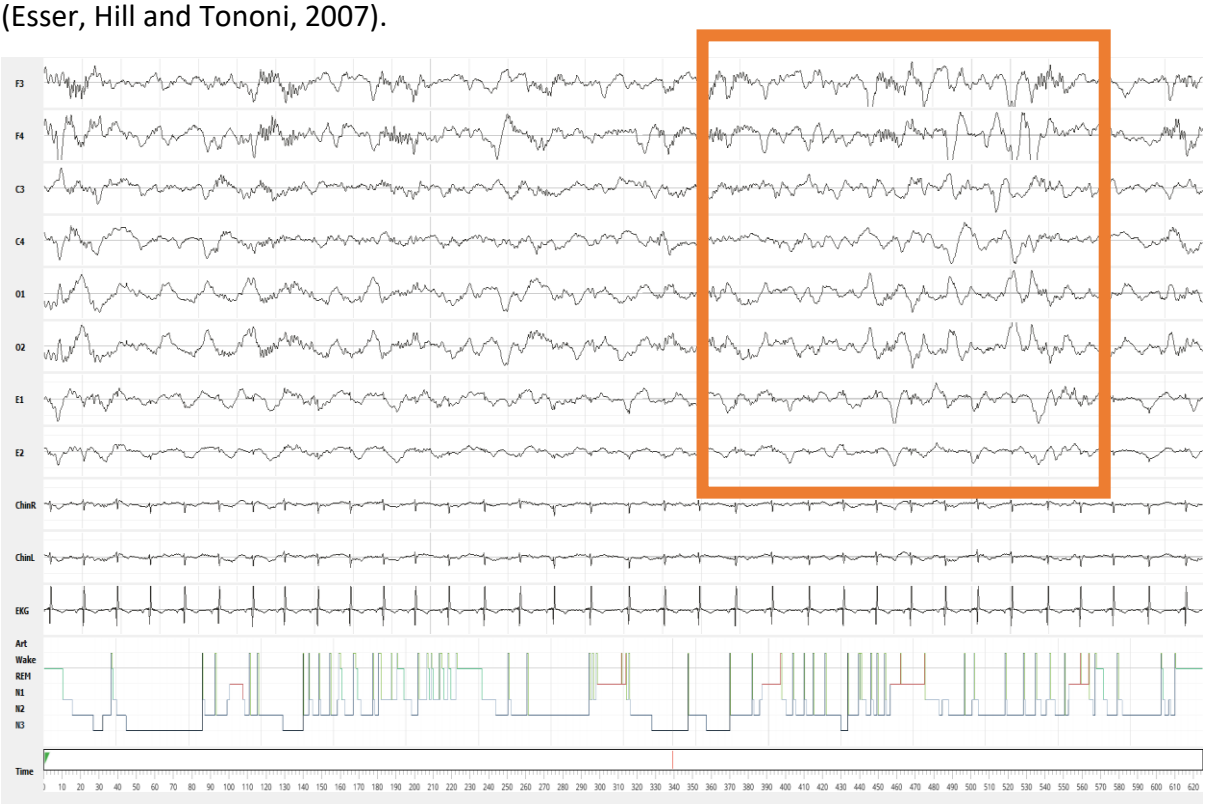


Figure 14 A 30-second epoch of NREM Stage 3 sleep. The orange box illustrates SWA. These are 30-second epochs from my own acquired data.

To score a 30-second epoch of sleep EEG as NREM Stage 3, brain activity must show 20% or more SWA in those 30 seconds. A healthy individual will spend around 10-25% of their total sleep time in slow wave sleep (SWS) (Ohayon *et al.*, 2010). SWA is dominant in stage 3 and

used to characterise this stage, however SWA is detectable through NREM and the 'deepness' of sleep can be characterised by the density of SWA: the deeper the NREM sleep, the more SWA is present and the fewer spindles present in the trace (Dijk and Archer, 2009). Throughout the 90-minute sleep cycle, the frequencies of waveforms present can reflect the deepness of sleep: the deepest sleep is NREM Stage 3 showing the lowest frequency band range of waveforms and the highest frequency, other than wakefulness, is identifiable in stage 1 and REM sleep.

7.1.1.5 REM sleep

REM sleep is characterised by rapid eye movements (outlined by the orange box in **Figure 15**) and saw-tooth waves (outlined by the red box in **Figure 15)**. They present as sharply contoured trains of waves which often look serrated and occur at a frequency band range of 2-6 Hz. Rapid eye movements can be derived from electrooculography (EOG) in which eye movements are bi-laterally measured by electrodes positioned close to the eyes (Berry *et al.*, 2016).

During REM sleep muscle atonia happens (loss of muscle tone) which is often likened to paralysis. This lack of muscle movement is required to confirm the presence of an epoch of REM sleep. Low muscle tone is assessed using electromyography (EMG) where either two or three electrodes are placed under the chin.

Figure 15 30-second epoch showing an example of rapid eye movement (REM) sleep. The orange box shows rapid eye movements and muscle atonia, and the red box illustrates saw-tooth waves. These are 30-second epochs from my own acquired data.

7.2 Rationale

There are currently no studies that have comprehensively described subjective or objective features of sleep in 22q11.2DS. There is scant literature that refers to the rates of sleep-related breathing disorders (SRBD) especially obstructive sleep apnoea (OSA) in 22q11.2DS (Heike *et al.*, 2007). These studies, which are predominantly based on clinical samples, have identified around 10% of individuals with 22q11.2DS have OSA, showing a higher preponderance than the typical young person population (O'Brien *et al.*, 2004).

However, there is evidence to suggest that there is a prevalence of up to 5% (on average) of young people in the age range investigated, with reported OSA or sleep-related breathing

problems (Capdevila *et al.*, 2008). This suggests that despite there being a higher prevalence in the 22q11.2DS young people, evidence of OSA across the study sample might not be completely unusual. Nonetheless, there have been no research studies that include subjective or objective measures of sleep in 22q11.2DS outside of case studies or clinical populations. However, there have been clinical cases of OSA or SRBD in individuals with 22q11.2DS which results into poorer sleep quality, reduced quantity, disruption to the sleep architecture and impairment of daytime cognition (Certal *et al.*, 2012). In the young people with 22q11.2DS, cognitive impairment especially is reported in the literature and supported by the findings in **Chapter 4.** Therefore, in the investigation outlined in this chapter, it is important to include objective assessment of sleep-breathing in addition to the electrophysiology to try and capture all potentially sleep-related effects in young people with 22q11.2DS.

Chapter 4 outlines these subjective parent-reported sleep problems showing that there is a higher prevalence of sleep problems in young people with 22q11.2DS compared to the typically developing sibling group. Furthermore, **Chapter 6** demonstrates differences in the objective actigraphy-derived sleep parameters showing that young people with 22q11.2DS spend longer in bed, a longer time asleep and have a higher sleep efficiency than the control sibling group. These current findings suggest differences in the sleep patterns and behaviours of young people with 22q11.2DS compared to typically developing young people.

The literature outlined in **Chapter 2** shows abnormal sleep physiology in individuals with NDDs and psychiatric outcomes that commonly present in 22q11.2DS. Individuals with 22q11.2DS are at a high biological risk for the development of SCZ and have elevated prevalence of ADHD, ASD and anxiety symptoms in addition to cognitive impairment within the neurodevelopmental phenotype. As a result, it was hypothesised that there could be

evidence of spindle and slow wave abnormalities during sleep. Evidence in the literature suggests that spindles might be an endophenotype of SCZ (Manoach *et al.*, 2016) and therefore, studying sleep physiology in a genetically high-risk population can add to the understanding of aberrant sleep physiology and the neurodevelopmental phenotype (see **Chapters 1** and **2**). Abnormalities in sleep physiology in NDDs also contribute to the rationale behind exploring sleep in 22q11.2DS (see **Chapters 1** and **2**). The unaffected siblings were a control sample that could be used to compare brain neurophysiology directly.

The overnight sleep EEG study was developed to identify the baseline sleep neurophysiology and from here, investigate subjective measures of sleep in young people with 22q11.2DS.

7.2.1 Aims of the EEG study

In **Chapter 2**, the third aim described exploring the sleep phenotype further in 22q11.2DS by conducting a sleep study in comparison to their unaffected sibling controls. The sleep study adopted the following three aims to address the main thesis aims:

1. to score the stages of sleep from the sleep EEGs and remove the artefacts from the sleep EEGs in young people with 22q11.2DS compared to their unaffected sibling controls;
2. to compare the objective PSG-derived sleep architecture between young people with 22q11.2DS and their unaffected control siblings;
3. to investigate the objective PSG-derived sleep architecture in relation to the neurodevelopmental phenotype in young people with 22q11.2DS

To achieve this, the full PSG with high density EEG 64-channel net was conducted in collaboration with another PhD student. The additional measures were used to gain a comprehensive understanding of the sleep phenotype including actigraphy watches with a

complementing sleep diary. My work aimed to derive objective assessments of sleep from these measures.

The sleep EEG assessments was based on the following hypotheses:

1. it was hypothesised that sleep problems would have an underlying physiological basis in 22q11.2DS;
2. it was hypothesised that individuals with 22q11.2DS will show more disturbed sleep compared to their siblings, shown by the objective sleep architecture such as reduced total sleep time;
3. objective measures of sleep in individuals with 22q11.2DS will show associations with the neurodevelopmental phenotype, but to what extent is unknown.

The scant literature regarding sleep in 22q11.2DS centres on SRBD and OSA. As part of the overnight sleep study, sleep-related breathing problems were measured however not analysed. Having a physiological signal could mean that potential sleep-related breathing problems could be accounted for to prevent confounding of results. Initially, a respiratory clinician was available to interpret any respiratory problems identified. However, unfortunately the clinician did not continue involvement in the study. The breathing traces were not analysed themselves. Instead they were used as a reference trace when analysing the EEG. Therefore, there is not adequate evidence to state that there was no evidence of OSA or SRBD in this sample of young people. Future investigation requires the assessment of the cardiorespiratory signals.

7.3 Methodology

The methods used in the main study were developed from the pilot study. **Chapter 5** outlines the basic EEG protocol. In this section, the protocol for acquisition of the data is outlined in

more detail identifying how the protocol evolved from the pilot study. Additionally, pre-processing of the EEG data will be addressed with reference to the pipeline used and the protocol for sleep EEG scoring and staging of sleep as well as the technique used to identify and remove the artefacts from the processed data. The cardiorespiratory data acquisition is outlined in this section. This was not analysed independently but used for interpretation of the EEG. This has been mentioned in the prior section.

7.3.1 Sample and Demography

Participants were part of the ongoing Experiences of CHildren with cOpy number variants (ECHO) study (see **Chapter 3, section 3.1, and Appendices 1** and **2**) (Niarchou *et al.*, 2014; Chawner *et al.*, 2017; Cunningham *et al.*, 2017). Recruitment for the ECHO Study can be found in **Chapter 4** and the recruitment process for the sleep study can be found in **section 6.4, Chapter 6.**

After pre-processing of the sleep EEG data and quality control (see **section 7.3.3**), the sample size reduced to n=28 young people with 22q11.2DS ($\bar{x}$ age=14.6, range = 6 years, ~8 months–20 years, ~5 months, s.d.=3.44, 50% females) (59.6% of the original sample) and n=17 age-matched unaffected sibling controls ($\bar{x}$ age=13.7, range=7 years, ~9 months–18 years, ~11 months, s.d.=3.36, 58.8% females) (77.3% of the original sample). This was due to the age of participants and the availability of overnight assessments with young people. For example, weekends and school holidays were the only available times. Family income information and highest parental education was obtained from primary carer questionnaires and can be found together with information of the age and gender of the participants in **Table 20**.

Table 20 Demographics of sleep EEG cohort

			%
Family ethnic background			
	Mixed		3.57
	British		92.9
	Other		3.57
Highest maternal education qualification			
	No qualifications		6.90
	Low: O-levels or GCSEs		24.1
	Middle: A-levels/Highers or vocational training		31.0
	High: University degree and/or other higher postgraduate qualification		34.5
	Other		3.45
	Unknown		0.00
Family income per annum (£)			
	£≤19,999		3.45
	£20,000-39,000		31.0
	£40,000-59,000		31.0
	£≥60,000		27.5
	Unknown		6.90
Age, years: mean (s.d.)			
Probands	14.6 (3.44)	*Siblings*	13.7 (3.36)
Gender, *n* (%)			
Probands	14 F (50)	*Siblings*	10 F (58.8)

In the 22q11.2DS cohort, n=12 (42.9%) of young people were on medication and n=4 of these young people were taking melatonin (14.3%) (see **Table 21**). There were n=3 sibling controls that were on medication. **Table 21** shows the number of individuals that were on medication at the time of assessment.

Table 21 Current medication taken by the individuals

Current medication for treatment of:	***Number of young people with 22q11.2DS***	***Number of siblings***
Hypothyroidism	2	1
Sleep problems	4	0
Gastrointestinal	4	1
Asthma	4	1
Supplements	1	1
ADHD	1	0
Cardiac problems	1	0
Migraine	0	1
Hormonal	1	0
Antibiotics	2	0
Hypocalcaemia	1	0
Mood	1	0

7.3.1.1 Study Logistics and Preparation

The study often required travel to the participant's home, which could be anywhere in the UK. **Figure 16** shows a map of the UK with markers denoting the locations of the sleep studies. **Figure 16** demonstrates the vast distances travelled across the UK to acquire the data for the sleep study.

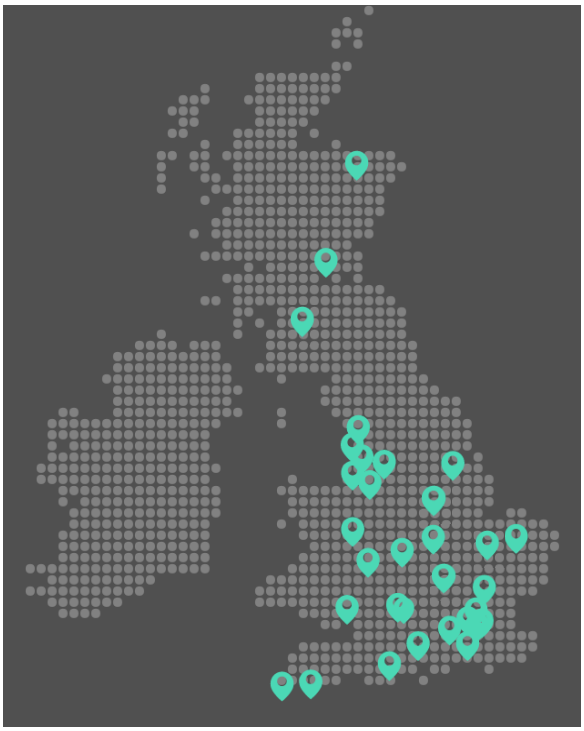


Figure 16 Map of the UK with pins of study locations.

The evening visit consisted of explaining the study and different pieces of equipment to the participant/s and primary carer such as the actigraphy watch (see **Chapter 6**), sleep diary (see **Chapter 6**) and the EEG net. Note, the sleep questionnaires had been sent to the family prior to the visit (see **Chapter 6**).

The methods used in the main study were outlined in a Case Report Form (CRF) for each participant. This document was produced for each study visit for each participant and a CRF for the primary carer was also produced. The CRF was divided into four sections: Visit Part A, evening activities; Visit Part B, morning activities; Visit Part C, midday battery change; Visit Part D, evening activities and Cardiff Activities.

The main CRF included a checklist of the equipment that was packed for every visit before leaving Cardiff. The Visit Part A section included a pre-set-up checklist and steps to ensure that all the pre-sleep activities had been completed accurately such as tick-boxes for each sensor that was applied and the equipment required for the bedroom setup.

Part B of the CRF included a section where events during the night could be recorded and ensured that any abnormalities during the night were recorded such as loose electrodes.

Parts C and the first section of Part D referred to part of the study that is not presented by my work but instead by Chris Eaton who was a PhD student on the project exploring seizure-related activity in 22q11.2DS. We both completed data acquisition for the full study protocol. Visit D included post-visit evaluations, a clean-up checklist, consumables re-stocking and the checklist of equipment to minimise loss of equipment in family's homes.

This document was developed during the pilot study and was modified throughout the pilot. This meant that by the end of the pilot, we had a comprehensive CRF with all the required information for the main study. Including sections for comments and notes was something we developed during the main study to ensure that all information regarding the participant and the family could be recorded during the study. It was a comprehensive document which ensures that the same protocol was conducted during each visit.

7.3.2 Polysomnography Assessments

The PSG includes the preparation of electrooculography (EOG), electromyography (EMG), electrocardiography (ECG), sleep EEG, pulse oximetry, respiratory inductance belts (RIP) and a nasal cannula. The EOG, EMG, pulse oximetry, RIP belts and nasal cannula were connected to an Embla Titanium, PSG ambulatory amplifier (Natus Neurology Supplies, Oxford, UK). The sleep EEG was acquired by a 64-channel high-density Geodesic net that was connected to an EGI EEG amplifier (Philips Company, 2018). The ECG was recorded by both amplifiers: one pair of electrodes was plugged into each amplifier. The ECG recordings were used to synchronise the two amplifiers and their outputs to allow interpretation of all measures together.

7.3.2.1 Electrooculography (EOG) and Chin Electromyography (EMG)

Surface electrodes were used to measure eye and muscle movements (see **Chapter 5** for explanation) (*Ambu® Neuroline 720*, 2018). These electrodes contain conductive gel and self-adhesive allowing for them to be easily applied directly to the skin and are relatively comfortable. The conductive gel enables low impedances and optimal signal quality. The connector at the terminus of the electrode was compatible with the inputs on the Embla titanium.

A preset setting was created for the Embla titanium that assigned inputs for the EMG and EOG to ensure consistency of measurements. The EOG was measured at a 128 Hz sampling rate with a low-frequency filter of 0.3 Hz and a high-frequency filter of 35 Hz; same as the EEG. The EMG was measured at a low-frequency filter of 10 Hz and a high-frequency filter of 100 Hz.

As outlined in **Chapter 5**, two EMG electrodes were positioned under the mandible lateral to the midpoint of the chin and two EOG electrodes were used, one positioned by the left eye and one by the right eye. These measures are essential in overnight sleep studies to allow accurate scoring of sleep stages throughout the night (see **section 7.3.3.1**).

7.3.2.2 Breathing and Cardiac measures

The measures used in the pilot study were continued in the main study (see **Chapter 5**). The pulse oximeter was used to measure the oxygen saturation of the haemoglobin in the blood. The pulse oximeter in the study was placed on a finger on the participant's non-dominant hand. A beam of infrared light passes through the finger and the blood, detecting the oxygen saturation. This measured by detecting the changes of light absorption in oxygenated blood. The pulse oximeter also provided the heart rate of the individual. This is required to measure

oxygen deprivation due to apnoeas or hypopneas occurring through the night. In a clinical setting, this would allow for the scoring of these events throughout the night also.

Electrocardiography (ECG)

The participants wore two pairs of ECG electrodes to monitor their heart rates. We know that individuals with 22q11.2DS have a higher risk for cardiac abnormalities (McDonald-McGinn *et al.*, 1999) that could result in abnormalities detectable on ECG. Where this was the case, the primary carer informed us and it was noted.

The ECG was used to be able to decipher between cardiac events and neurophysiological events and proved to be essential in the matching of the different datasets. One pair of ECG electrodes was inserted into the EGI amplifier whereas the other was attached to the Embla titanium amplifier.

The participants wore four single-use ECG electrode pads (Ambu, 2018) that had a stud connection for easy application of the ECG snap electrodes) which were attached to the pads. The pads were pre-gelled like the EOG and EMG electrodes and could be directly applied to the skin. Two pads were applied to the participant's skin under their left clavicle, and the other pair applied underneath their right clavicle in proximity to the anterior auxiliary line so that ECG was detectable from the auxiliary artery. The ECG was measured at a sampling rate of 128 Hz with a low-frequency filter of 0.3 Hz and a high frequency filter of 70 Hz.

Respiratory Sensors Application

The nasal cannula was used to measure the pressure of inspiration and expiration, with an output showing the respiratory rate and transference of air. The nasal cannula was the most invasive and uncomfortable piece of equipment from the feedback of the pilot study. As a

result, there were individuals that wore the nasal cannula throughout the night; there were some who wore it initially however removed it during the night and others that did not wear the cannula. Therefore, there was a varied amount of data acquired from the nasal cannula. This itself must be acknowledged as contributing to potential underestimation of significant sleep-related breathing problems: without the nasal cannula measure, there would be insufficient data acquired to recognise changes in the respiratory pressure and airflow. The other respiratory measures, the pulse oximetry and RIP belts were tolerated better and could provide limited information regarding respiratory events during the night's sleep.

The RIP belts measure the expansion and deflation of the thoracic cavity and the abdomen during the process of breathing: expansion of the thoracic cavity and narrowing of the abdomen during inhalation and deflation of the thorax and extension of the abdomen during exhalation. The movement of the thorax and abdomen is measured by the physical expansion of copper wire that is contained within elasticated belts that sit under the participant's armpits and above their navel (see **Chapter 5** for an example). The RIP belt signals were recorded at a 0.1 Hz low-frequency filter and a 15 Hz high-frequency filter.

The respiratory measures require clinical input for the analysis of SRBD and potential incidences of OSA. The protocol used in the sleep study with the young people with 22q11.2DS and the sibling control group does not adequately record the breathing parameters required to state the prevalence of OSA or SRBD in this sample. It is important to identify the rates of OSA in these young people but it is also important to recognise that to do so, further input is required from external clinical experts and this will be required before further investigation of the sleep physiology can take place in the future.

Bio-calibrations

All signals were acquired and analysed initially by Embla REMLogic software (Natus Neurology Supplies, Oxford, UK). Embla REMLogic software is a sleep acquisition and diagnostic software which was also used to review the EOG, EMG, ECG and respiratory measures. It was used during setup, bio-calibrations and subsequent ambulatory acquisition.

Standard bio-calibrations were used to ensure that the equipment was accurately detecting the correct signal. It was important to identify that the EOG was detecting eye movements by testing movements and assessing the output on the EOG trace. This was conducted for the EMG and airflow also. To bio-calibrate the ECG, a heart rate signal is assessed on the trace and compared to a baseline ECG.

The geodesic net was not compatible with the Embla Titanium; the EGI amplifier which used the Galileo software to acquire and review the EEG signals was compatible with the net.

7.3.2.3 Sleep Electroencephalography (EEG)

The sleep EEG was acquired using a 64-channel, high-density geodesic net (Electrical Geodesics, Inc. Eugene, Oregon, USA). This 64-channel net was a relatively new piece of equipment which was designed for ease of use for EEG application and detection of brain activity derived across the cortex. EGI provided the equipment along with the EEG interface programme Galileo Suite (EBNeuro S.p.A, Florence, Italy) used to acquire the data and for monitoring and calibration of the data.

EEG Net Application

The application of the net was outlined in **Chapter 5** with images, showing the matrix of electrodes. The net was used in the acquisition of data from all participants apart from one

individual. This individual wore a continuous positive airway pressure (CPAP) mask during the night as a result of previous obstructive sleep apnoea having been identified. This meant that the net could not be used in this case as both the mask and net could not sit comfortably on the individual's head. The free silver-chloride electrodes were used for this individual with 22 electrodes measuring the brain activity of this one individual overnight. The EEG net however was used during the day for the daytime assessment. The protocol used is outlined in **Chapter 5** for the free electrodes.

The first step in the application of the net was to measure the participants head. The circumference was recorded as was the distance between the nasion (bridge of the nose) and the inion (the raised, curved part at the posterior of the cranium), with a midline on the scalp and the distance from left to right preauricular with midline on the scalp which should overlap to create a cross at the central point of the scalp. The cross demonstrates the overlap of the midline and the interaural line and where the Cz electrode on the net is aligned to.

Once these positions were measured, the appropriate net was chosen: we had three differently sized nets. A particular technique was used for applying the net: the matrix of pedestals had to be expanded using both hands to ensure the net could fit over the participant's head and sit snuggly on their scalp. The Cz central electrode was positioned in alignment with the central cross that was marked on the participant's head. The pedestals were spread across the head to ensure best fit. Straps were tightened under the chin, positioning the net correctly and keeping it in place during sleep.

Once the net was positioned, the pedestals could be filled with electro-conductive gel to decrease the impedance between the electrode and the scalp to ensure best detection of brain activity across the cortex.

There were 60 pedestals that we needed to gel. The pedestals were numbered and we used a PDF map of the net to match the number of the pedestal with the anatomical position such as pedestal 1 was FCz. Once we had matched each pedestal with the anatomical location, we could use the surface map of electrode locations to monitor the recorded signal. This map showed the impedances for each electrode whilst gelling so we could ensure that we had the best signal (impedance below 30 kΩ). We performed a separate bio-calibration for the EEG signals using the Galileo software**.** Bio-calibrations were marked in the software for later inspection.

Acquisition of Data

The Galileo Suite was used to acquire the data but the traces were transferred to REMLogic for convenience of interpretation.

The design of the equipment also meant that the EGI amplifier had to be plugged in overnight as the battery pack only lasted for eight hours of constant data acquisition. This meant that we depended on the primary carer, and/or participant to remember to plug the equipment in before they went to bed and when they were inactive such as sitting in the living room or in bed watching a film. **Figure 17** shows an example of the image that was attached to the front of the amplifier and included an instruction booklet to ensure that the amplifier was plugged in overnight. This was included in the methods of data

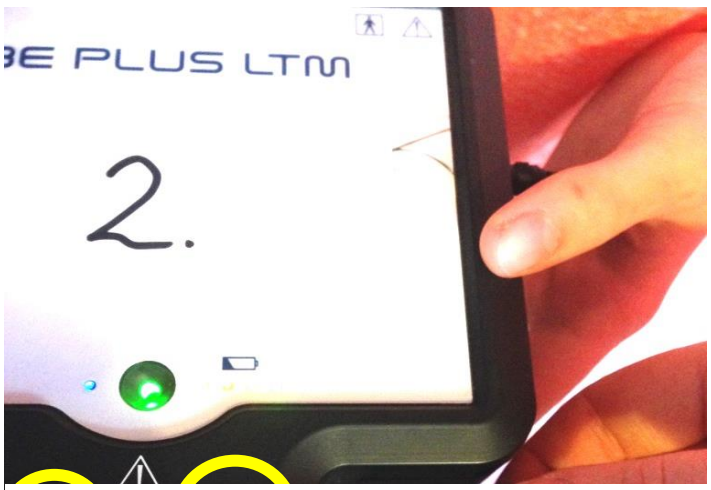


Figure 17 An example of the image stuck to the front of the EGI EEG amplifier to ensure the charging cable was inserted correctly before lights out.

acquisition as we learnt from the pilot study and early studies in the main study that the charging wire could be easily forgotten.

7.3.3 Data Analyses

7.3.3.1 Sleep EEG Interpretation

The two amplifiers (Embla titanium and EGI) were combined to generate a reduced EEG dataset from 60-channels on the EEG net. There were four channels that did not detect a physiological signal. This new reduced EEG dataset was merged with the cardiorespiratory signals measured by the Embla Titanium amplifier to generate a complete PSG dataset. However, because the EEG signals were acquired using a different amplifier, the two groups of signals had to be merged. During the setup, there were two lots of ECG: one connected to the EGI amplifier and one connected to the Embla titanium. This meant that the participants wore four individual ECG electrodes but each pair led to one of the amplifiers. When combining the signals, the ECG could be used to align the signal traces as the ECG signature would be identical. This meant that all the signals could be combined into one data file and analysed together.

Once the files were combined using MATLAB R2017a (MathWorks, 2017), *EEGLAB V14.1.2* (Delorme and Makeig, 2004) toolbox was used to generate the '.edf files' that allowed for the data to be transferred and imported into any software for further analysis.

The EEG data file required pre-processing before analyses could be conducted to ensure that the signal to noise ratio was minimised and the signals across the channels were clean. From the 60-channels that were acquired, there were six channels in the scoring set and used for sleep analyses: frontal (F3 and F4), central (C3 and C4) and occipital (O1 and O2). The odd number refers to the left hemisphere and the even, the right hemisphere of the brain.

Electroencephalography (EEG) Pre-processing PREP pipeline and Sleep Montages

For the pre-processing, MATLAB R2017a (MathWorks, 2017) was used. The raw EEG data was pre-processed by Dr Nick Donnelly at the University of Bristol using a standardised early-stage EEG processing pipeline (PREP) which is freely available from a MATLAB library on a software development platform, 'GitHub'. The plug-in for the PREP pipeline was downloaded and could be used in the *eeglab* toolbox (Delorme *et al.*, 2011). The fundamental reasons for the pre-processing were to reduce the signal-to-noise ratio and removal of unwanted artefacts in the EEG data. The PREP pipeline worked to robustly re-reference the signals to allow for the data to be standardised, retaining the cleaned data.

To summarise the PREP pipeline (quoted verbatim from (Bigdely-Shamlo *et al.*, 2015)), the pipeline aims to:

1. "*Remove line-noise without committing to a filtering strategy.*
2. *Robustly reference the signal relative to an estimate of the 'true' average reference.*
3. *Detect and interpolate bad channels relative to this reference.*
4. *Retain sufficient information to allow users to re-reference using another method of to undo interpolation of a particular channel."*

All the sleep EEGs were pre-processed meaning that there were n=47 22q11.2DS and n=22 sibling controls. During the pre-processing phase, it could be determined whether the sleep EEGs were of sufficient quality to pass quality control and therefore be analysed further. This resulted in the total output of n=28 22q11.2DS and n=17 sibling control sleep EEGs that were of sufficient signal-to-noise quality and could be robustly referenced and interpolated. Montages of the sleep EEGs were produced using markers of the 'lights off' and 'lights on'

conditions that were marked post-acquisition on each of the participants sleep EEG using the Galileo programme.

Sleep EEG Scoring – Staging Sleep

For sleep staging, Windows Python Programming Language (version 3.6.5 release) (https://docs.python.org/3.6/reference/) was used. The processed EEG data files were scored before PSG sleep parameters could be derived and analysed.

The n=45 sleep EEGs were recorded and analysed in accordance with the American Academy of Sleep Medicine (AASM) Manual for the Scoring of Sleep Events and Associated Events Version 2.3 (Berry *et al.*, 2016) using an open-source module 'Sleep' from an open-source Python software package, 'Visbrain' (Combrisson *et al.*, 2017) downloaded from GitHub. Windows Python Programming Language (version 3.6.5 release) (https://docs.python.org/3.6/reference/) was used to open the Sleep package and analyse the data.

The sleep EEGs were manually scored based on my experience gathered prior and throughout the study. Previously mentioned in **Chapter 5**, I undertook a polysomnography three-day course, assisted with the sleep staging of 10 sleep nights as part of the University of Bristol ZNF804a study in addition to practicing scoring of EEG with an electrophysiologist at the University Hospital Wales at the Heath. In addition to this, open source EEG data was available and I practiced my scoring using this data which was then reviewed by an experienced polysomnographer at the University of Bristol. Nonetheless, I recognise that I am not certified nor have expertise in the staging of sleep physiology. The staging that I completed was secondly staged by an automated sleep staging module in addition to 10% of these being scored by a colleague at the University of Bristol who had experience of conducting a clinical sleep study using two-night EEG measurements. Nonetheless, before further exploration of

the sleep physiology in the young people with 22q11.2DS, we intend to have 10% of the staged sleep EEGs to also be staged by a registered sleep physiologist.

It is important to note that there is observer variation in sleep staging: this occurs amongst different individuals with the most experienced polysomnographers aiming for 80% agreement in their sleep staging in most sleep laboratories (Schaltenbrand *et al.*, 1996). The 80% agreement was applied to the comparisons between my staging and the automated scoring script. This will also be adhered too when the registered sleep physiologist analyses a 10% selection of the EEGs before future work.

The EEG channels were measured at 254 Hz but when undergoing pre-processing were reduced to a sampling rate of 128 Hz. This is enough for measurement of the sleep channels. During acquisition of the EEG and EOG, the frequency filters were 0.3 Hz low-frequency and 35 Hz high-frequency. On analysing the sleep EEG channels, an amplitude of 150 µV was used for visualisation and 200 µV for the EOG channels. The EMG was acquired using a low-frequency filter of 10 Hz and a high-frequency of 100 Hz with a 200 µV amplitude applied to the channel during analysis. The ECG was acquired using 0.3 Hz as the low-frequency filter and 70 Hz as the high-frequency filter. An amplitude of 700 µV was used for visualisation of the ECG channel during analyses. The Respiratory Inductance Belts were measured at a 0.1 Hz low-frequency filter and a 15 Hz high-frequency filter. On using these channels during analyses to ensure that artefacts in the EEG weren't a consequence of respiratory changes, an amplitude of 250 µV was used for visualisation. The nasal pressure, if measured, was acquired with a low-frequency filter of 0.03 Hz and high-frequency filter of 100 Hz. These filter frequency bounds were recommended by the AASM guidelines and were changed before the acquisition of the data. The stages of sleep were scored based on the assessment of a 30-

second epoch of sleep EEG that was either deemed to be stage 1,2,3 of NREM, REM, wake or an artefact usually as a result of movement or a consequence of an underlying cardiorespiratory problem. The sleep EEGs were double scored. See **section 7.1.1** for figures demonstrating characteristic EEG signatures for the stages of sleep and for wake. **Figure 18** demonstrates a 30-second epoch captured from the analysis.

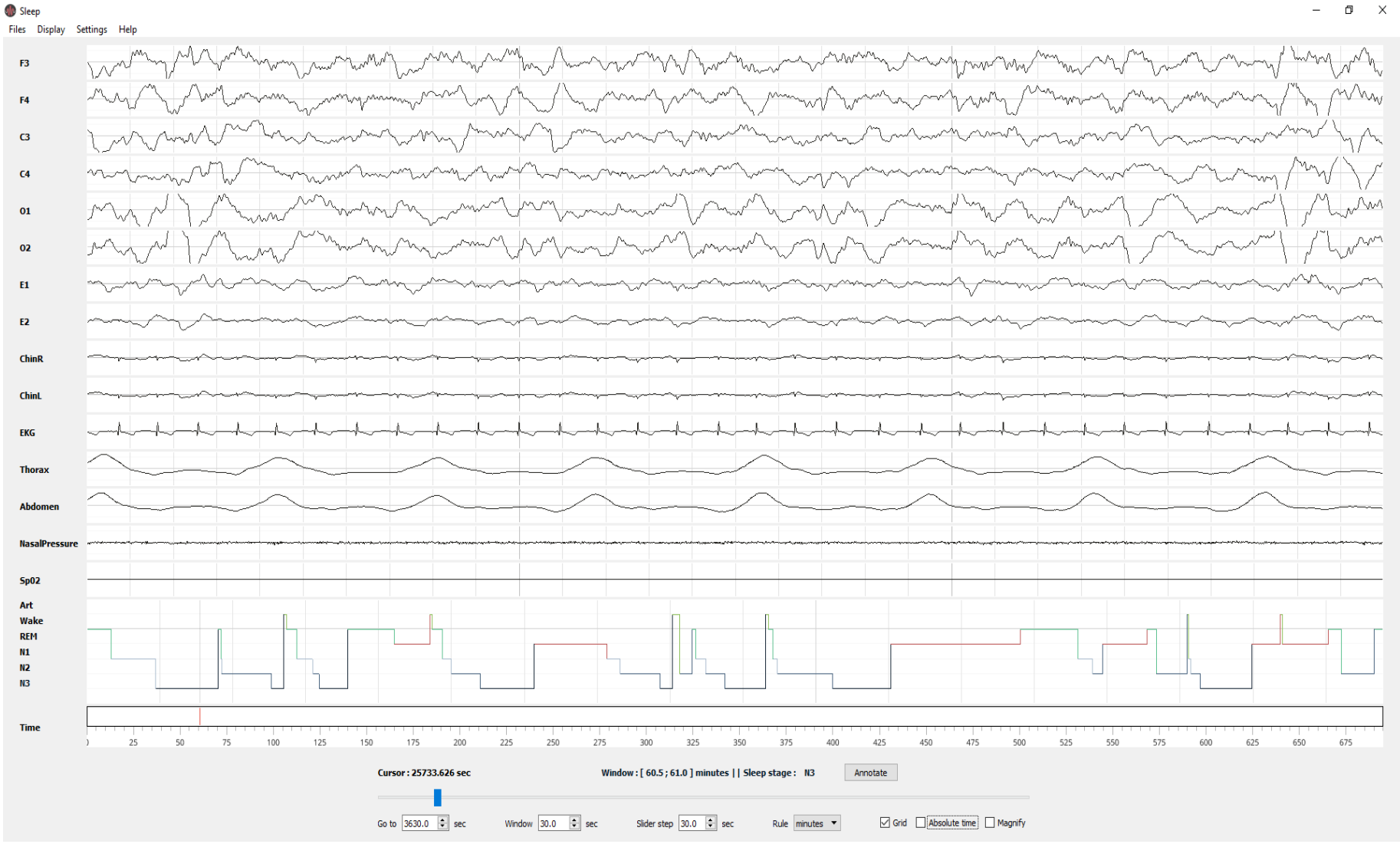


Figure 18 An example screenshot of the Visbrain - Sleep interface showing a 30-second epoch of stage 3 NREM sleep. The hypnogram is displayed at the bottom of the screen. This is a 30-second epoch of my own acquired data

Once the sleep EEGs were scored and all stages allocated, the data was cleaned once more to ensure that all areas of artefact, wake and arousals were removed before the sleep architecture parameters were derived ready for analysis.

Artefact and Wake Epoch Removal

Once the sleep EEG data files had been scored and each 30-second epoch had an allocated stage, the data was reviewed for artefact removal. The PREP pipeline removed significant artefacts that would make the EEG uninterpretable, but during the sleep scoring process, stages were identified as an artefact where there were movements shown by complete noise across the signals as well as the disruption to respiratory signals and the EMG.

The sleep EEG data files were converted into a '.set' and '.fdt' data type by co-investigator Dr Ullrich Bartsch, from the University of Bristol so that they could be opened in MATLAB R2017a (MathWorks, 2017) and analysed using the *EEGLAB V14.1.2* (Delorme and Makeig, 2004) toolbox. I cleaned the scored sleep EEG montages using the '*eeglab'* command to load the '. set' data files as existing datasets into the package. Using the 'plot' option, the six sleep electrodes (F3, F4, C3, C4, O1, O2) separated into 30-second epochs were scrolled through at a sampling rate of 128 Hz. The amplitude of the typical six sleep channels was set at 150 µV.

Each of the 30-second epochs were assessed as to whether there were any artefacts present. Take **Figure 19**, there is an evident noisy signal across all six channels suggesting that there is an artefact. However, looking at the stage of 'sleep', it is obvious that all the epochs shown in the figure are wake. Therefore, all five of these 30-second epochs would be highlighted and 'rejected', removing the wake periods and artefacts from the overall EEG signal.

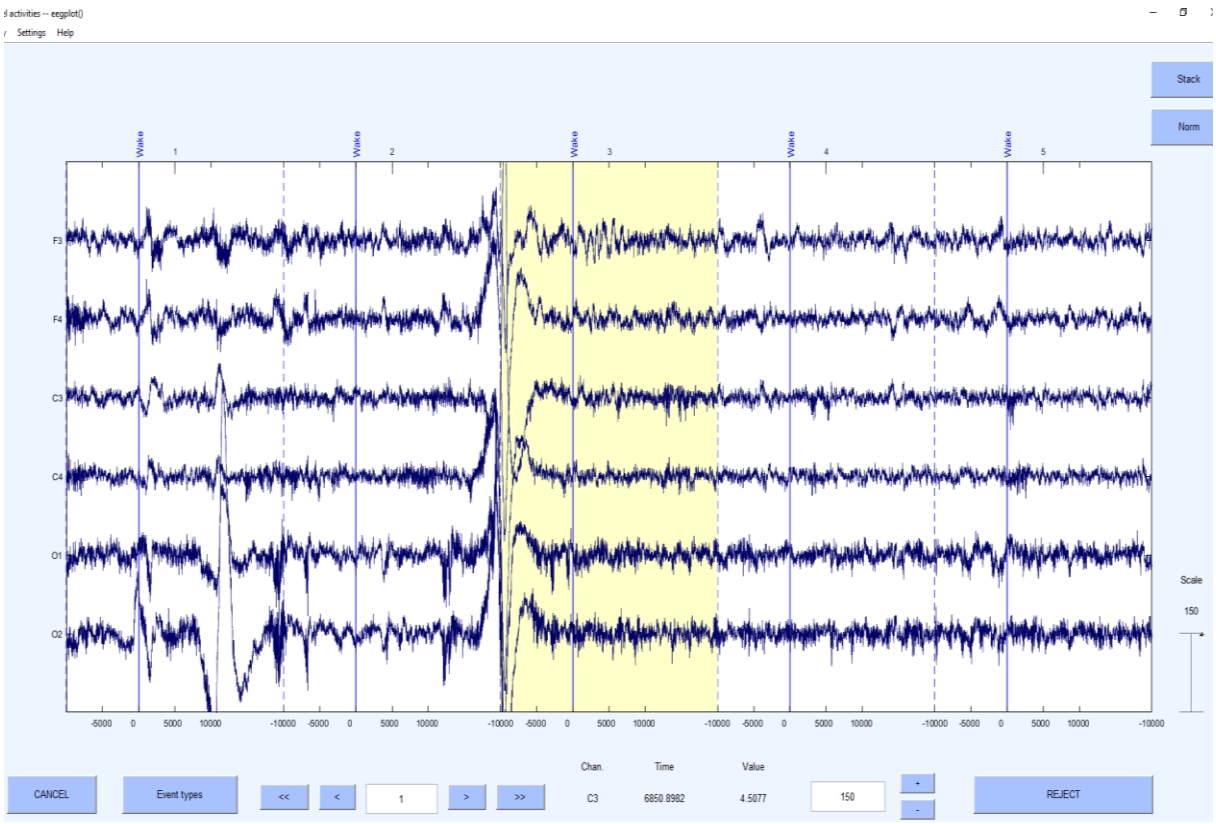


Figure 19 An example of wake EEG epochs that would be rejected from the EEG dataset before waveform analyses. The 30-second epoch highlighted in yellow shows noise across all channels showing an artefact. This is a 30-second epoch of my acquired data.

7.3.4 Statistical Analyses

The first aim of the PSG was addressed in the data analysis as this did not require statistics. To address the second and third aims multiple linear regression analyses were used to examine the differences in the PSG-derived sleep architecture between groups including covariates, and to investigate the relationship between the neurodevelopmental phenotype and the PSG-derived sleep architecture. Box-plots were generated to demonstrate differences in PSG-derived sleep parameters.

All regressions were controlled for age, gender, highest maternal education and family income which highlighted no differences between the groups.

The psychiatric and the cognitive assessments that were used to derive the symptom scores and the cognitive outcomes are outlined in **Chapter 3, sections 3.5-3.9**. These outcomes were used to explore the relationships between the PSG-derived sleep architecture after the pre-processing, scoring and cleaning of the sleep EEG data.

All statistical analyses were completed using STATA (version 13.1) (https://www.stata.com/stata-news/news28-4/stata13.1) and R (version 3.5.0) (*R: The R Project for Statistical Computing*, 2018). To correct the analyses for multiple comparisons, we used a Benjamini–Hochberg false discovery rate (FDR) rate of 5% (Benjamini and Hochberg, 1995). The Benjamini-Hochberg method ranks the individual p-values from smallest to largest, with the smallest p-value ranking as 1. By comparing each individual p-value to the Benjamini-Hochberg critical value ((rank/total number of tests) * FDR (i.e., 5%)), if the largest p-value in our analyses before correction is smaller than its critical value, it is considered significant. Any p-values smaller than the largest p-value are also interpreted to be significant.

7.4 Results

Results were derived from the sleep EEGs sampled from n=28 individuals with 22q11.2DS and n=17 sibling controls. The following section includes comparisons between 22q11.2DS and sibling controls and explores psychopathology in relation to 22q11.2DS sleep EEG measures.

7.4.1 Sleep Architecture – 22q11.2DS vs. Siblings

From the scored sleep EEG, there were several PSG-derived sleep architecture parameters for all individuals: total sleep time; time in bed; wakes after sleep onset; sleep efficiency; percentage of time spent in NREM Stage 1, 2 and 3 and percentage of time spent in REM sleep. Sleep architecture was recorded over a single night. This must be considered when

exploring the differences seen between the 22q11.2DS young people and siblings: findings are to be considered loosely and preliminary with a further, more robust study needed to be conducted.

It was important to recognise that the individuals in the study only undertook a single night of sleep EEG. As a result, there was no way of accounting for the 'first-night effect' of wearing the EEG equipment and various sensors (Agnew, Webb and Williams, 1966). These young people, especially the young people with 22q11.2DS, are regarded at elevated risk for anxiety and therefore, it would be important to realise that these young people would sleep differently during the night when their sleep was being assessed. Additionally, night to night variability is common and therefore multiple nights would be important to assess to control for this in addition to the 'first-night effects.' However, the current study could not logistically be conducted across multiple nights and there were additional confinements to the reason why two-nights could not be assessed (controlling for the 'first-night effects'). There is further information in the Strengths and Limitations on **pages 248-250.**

7.4.1.1 Hypnograms – Stages of Sleep

Hypnograms were derived showing the composition of the night's sleep for all participants: the stages of sleep and the percentage of time spent in each stage could be derived (**Figures 20** and **21**).

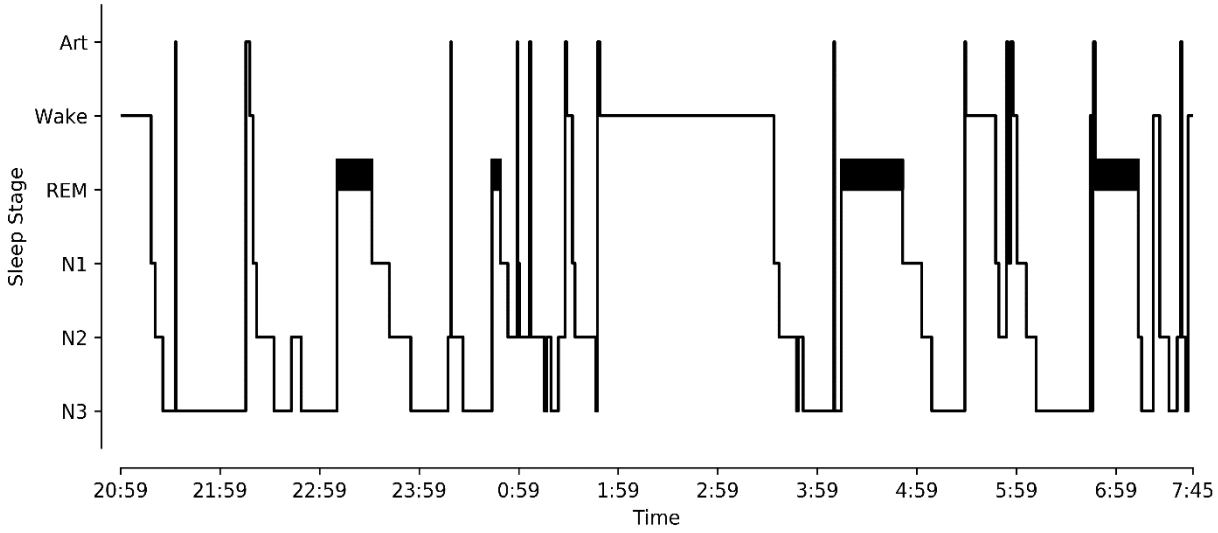


Figure 20 Hypnogram showing the stages of sleep and transitions in a young person with 22q11.2DS

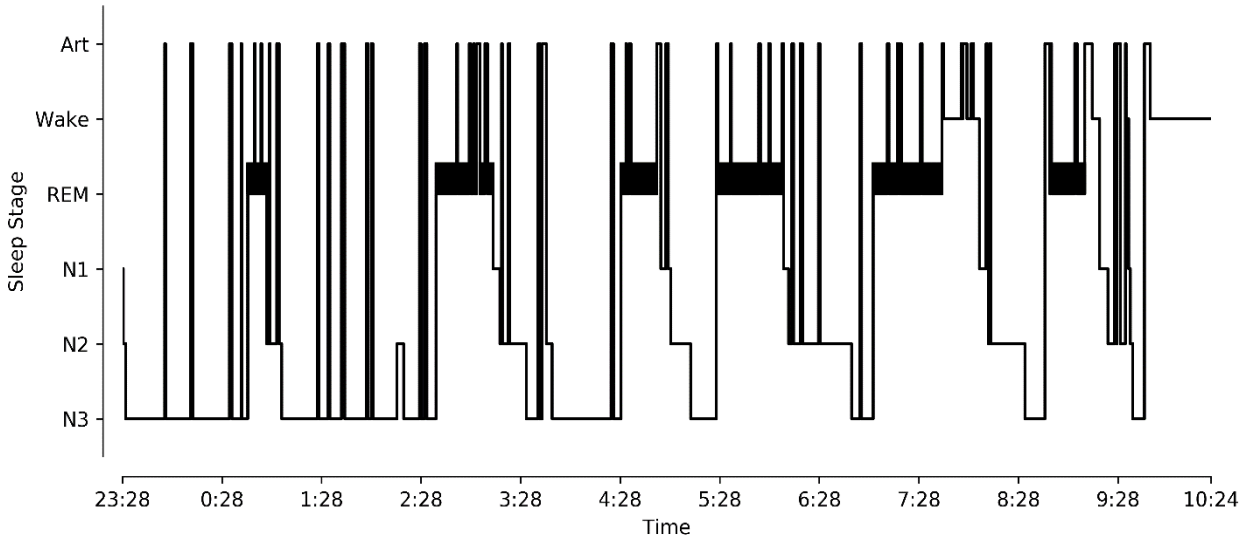


Figure 21 Hypnogram showing the stages of sleep and transitions in an unaffected sibling control

7.4.1.2 Percentage of time spent in NREM and REM sleep stages

Comparisons between the percentage of time spent in the stages by individuals with 22q11.2DS and siblings excluded the n=4 individuals with shortened sleep EEGs.

Differences in the percentage of time spent in NREM Stage 1 (B=-3.23, p=0.04) and REM sleep (B =-3.33, p=0.04) were found in individuals with 22q11.2DS compared to the sibling controls. The differences in the percentage of time spent in NREM Stage 1 and REM sleep remained even when accounting for covariates: age (B=0.219, p=0.364); gender (B=-0.771, p=0.627), and highest maternal education level (B=-0.878, p=0.260). Age showed an association with the percentage of time spent in REM (B=0.514, p=0.039) in the total cohort however was lost when accounting for group status (22q11.2DS or sibling control).

There was a non-significant trend in the time spent in NREM Stage 3 sleep (B=4.12, p=0.055) however showing young people with 22q11.2DS spent a longer percentage of time in NREM Stage 3 but there was no difference in the percentage of time spent in NREM Stage 2 sleep (B=-0.659, p=0.784) (see **Figures 22-25).**

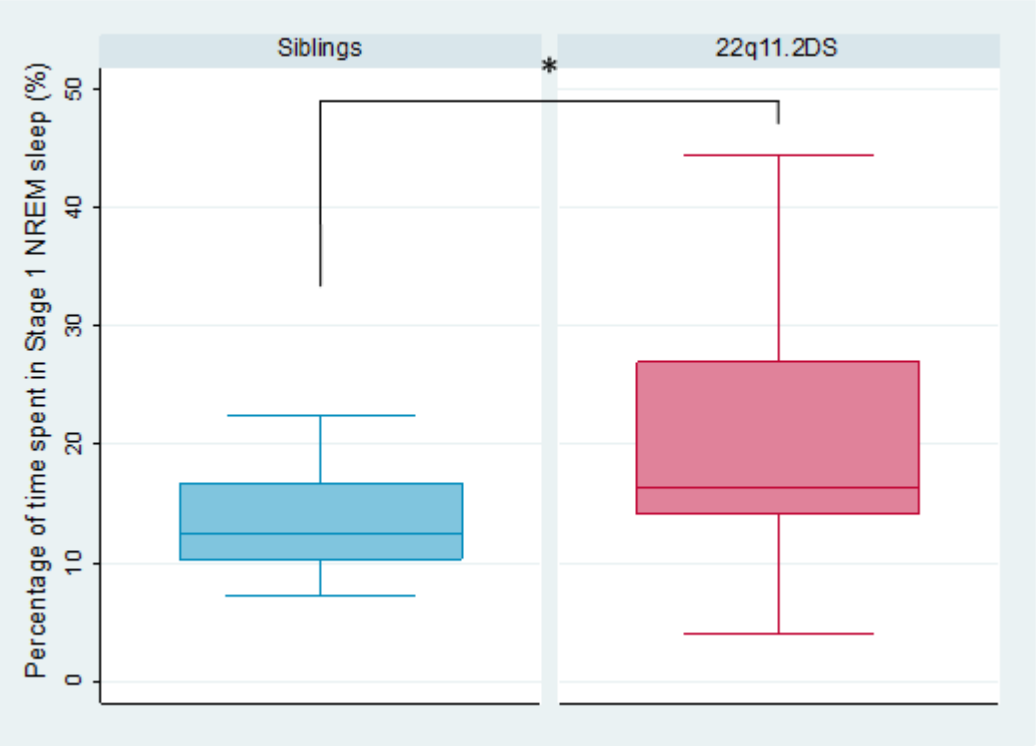


Figure 22 Boxplots of percentage of time spent in Stage 1 NREM sleep in 22q11.2DS compared to siblings

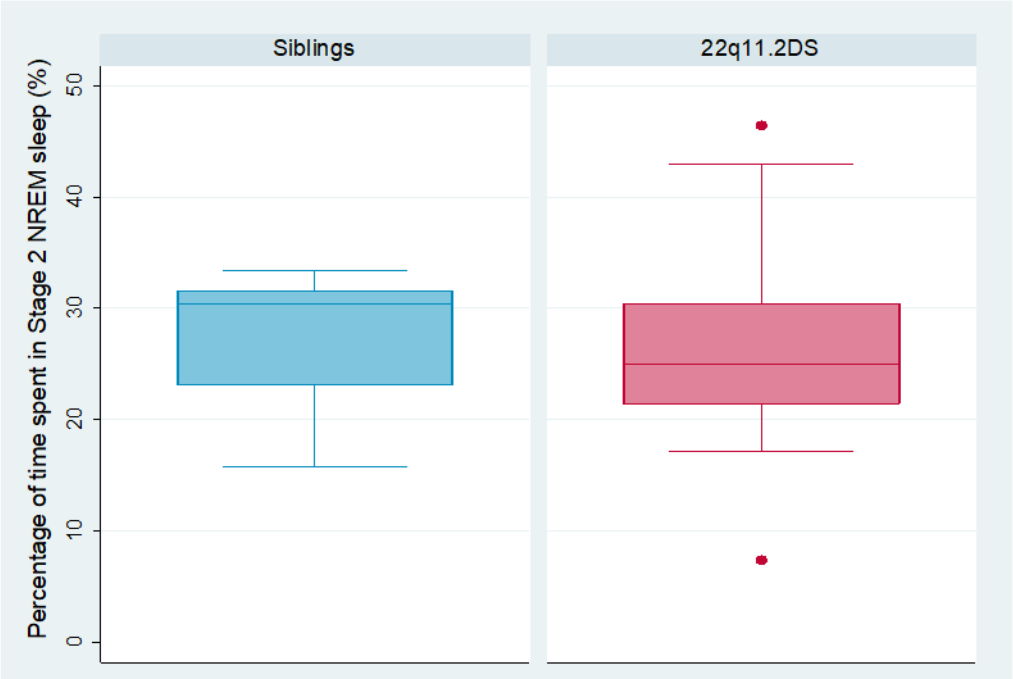


Figure 23 Boxplots of the percentage of time spent in Stage 2 NREM sleep in 22q11.2DS compared to siblings

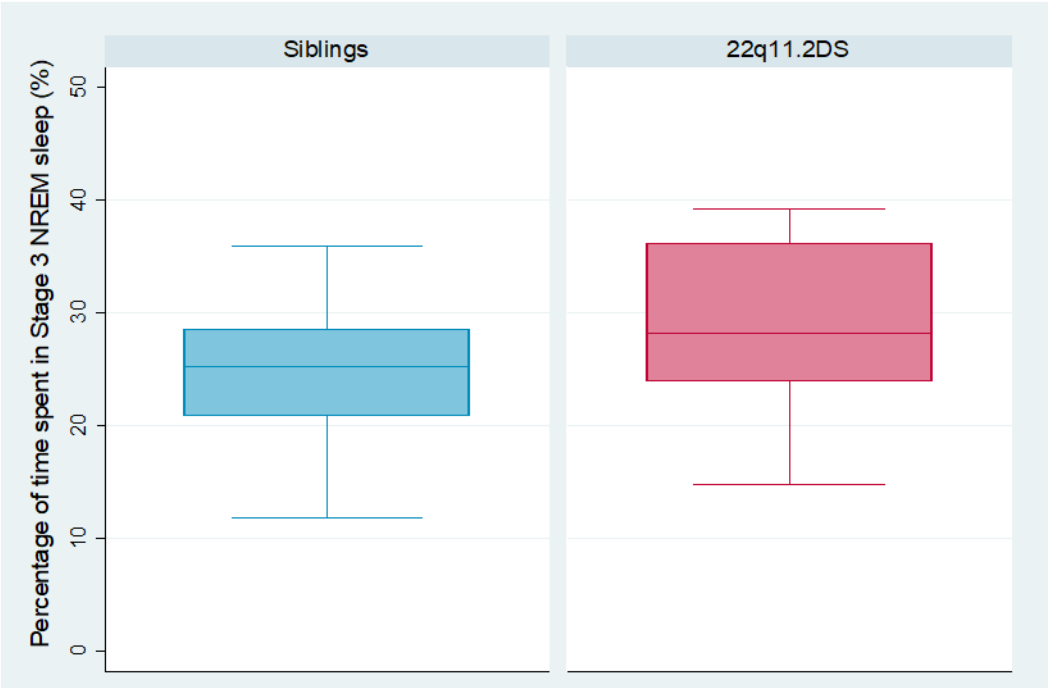


Figure 24 Boxplots of the percentage of time spent in Stage 3 NREM sleep in 22q11.2DS compared to siblings

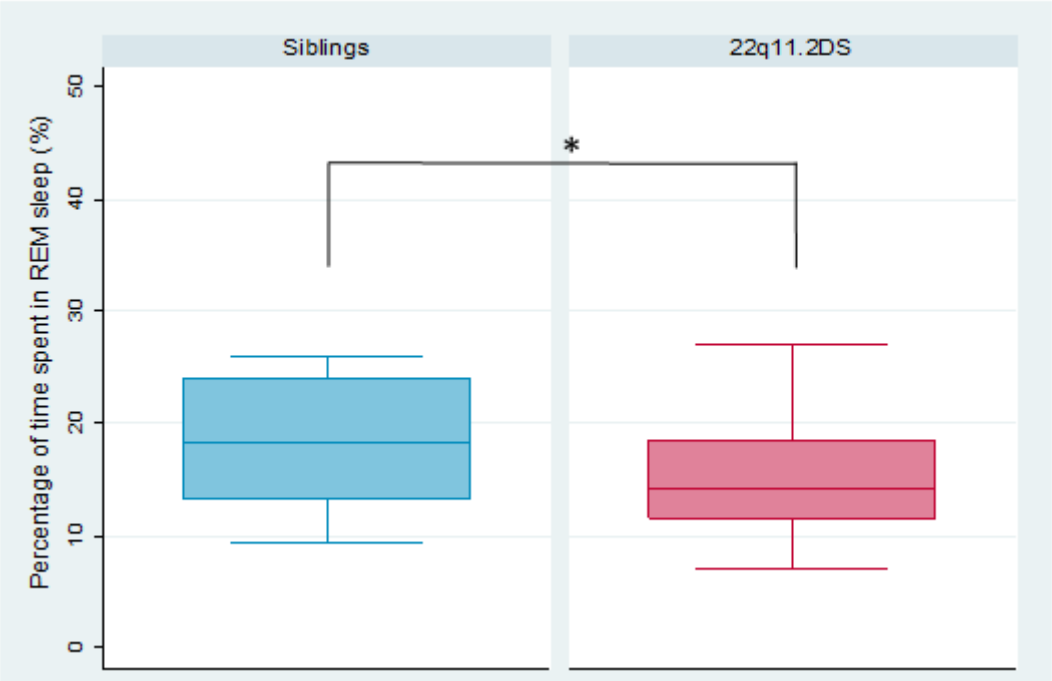


Figure 25 Boxplots of the percentage of time spent in REM sleep in 22q11.2DS compared to siblings

7.4.1.3 Other PSG-derived Sleep Architecture

The additional PSG-derived sleep architecture was compared between 22q11.2DS and siblings, showing no differences (see **Table 22**).

Table 22 No associations were found between 22q11.2DS and unaffected sibling controls

Outcome variable	***22q11.2DS (mean)***	***Siblings (mean)***	***B***	***SE***	***p***	***95%CI***
Total Sleep Time (TST) (hours)	08:12 ±01:28	08:07 ±01:20	7.31	26.7	0.785	-46.6-61.3
Time in Bed (TIB) (hours)	9:26 ±01.17	9:27 ±01:22	24.0	25.1	0.345	-26.7-74.7
Wake After Sleep Onset (WASO) (mins)	00:41 ±00:04	00:42 ±00:04	5.49	14.4	0.706	-23.7-34.7
Sleep Efficiency (%)	87.1	88.9	-1.75	2.76	0.530	-7.34-3.83

There were n=4 individuals with 22q11.2DS taking melatonin for their sleep problems. No differences between TST (p=0.188), TIB (p=0.930), WASO (p=0.097) or SE (p=0.06) in those on melatonin compared to individuals not on melatonin were found. Melatonin was therefore not incorporated as a covariate into further analyses exploring the PSG-derived sleep architecture.

7.4.1.4 Sleep Architecture in 22q11.2DS – How Does this relate to the Neurodevelopmental Phenotype?

An assessment of the sleep study cohort of individuals with 22q11.2DS showed that it was not representative of the overall ECHO study cohort outlined in **Chapter 4**. **Table 23** shows the differences in diagnoses based on published work from the ECHO study (Niarchou *et al.*, 2014; Cunningham *et al.*, 2017).

Table 23 Sleep study cohort vs published proportions of psychopathology in ECHO Study cohorts

	Sleep Study cohort	***Niarchou, M. et al. (2014)***	***Cunningham, A.C. et al. (2017)***	***Chapter 4***
Sleep problems	47.6%	/	/	60%
ADHD	4.76%	40.5%	/	38.7%
Putative ASD	11.1%	26.0%	/	36%
Anxiety	14.3%	26.3%	/	25%
Indicative DCD	47.1%	/	80%	81.4%

Presence of diagnoses did not necessarily reflect symptomology and therefore it was important to explore whether there were any associations between the sleep architecture and the neurodevelopmental phenotype in these young people with 22q11.2DS.

Similarly, to the analyses conducted in **Chapter 6**, it was important not to over-interpret the findings and therefore the sleep architecture was investigated in relation to the statistically significant findings in **Chapter 4**, reflecting analyses undertaken in **Chapter 6**. In this chapter, it was important to explore whether there were associations in the psychopathology and the cognitive outcomes only in the differences seen between the young people with 22q11.2DS

and the sibling control group. This further reflected the analyses conducted in **Chapter 6** and helped to limit the over-interpretation. The findings were corrected for multiple testing.

The CAPA and cognitive assessments were, on average, conducted ~9.6 months from the EEG assessment. As a result, the time difference needed to be controlled for.

Where associations were found between the neurodevelopmental phenotype and the stage of sleep, the age difference was added as a covariate to test the robustness of association. There were no associations between the percentage of time spent in Stage 1 NREM sleep for the young people with 22q11.2DS (**Table 24**).

Table 24 Percentage time spent in Stage 1 NREM in relation to psychopathology and cognition in 22q11.2DS

% time spent in Stage 1 NREM	***B***	***SE***	***p***	***95%CI***
ADHD symptoms (n=24)	0.068	0.182	0.711	-0.309-0.446
Any anxiety symptoms (n=24)	0.189	0.124	0.143	-0.069-0.447
Indicative DCD symptoms (n=22)	0.040	0.086	0.646	-0.139-0.219
CD symptoms (n=24)	1.21	0.898	0.191	0.652-3.07
Set shifting ability (n=22)	-0.024	0.0484	0.623	-0.125-0.077

Table 25 Percentage time spent in REM in relation to psychopathology and cognition in 22q11.2DS

% time spent in REM	***B***	***SE***	***p***	***95%CI***
ADHD symptoms (n=24)	-0.127	0.163	0.445	-0.465-0.211
Any anxiety symptoms (n=24)	0.006	0.118	0.963	-0.240-0.251
Indicative DCD symptoms (n=22)	0.043	0.078	0.585	-0.189-0.205
CD symptoms (n=24)	0.942	0.821	0.263	-0.760-2.65
Set shifting ability (n=22)	0.086	0.040	**0.045**	0.002-0.173

A better performance on the set shifting on the WCST was shown to associate with a higher percentage of the night spent in REM sleep (B=0.086, p=0.045) (see **Table 25**). This association remained when correcting for the age difference in the participant between the cognitive assessment and the sleep EEG (B=0.106, p=0.031), however it did not remain when correcting for multiple testing.

7.5 Discussion

This is the first EEG study to assess the neurophysiology of young people with 22q11.2DS during sleep. The percentage of time spent in the different stages of sleep showed that young people with 22q11.2DS spend a longer percentage of their night's sleep in NREM Stage 1 and a shorter percentage of the night in REM sleep compared to their siblings.

7.5.1 Differences in percentage time spent in stages of sleep between young people with 22q11.2DS and siblings

There were differences in the time spent in the NREM Stage 1 and REM in the young people with 22q11.2DS and the sibling control group. The boxplots show differences however there are large overlapping confidence intervals which is related to the small sample sizes of the groups. Therefore, as a result there is large variability in the findings and it suggests that the differences seen do not map onto clinically relevant differences in the sleep stages. It is important to understand that individual sleep fluctuations and night-to-night changes themselves could contribute to these differences rather than clinical implications (Juda, Vetter and Roenneberg, 2013). Despite the statistically significant findings, the current data that has been collected cannot provide information that can be interpreted as clinically significant. It is important to recognise that these findings are taken from a single night of

assessment. They provide a snap-shot of the sleep architecture of the individuals and exploring this further would be over-interpreting the findings. There is evidence in the literature to suggest that a single night of PSG cannot provide an accurate projection of what the sleep architecture of an individual looks like (Verhulst *et al.*, 2006). It is important to account for variability in a night's sleep and as a result, the single night that is measured in this study is insufficient to draw conclusions about the differences in the percentage of the night's sleep spent in particular stages between the young people with 22q11.2DS and the sibling control group.

Furthermore, it is important to recognise that these single nights of sleep were scored by an inexperienced individual and there is need for confirmation from a well-trained and qualified polysomnographer. This will ensure that any further findings from the data are not over-interpreted or misconstrued. Work to explore sleep physiology and architecture further can be developed from this initial work.

7.5.2 Association with better set-shifting performance on the WCST

Correcting for multiple testing accounted for the association between spending longer time in REM sleep and better performance on set-shifting on the WCST, and therefore was not maintained. This is important to recognise as by not controlling for the multiple testing could lead to over-interpretation of the cognitive findings. Additionally, there is a problem with selection of positive associations. For example, the better performance on the WCST was associated with higher percentage of the night spent in REM sleep. This in itself is problematic as the sleep architecture was derived from the single night of sleep and is subject to variability as a result of the night-to-night variability and the 'first-night effects'. Additionally, the particular outcome which showed an association was a cognitive-related variable: cognition

varies daily and performance on cognitive tests is subject to great variability (Van Herwegen *et al.*, 2011). Therefore, this could form the reason for the seen associations: that variability in both the measures map onto each other when only accounting for a snap-shot of the sleep architecture and the cognitive ability of the young people with 22q11.2DS.

7.6 Strengths and Limitations

Current literature shows that this is the only study to have assessed sleep in young people with 22q11.2DS. The sample in this study is modest in size however it remains the only study to have acquired sleep EEG in 22q11.2DS. It is therefore novel in its nature and includes a valuable sibling control group that allows for comparisons to typically developing children to be made.

This study was conducted in the homes of the young people and their families. This was done to try and reduce the amount of stress experienced by the family. It allowed the participant to be comfortable in their own environment although this resulted in difficulties regarding protocol consistency, ensuring that the equipment was operating and ensuring availability of researchers to attend domiciles across the UK. Working in a sleep clinic would have ensured that the environment was consistent, but the young people would have had to travel to stay overnight in a clinic, away from their family and their environment.

Gold-standard sleep measures and protocols were used to acquire an objective assessment of sleep in these young people. The cardiorespiratory measures in this study also ensured that confounding problems regarding breathing and cardiac problems could be accounted for when analysing the sleep EEG. However, this study does not include an objective analysis of the cardiorespiratory measures as there was no clinician available to assist in the

interpretation of the respiratory oscillations. The cardiorespiratory measures however did pose an issue: the sensors did not connect to the amplifier that was used for the EEG net. This resulted in the need for two different amplifiers for data acquisition.

It is important to recognise that the sleep EEG analyses were undertaken by an individual without a specific qualification or expertise in the area. I was personally responsible for undertaking the sleep staging and analyses and as a result, there is a need for confirming the scoring before definitive results can be concluded and before future investigations into sleep problems in young people with 22q11.2DS can be conducted. Despite attending training courses and undertaking hours of practice scoring, it cannot go unnoticed that a qualified somnologist must confirm these findings to inform further investigation into the physiology of sleep in young people with 22q11.2DS.

The sleep EEG was acquired in conjunction with the other measures as part of the wider sleep study that included the actigraphy and the sleep questionnaires. Nonetheless, this study despite including a multidimensional approach to assessing the sleep phenotype in young people with 22q11.2DS was cross-sectional so it remains difficult to comment on the aetiology of the sleep problems and whether sleep problems emerged before the associated neurodevelopmental phenotype.

The number of sensors used for the assessment of the cardiorespiratory measures should be addressed in further studies as the use of the nasal cannula caused issues with tolerability for some of the young people. The number of different tasks that the young people were asked to complete and the management of the family's expectations should be reassessed in future studies to avoid any misunderstanding or questions from parents regarding the nature of the assessment.

Statistical comparisons were corrected for multiple comparisons and as a result, the significant findings did not remain.

Additionally, a limitation to the study and findings is that there was only one night of sleep EEG acquired. This means that the participants did not have time to become accustomed to wearing the equipment and there was no baseline night to make comparisons to. As a result, the findings need to be interpreted with caution as the single night could have impacted on the night's sleep that the individual experienced and resultantly, affected the derived objective measures of sleep. During analyses, wake periods and artefacts were removed and percentages were used over raw data to account for this, however in the literature the norm is for a baseline night to be completed.

7.7 Clinical Implications

This objective assessment of sleep in young people with 22q11.2DS has contributed to the understanding of sleep problems in this population adding to the cross-sectional assessment of sleep problems from interview data, objective actigraphy measurements and assessment of subjective sleep measures from sleep questionnaires. However, these findings are insufficient to suggest any features of clinical importance. The normal distribution of sleep problems and fluctuation between nights is most probably of relevance to these current findings rather than clinical problems in young people with 22q11.2DS.

To be able to draw clinical findings, further work must robustly explore sleep physiology in young people with 22q11.2DS as replicable and reliable outcomes could potentially act as biomarkers in relation to sleep problems and the neurodevelopmental phenotype.

7.8 Future Work

Qualified somnologists, sleep physiologists and respiratory clinicians should be involved in the development of further work regarding sleep in young people with 22q11.2DS. It is important to investigate the prevalence of SRBD in these young people and in order to achieve this, clinicians are required to be involved in the study from the onset, to its completion.

A larger sample of individuals should be assessed to test how robust the results are. If there are potential physiological sleep differences in young people with 22q11.2DS compared to typically developing children after corrections, this would be a significant finding which could have an impact on intervention development.

Further analyses regarding waveform characteristics including the frequency, amplitude, density and length need to be undertaken to investigate whether differences in specific features of the waveforms differ between the groups. These analyses take the form of linear mixed models and are in the preliminary stages. With additional time, the models can be completed to supplement the current findings.

Future investigation would need to be conducted with a much larger sample of individuals and they would need to be followed-up longitudinally to determine whether sleep electrophysiology could be used to assess the prognosis of the neurodevelopmental phenotype of 22q11.2DS. Investigating objective sleep measures in younger individuals with 22q11.2DS such as toddlers could help to identify whether there is a physiological underpinning to the sleep problems. Additionally, adult individuals with 22q11.2DS could help to identify whether there is persistence of such problems identified using PSG.

For future PSG assessment, including a baseline night for the sleep EEG to allow the participants to be accustomed to the wires and the equipment they wear is essential, to remove first-night effects that can confound the findings from the PSG. The first night wearing the equipment often provides an alternative sleep architecture to a second night (Agnew, Webb and Williams, 1966). In young people susceptible to neurodevelopmental and anxiety problems (Saletu *et al.*, 1996), this could further impact their participation in the study and their sleep that is measured. The second night of assessment would provide a more realistic picture of the sleep architecture and physiology of the young person. Ideally, there would be multiple nights of assessment that would also demonstrate the natural night-to-night variability. This would benefit analysis as recognition of the baseline changes in the sleep night-to-night could be accounted for when exploring the sleep architecture and physiology of the individual.

Furthermore, sleep problems are not isolated to 22q11.2DS. In the IMAGINE-ID Study (see **Chapter 3**) there are several different CNV genetic syndromes that are characterised by intellectual disability. Sleep problems are commonly reported in these individuals therefore, the sleep EEG study could be extended to include other CNV genetic syndromes to compare outcomes and develop further understanding of the sleep phenotype in these rare genetic syndromes.

7.9 Summary

This results chapter has shown that there are potential differences in PSG-derived sleep parameters in young people with 22q11.2DS compared to their unaffected sibling controls. This study is novel in assessing sleep architecture of these young people and has worked to

contribute to the understanding of sleep problems in 22q11.2DS. However, it is important to note that this work should not be over-interpreted: it is important to further explore the physiology of sleep in the young people with 22q11.2DS and the siblings. Findings also did not survive correction for multiple comparisons either. Understanding normative and typical findings of their sleep physiology, potentially by assessing more than one night, can help to provide the foundations to build upon when making conclusions about the differences in sleep problems between young people with 22q11.2DS and unaffected siblings.

The electrophysiology of sleep in young people with 22q11.2DS requires further investigation to begin to better understand how the sleep phenotype could relate to the neurodevelopmental phenotype.

These findings have begun to explore the architecture of sleep in young people with 22q11.2DS but are heavily reliant on further investigation with more rigour and involvement of sleep experts and clinicians.

Chapter 8 – General Discussion

8.1 Overview

This thesis aimed to examine sleep problems in young people with 22q11.2DS by using subjective and objective measures of assessment to comprehensively assess sleep in these young people. Sleep problems can significantly impact day-to-day life and in individuals at elevated risk of neurodevelopmental disorders, how sleep affects their prognosis is of great importance. In 22q11.2DS, investigation into sleep problems is scant and the aetiology of sleep problems in this high-risk population remains to be elucidated. In neurodevelopmental disorders such as ADHD and ASD, sleep problems are regularly accounted for in treatments and the behavioural phenotype. However, focus on sleep in 22q11.2DS is lacking; masked by other aspects of the syndrome.

8.2 Collating Findings

In the first experimental chapter (**Chapter 4**), sleep problems were preponderant in 22q11.2DS compared to their siblings, with parent-reports suggesting around 60% of young people experienced sleep problems compared to 23% of their unaffected sibling controls ($p<0.001$). Moreover, it was determined that there were specific patterns of sleep problems in young people with 22q11.2DS: tiredness-related sleep and insomnia-related sleep. Abnormalities in these patterns were seen in 22q11.2DS with 44.7% of young people endorsing a tiredness-related sleep pattern, and 21.1% an insomnia-related pattern. Furthermore, there were a number (n=17) of young people with 22q11.2DS who were taking

melatonin for treatment of their sleep problems. However, 16 of the 17 individuals taking melatonin still reported sleep problems.

The patterns of sleep problems showed associations with the neurodevelopmental phenotype of 22q11.2DS: tiredness-related sleep was associated with symptoms of ADHD, indicative DCD, anxiety disorders and impaired executive functioning (explored as set shifting ability). Furthermore, an association was seen between insomnia-related problems and symptoms of anxiety disorder, indicative DCD and conduct disorder. These findings suggested that sleep problems in 22q11.2DS are associated with concurrent psychiatric symptomology and cognitive impairment. These findings survived multiple testing corrections.

A small sample of young people with 22q11.2DS (n=87) and unaffected sibling controls (n=39) that were assessed at wave 1 and wave 2 of the ECHO Study were investigated to look at the persistence of sleep problems over time. A comparative prevalence of sleep problems of sleep problems in young people with 22q11.2DS reached 57.5% and in siblings, 20.5% respectively (p=0.001) at wave 2. There was no overall change across the two waves in the prevalence of sleep problems shown in the young people with 22q11.2DS (p=0.835) or the unaffected sibling controls (p=0.739). The small group of toddlers with 22q11.2DS (n=10) and unaffected sibling controls (n=3) showed that 60% of the toddlers with 22q11.2DS had parent-reported subjective sleep problems compared to 33.3% of the unaffected sibling controls (p=0.559). This mirrored the preponderance in the larger, better powered cohort of young people with 22q11.2DS investigated earlier in **Chapter 4**. Furthermore, the small subsample of 22q11.2DS adults (n=23) who reported sleep problems objectively, excluding a small few individuals that required an advocate to respond on their behalf, reported sleep problems at a prevalence of

56.5%. These findings suggested potential persistence of sleep problems throughout the life course of individuals with 22q11.2DS.

Chapter 5 outlined the pilot study that was completed with typical young people from the general population (n=12) to assess the feasibility of the sleep study protocol that had been designed for use with the young people with 22q11.2DS. The aim of the pilot study was to assess the feasibility of the study including the tolerability of the equipment and methods that were used to assess sleep. The n=12 young people had varying experiences of the protocol but ultimately, there was a preference for the Geodesic EGI net. This resulted from the reduced time taken to setup the equipment compared to the traditional electrodes, the ease of application and subsequent removal, convenience and the lack of need for solvents to be transported around the UK.

The protocol had to be refined before implementation in the main sleep study, to ensure that the highest yield of data could be acquired but not to the detriment of the comfort and understanding of the families involved.

The protocol and the main sleep study were developed successfully resulting in n=50 young people with 22q11.2DS and n=29 unaffected sibling controls completing the actigraphy protocol, sleep diaries and sleep questionnaires. While this smaller that was included in the sleep study is likely underpowered, this sample was the first individuals with 22q11.2DS to complete objective and subjective assessments of sleep as part of a research study. Therefore, any findings were considered novel and could be built upon with further work.

After controlling for multiple comparisons, the actigraphy data demonstrated that young people with 22q11.2DS spent longer time in bed than their siblings (p=0.001) and slept for

longer also (p<0.0001). Young people with 22q11.2DS also had better sleep efficiency than their siblings as measured objectively by the actigraphy watches (p<0.0001). There were no differences however in the nocturnal activity measures between groups.

No associations were found between the objective actigraphy and the neurodevelopmental phenotype.

In the final experimental chapter (**Chapter 7**), PSG was obtained and was preliminarily analysed for n=28 young people with 22q11.2DS and n=17 unaffected sibling controls. There were several limitations with regards to these analyses. Namely, the PSG was obtained over a single night and 'first-night effects' were not adequately controlled for. Additionally, the young people with 22q11.2DS were not accustomed to the equipment and therefore, further disruption was introduced. Furthermore, the cardiorespiratory measures were not adequately interpreted and therefore, no conclusions referencing SRBD could be made from this work. However, what could be shown was the baseline percentages of time spent in different stages of sleep in 22q11.2DS young people and their siblings. Nonetheless, it was important to recognise that individual variability and night-to-night fluctuation could also provide reasons for the minimal differences observed between the young people with 22q11.2DS and their siblings.

Differences in the percentage of the night that young people with 22q11.2DS spent in NREM Stage 1 (p=0.04) and REM sleep (p=0.04) were seen compared to their unaffected siblings however, nothing could be definitively derived from this as first-night effects and general night-to-night variability in individuals was not accounted for. The overlapping nature of the 95% confidence intervals in the analyses also suggested a wide range of percentages of sleep stage. Therefore, despite the difference, it would be difficult to derive clinical meaning from

these analyses. Despite conditions for the acquisition of data using different objective and subjective sleep methods not being optimal, this thesis describes a unique and novel study; the first to explore sleep problems in young people with 22q11.2DS. It is the first to explore how sleep problems relate to the neurodevelopmental phenotype in these young people and can potentially provide foundations for further work into sleep problems in individuals with 22q11.2DS in the future. This is a novel, unique study that is the first of its kind, and therefore has contributed to an understanding of sleep in individuals with 22q11.2DS that can be built upon and developed further.

8.3 Implications for Research

Young people with 22q11.2DS have a higher prevalence of sleep problems than their unaffected siblings, and these sleep problems show some associations with psychopathology and cognition. Nonetheless, when exploring the sleep phenotype further using validated sleep assessment methodology including subjective and objective measures, these associations were not replicated. Mentioned previously, there were a few problems with the objective PSG data acquisition and subsequently the analyses that could be undertaken. Furthermore, the sample sizes were not comparable with the subjective parent-reported sleep problems outlined in **Chapter 4**. Nonetheless, it was interesting to observe concordance between the subjective parent-reported sleep problems both on the CAPA and the sleep questionnaires. However, the suggestion that parent-reports do not accurately define older adolescents' sleep is of interest for further research and has a wider sleep medicine implication. Furthermore, individuals with more severe psychopathology were unable to complete the PSG therefore, a representative sample was not assessed objectively.

Currently, there is no other research into sleep in 22q11.2DS. Sleep problems in NDDs such as ASD have been shown to have a biological aetiology but not in all individuals (Veatch *et al.*, 2015). The underlying mechanisms that could relate to the manifestation of sleep problems in 22q11.2DS require further investigation. In individuals with numerous problems, including both physical and neurodevelopmental, it is important to not underestimate the importance of gene-environment interactions and how environment can impact upon sleep (Barclay *et al.*, 2012) as well as the contributions that familial or social influences can have (Poisson *et al.*, 2015). In this thesis, the investigations have included family income and highest maternal education to test for confounding. This provides a basis to build from with regards to exploring socioeconomics and the influence of sleep-related behaviours (Grandner *et al.*, 2010). Further research should be carried out to better understand these relationships for individuals with 22q11.2DS but also for the wider paediatric population.

8.4 Limitations and Experiences

The ECHO Study aims to recruit a representative sample from the UK through NHS Genetics Clinics and charities: there is no exclusion criteria. However, the study does depend on families having free-time to complete the assessments. This has resulted in the slight skew of the sample towards including families from an above average socioeconomic background. Parents and primary carers with access to supportive medical professionals and who are motivated to engage with the study are more likely to volunteer. This interaction with medical professionals could derive from their child/ren having a more severe phenotype and therefore they want answers regarding this for example.

Families where a parent has a CNV also could have limited ability to undertake assessments or find it difficult to do so. This should be accounted for when interpreting results. There could also be an ascertainment in recruitment through NHS Genetics Clinics as these individuals present with an impairment such as an NDD which would result in their referral to the genetics clinic. Therefore, the ECHO Study might not be a representative sample of the 22q11.2DS population but regardless, it does provide the best sample that can be acquired for examining the relationship between sleep problems and the neurodevelopmental phenotype.

With regards to the sleep study, the sample was not representative of the general ECHO Study. Young people with fewer severe problems completed the full sleep study. In those young people with psychotic-like symptoms, parents reported that they would be unable to undertake the study. Drawing conclusions from this work was therefore difficult and it remains important that over-interpretation is not conducted.

The ambulatory study allowed for us to reach families across the UK however it resulted in a small number of incomplete studies because of technical problems or equipment failure. There are smaller technologies that can acquire EEG data and if more PSG studies were conducted, considering less invasive techniques which could increase tolerability could be an option. However, travelling to the young people's homes and engaging with the families in their comfortable environment was a strength of the study. The concerns of the parents and families were prioritised.

There is the issue of power also for the sleep study. The larger sample completed the actigraphy and sleep diaries however there was a smaller subsample of EEGs that survived quality control. This was due to poor signal-to-noise ratios in the EEG data. This occurs from

interference in the room which occurs at similar frequencies to the acquisition frequency however this was limited by the software which applied a filter when acquiring the data. Additionally, artefacts include movement of the person and the equipment itself. This is unavoidable especially in novel environments where baselines cannot be adopted.

All individuals were genotyped and individuals with the 3-Mb and the 1.5-Mb deletion were included. Further work could potentially explore deletion size and effects on sleep in individuals with 22q11.2DS.

Possibly the most pertinent and important limitation is that I am not a trained respiratory clinician or neurologist, nor am I qualified to interpret sleep EEG as I do not hold sleep physiologist or somnologist qualifications. Therefore, I was unable to make a clinical assessment of any sleep-related breathing disorders, or sleep disorders in general, that could have been detected by the PSG. These facts need to be considered when assessing the PSG data. It would be important to conduct a comprehensive study in the future with the necessary clinical expertise involved in the study to allow for clinical conclusions to be made, especially as they could have real-life effects on the young people with 22q11.2DS.

The following **Table 26** demonstrates differences and similarities between the subjective and objective measures of sleep problems used in this thesis.

Table 26 Comparing the methods of sleep assessments - subjective vs. objective measures

Subjective vs. Objective Sleep Assessments	
Similarities	***Differences***
Dependent on honest reporting: written for subjective, and wearing the equipment with the objective measures	Self or parent-report can include biases whereas objective measures uses physiological changes
There are standards to adhere to when analysing and interpreting the data	Objective measures depend on predetermine algorithms for data acquisition whereas subjective measures can provide varied day-to-day responses
Measures have been validated on appropriate population samples	The scoring of the sleep electroencephalography was analysed using subjective interpretation whereas the actigraphy wasn't, and questionnaires include closed questionnaires
Can acquire a large wealth of data regarding sleep behaviours, patterns and physiology	Questionnaires do not provide a direct narrative regarding sleep problems; usually there are boxes to tick, and scales to choose from whereas the objective measures can have a wider range of measured responses
Should be acquired by trained professionals who are experienced in conducting the particular methods of measurement: questionnaires need to be explained and actigraphy interpreted for example by a trained individual	Parents are involved more in the subjective measures whereas participants alone can provide the data acquired from the objective methods of assessment
	Questionnaires and sleep diaries are historical techniques of measurement, whereas actigraphy and EEG are now becoming more common and accessible in research studies

Multiple comparisons were controlled for throughout the thesis. However, it is important that the work is replicated and progressed before conclusions can be made from the objective actigraphy and PSG data in the young people with 22q11.2DS.

8.5 Future Work

The work outlined in this thesis has created a foundation that can be further developed to explore the overlooked and important area of sleep in young people with 22q11.2DS. This thesis provides insight into the high prevalence of sleep problems in these young people and the wide-ranging impact that these can have on the individual and their family's day-to-day lives. This work was instigated partly because of the frequent requests from parents of young people with 22q11.2DS to explore sleep in their children because of the debilitating effect on the child and the family. This thesis addresses the need to look more into the sleep phenotype of 22q11.2DS at all ages and indicates that this is likely to be a fruitful area for future work.

Further research including longitudinal assessments and analysis can help to understand whether there is a biological component to the aetiology of sleep problems. Studies in other CNV genetic syndromes have discovered biological aetiologies (S. R. Williams *et al.*, 2012), whereas others have suggested that it is the concurrent neurodevelopmental problems that relate to the sleep problems in that population (Robinson-Shelton and Malow, 2016).

Furthermore, physical problems have not been identified in this study as it was not part of the main hypotheses. There are several physical problems such as cardiac, respiratory and physical limb problems. Limb pain and discomfort could have an impact on the quality of sleep gained and therefore, could influence sleep problems (Tudor *et al.*, 2014).

Chapter 4 did not include investigation into whether sleep problems were associated with psychosis and it is known that individuals with 22q11.2DS are at a 25-30% risk for schizophrenia (Schneider *et al.*, 2014). In the wave 1 cross-sectional assessments, there were a limited number of individuals reporting psychotic experiences and therefore it was not practical to assess this relationship. The sleep study did include individuals who were older and therefore psychotic symptoms could be assessed. Further work however could be done as part of the longitudinal ECHO Study to explore whether there is an association between actigraphy-derived sleep architecture parameters and psychotic symptoms.

Persistence of sleep problems over time was suggested from the prevalence at wave 2 (**Chapter 4**), and supported by the toddler and adult studies. Assessing the stability of the objective measures of sleep over time could help to understand how sleep problems could confound problems in later life and in adults with 22q11.2DS, especially in those who develop Parkinson's disease. Sleep problems such as REM behavioural disorder are prevalent in Parkinson's disease (Van, Boot and Bassett, 2017). Individuals with 22q11.2DS are at an elevated risk and it is important to identify whether sleep problems throughout life could be associated with this outcome.

Assessing sleep problems over time can also add to an understanding of when it is best to intervene with treatments. There are currently no research studies into sleep interventions in individuals with 22q11.2DS but there is evidence of the effective practise of sleep hygiene and cognitive behavioural therapies in other chromosomal disorders and NDDs (Blackmer and Feinstein, 2016).

An investigation into the relationship between the sleep of the young person with 22q11.2DS and their primary carer could help to better understand the bedtime behaviours and

potentially identify activities detrimental to establishing good quality and quantity of sleep. Some parents of these children experience mental health problems and poor sleep themselves. Examining how parental sleep could be related to the young person's sleep could have benefits for the entire family and act as a whole-family project and intervention could work to develop the best night's sleep for all.

Future PSG studies in these young people should be conducted in a controlled environment. It is important to account for the 'first-night effect', the anxiety of the young people, the baseline night comparison to a second night and the time that is required for the young people to become accustomed to the equipment. Qualified and experienced sleep physiologists are required to help with the interpretation of the data and reliability testing. Work into the sleep physiology is required in the future for example waveforms during the sleep period. Larger samples need to be assessed using objective methods to draw conclusions that could help inform families, schools and clinical practice.

8.6 Conclusions

Sleep problems are prevalent in young people with 22q11.2DS. Sleep problems are not dissimilar from other aspects of the syndrome in their variability amongst individuals in their presentation. This thesis provides evidence for sleep problems outside of subjective measurement. Objective measures have suggested that there are differences between young people with 22q11.2DS and their unaffected siblings. This work has showed that is important to include both subjective and objective methods of sleep assessment to best explore the sleep phenotype in young people with 22q11.2DS. Evidence showing minimal agreement between the objective and subjective measures reflects this. However, showing agreement

between subjective and objective methods of sleep assessment in individuals with 22q11.2DS is an underrepresented area of research.

Future work should broaden its focus on how sleep problems affect the individual with 22q11.2DS and their surroundings including family, parents, social interactions and gene-environment relationships. Work into sleep problems should aim to address the substantial impact sleep problems have on individuals with 22q11.2DS throughout their lives, and how these interact with their neurodevelopmental phenotype. Treatments for sleep problems in individuals with 22q11.2DS must be developed for the immediate wellbeing and fruitful futures of every single individual.

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Appendix 1: A flow-chart of the ECHO Study and its relationship with the IMAGINE-ID study

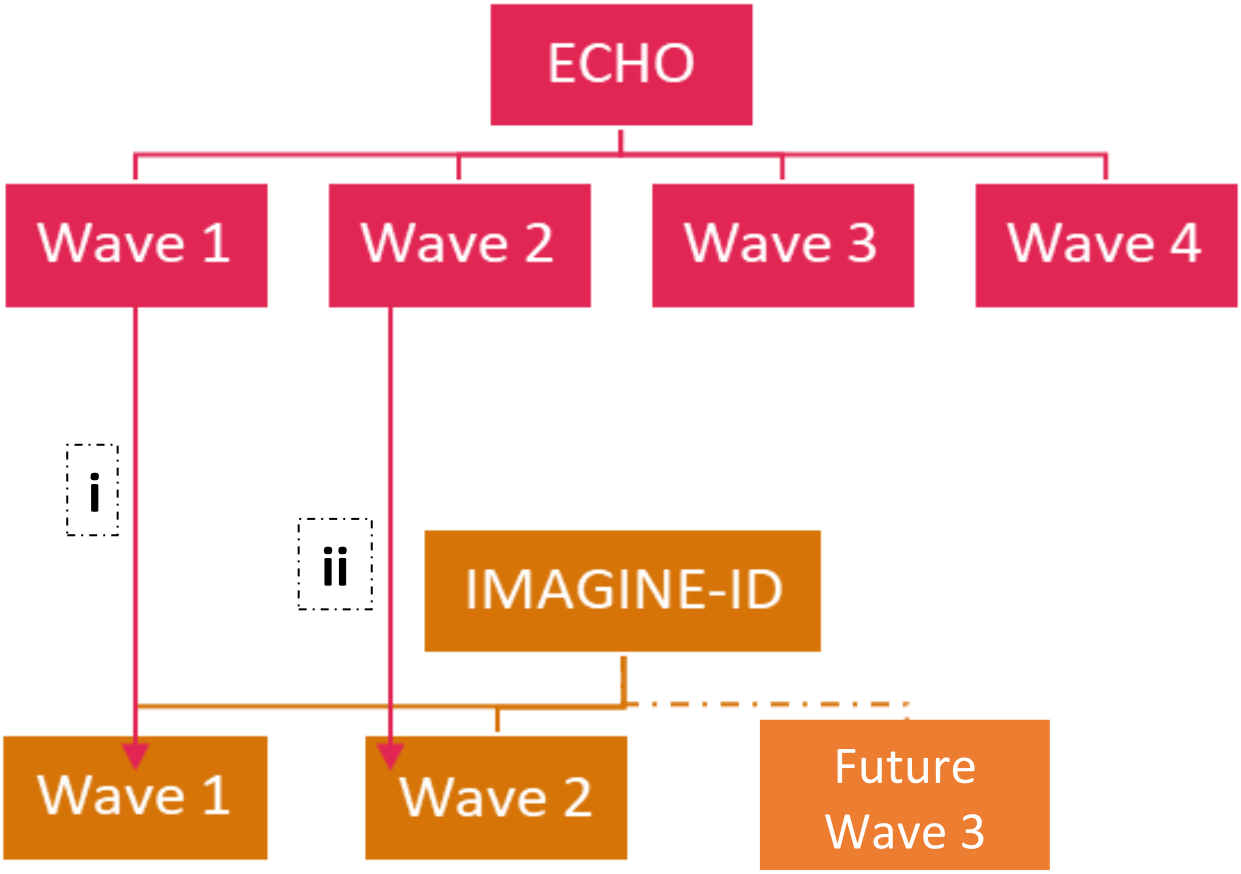


Figure 26 How the ECHO and the IMAGINE-ID Studies relate to one-another

i) An individual could be recruited into the ECHO Study at wave 1. This was the first time point for assessments. At the same time that an individual was recruited into the ECHO Study, the primary carer could consent to being informed of other projects: in the case of the ECHO Study, it was namely the IMAGINE-ID Study. The ECHO Study worked to investigate 22q11.2 Deletion Syndrome whereas the IMAGINE-ID Study includes 22q11.2 Deletion Syndrome in addition to other copy number variants including 22q11.2 Duplication, 15q11.2 Deletion and Duplication, 16p11.2 Deletion and Duplication and 3q29 Deletion.

ii) The ECHO Study commenced in 2010. The first families that were seen completed assessments as part of wave 1. After ~2.5 years, the same family became eligible for a follow-up assessment and they were invited to participate in wave 2 of the ECHO Study.

However, after the inception of the IMAGINE-ID study in 2014, young people eligible for wave 2 follow-up in the ECHO Study were instead (if the primary carer had consented) followed-up as part of the IMAGINE-ID Study. This in turn resulted in some young people having participated in both the ECHO and IMAGINE-ID studies. It also resulted in fewer young people being newly recruited through the ECHO Study from 2017 onwards as the IMAGINE-ID Study was then responsible for the recruitment of new young people including young people with 22q11.2 Deletion Syndrome.

Appendix 2: A flow-chart of the different studies and samples outlined in the thesis with sample sizes included

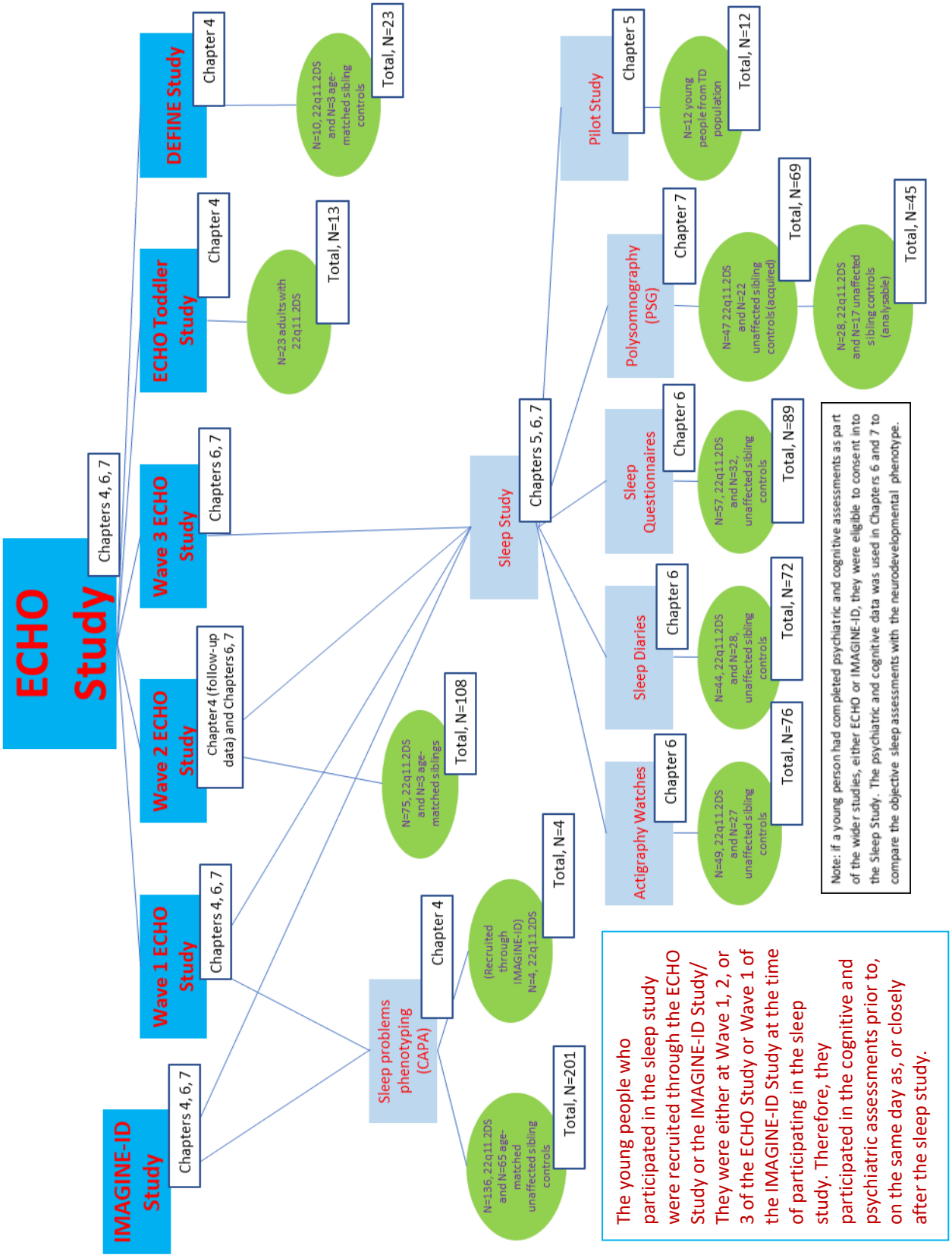


Figure 27 A flow-chart of the different studies and samples in this thesis

Appendix 3: Recruitment and Ethics Protocol for the ECHO Study

The ECHO Study

Young people with 22q11.2DS were recruited through NHS Medical Genetics clinics in the UK, British 22q11.2DS charities (Max Appeal! 22qCrew and Unique), social media advertisements and family networks. Where available, a sibling control closest in age to the child with the deletion was invited to take part in the ECHO study. Carriers of the deletion and sibling controls were aged 6 or older and the presence (in carriers) or absence (in sibling controls) of the deletion was confirmed either by a Medical Genetics laboratory providing a medical genetics report, and/or a microarray analysis in the MRC Centre for Neuropsychiatric Genetics and Genomics laboratory at Cardiff University.

Timepoint Two: wave 2

The recruitment process for the wave 2 assessment was dependent on whether a primary carer had provided consent for their families to be contacted again after completing the wave 1 assessments. If there was consent, the family were contacted and asked whether they would be happy to participate in the follow-up assessments. The same assessments that were completed at the first timepoint, were repeated at the second timepoint. Further information regarding the actual assessments that were undertaken can be found in **Chapter 3**.

The Toddler Study

The cross-sectional toddler study involved families with a toddler with 22q11.2DS and any age-matched sibling controls. The families either attended the clinic at Cardiff University or were visited by a psychology team in their own home, where they participated in psychiatric, cognitive and other assessments. These assessments were based on the assessments in the

ECHO Study and outlined in **Chapter 3**. For the purpose of this thesis, the assessments that are of relevance are outlined in **Chapter 3**. The families that participated in the Toddler study were recruited predominantly through social media. There were some families that had previously contacted the ECHO Study as they had been interested in participating. However, there child/ren were too young at the time. These individuals were now able to participate in a study.

DEFINE: The Adult Study

The cross-sectional adult data were collected via a similar method of in-home psychiatric and cognitive assessments to the ECHO and toddler studies (see **Chapter 3**).

Adults with capacity consented into the study and reported on their own psychiatric morbidities and completed the cognitive tasks. For participants who lacked capacity an advocate or personal consultee completed questions on behalf of the participant with 22q11.2DS regarding their psychiatric morbidities, but the individual, if able to, completed their own cognitive tasks. This ensured that the most accurate responses were provided.

The IMAGINE-ID Study

Young people with rare chromosomal genetic syndromes were able to participate in the IMAGINE-ID Study which is a collaborative project between Cardiff University, the University of Cambridge and University College London. The protocol that was undertaken in the IMAGINE-ID Study was developed in line with the assessments that are undertaken in th ECHO Study. This allowed for cross-comparability. The IMAGINE-ID Study included young people with 22q11.2DS and these were recruited through NHS Medical Genetics clinics in the UK, Unique, social media advertisements and family networks. Where available, a sibling control

closest in age to the child with the deletion was invited to take part in the ECHO study. Carriers of the deletion and sibling controls were aged 6 or older and the presence (in carriers) or absence (in sibling controls) of the deletion was confirmed either by a Medical Genetics laboratory providing a medical genetics report, and/or a microarray analysis in the MRC Centre for Neuropsychiatric Genetics and Genomics laboratory at Cardiff University.

Appendix 4: Example of Sleep Diaries

Please note that the sleep diaries shown in **Appendix 4** have not been included as part of the analyses undertaken in this thesis. This is due to the inconsistencies with recording who was responsible for completing the sleep diaries and therefore, there was no validation or reliability data available to ensure that the sleep diaries could be accurately compared to the objectively-derived actigraphy data.

The sleep diaries were developed to be implemented in the Pilot Study (**Chapter 5**). By testing the sleep diaries in the Pilot Study, changes could be made before introducing them into the Main Sleep Study (**Chapter 6**). As mentioned in the main thesis text, there were different sleep diaries developed for different age categories. The different sleep diaries have been included in this appendix to demonstrate:

i) What was included in the sleep diaries and to observe how the data was acquired;

ii) To identify the differences in the sleep diaries for the younger and older young people that participated in our study;

iii) To demonstrate the parent diary that was developed to compliment the sleep diary that was given to the younger children and the young people who were not considered to have capacity.

Please note that all participants and parents were informed how to complete the sleep diary and an example day was completed with them during the overnight sleep study. Families were informed about not having to be precise in their time measurements and were verbally advised to ensure either accuracy or rounding to the nearest 0, 5 or 10 minutes. I recognise that this was not written down and therefore created some discrepancies between the sleep

diaries and the actigraphy. This contributed to the overall decision of excluding the sleep diaries from the analyses in this thesis.

The sleep diaries are included in **appendices 4, a**: The Child Sleep Diary; **4, b**: The Teenage Sleep Diary and **4, c**: The Parent Sleep Diary (on behalf of their child/ren).

NAME: ID:

Sleep Diary

ECHO STUDY

Hello my name is Hayley. I am a researcher at Cardiff University. I work on the ECHO Study.

I study sleep in children and teenagers.

I am hoping to measure your sleep!

Lots of children have trouble getting a good night's sleep. Some find it hard getting to sleep, and some wake up really early! Getting a good night's sleep is really important!

It's sometimes hard to get out of bed in the morning. If you're tired after waking up, it might mean you fall asleep at school.

Your parents told us that you and your family were interested in helping me find out a bit more about how you sleep.

This is your **sleep diary!** It is a fun way to find out a bit more about how you sleep. It will help you, your family and me to better understand what your sleep is like.

If you have any questions Mum or Dad doesn't know the answer to, you can ask me:

Hayley Moulding
(029 20 688067 or MouldingHA@Cardiff.ac.uk).

Have fun and thank you for helping me!

A sleep diary is a place where you write down information about your sleep.

The first thing to do is to complete the table for week 1. Fill in:

1. **Wake up time**
2. **Out of bed time**
3. **Turn light off time**
4. **Fell asleep time**
5. **Did you take the watch off?**
6. **Was today a normal day? .i.e. Were you ill?**
7. **How many caffeinated drinks? .i.e. Tea, Coca-Cola**
8. **Did you have a nap?**
9. **Any exercise today?**

For example:
I went to bed on Monday night at 7:30PM and read my book for an hour.
I turned out my light at 8:30PM and fell asleep at 9:00PM.
I woke up on Tuesday morning at 7:30AM but didn't get out of bed until 8:00AM.

I would write the following in the table section my sleep diary:

	Monday	Tuesday
Time Wake Up	-	*7:30 am*
Time Out of Bed	-	*8:00 am*
Time Into Bed	*8:30 pm*	
Time Fell Asleep	*9:00 pm*	

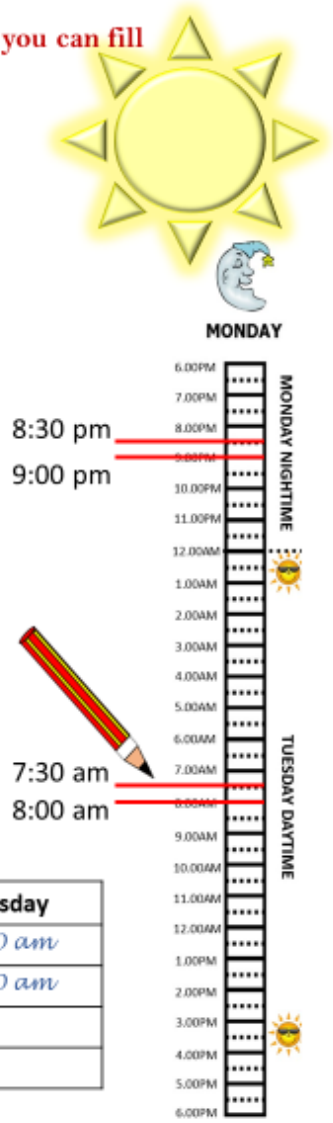
Once the table is finished for the day, you can fill out the rest of your sleep diary!

Next, you can do some drawing and colouring!

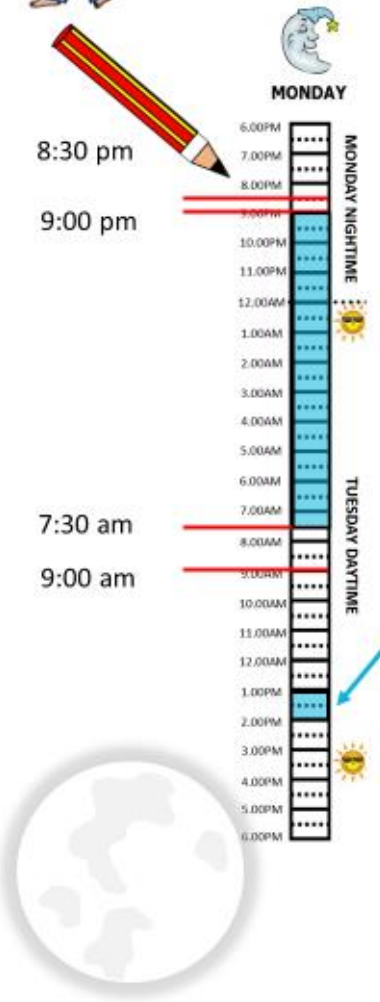
So I can work out when you slept, please draw lines on the charts in this diary. All you have to do is draw a line on the chart when each of the four things in the table happened:

1. Time Wake Up
2. Time Out of Bed
3. Time Into Bed
4. Time Fell Asleep

The times you write down **DO NOT** need to be exactly right. For example 8.30am is fine instead of 8.32am



	Monday	Tuesday
Time Wake Up	-	7:30 am
Time Out of Bed	-	8:00 am
Time Into Bed	8:30 pm	
Time Fell Asleep	9:00 pm	



Don't forget the most important and fun part.

Once you have your four lines, colour in the time between when **YOU GO TO SLEEP** and **WHEN YOU WAKE UP**.

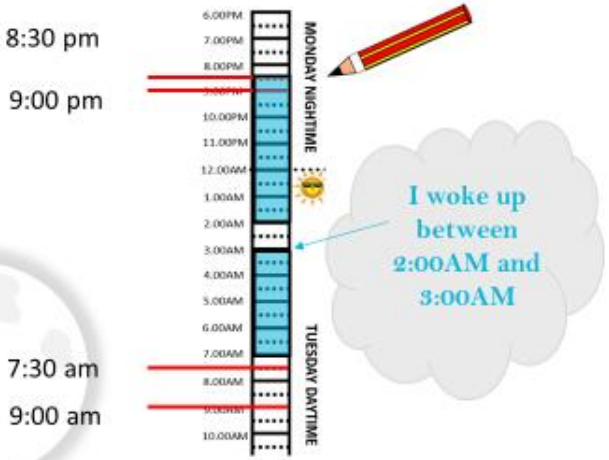
If you have a nap in the day, please colour in it e.g. if you fell asleep between 1:00PM and 2:00PM you would colour in the squares between 1:00PM and 2:00PM (see arrow pointing to blue square on diagram)

You can colour in with anything; make it as colourful as you like!

DON'T FORGET:

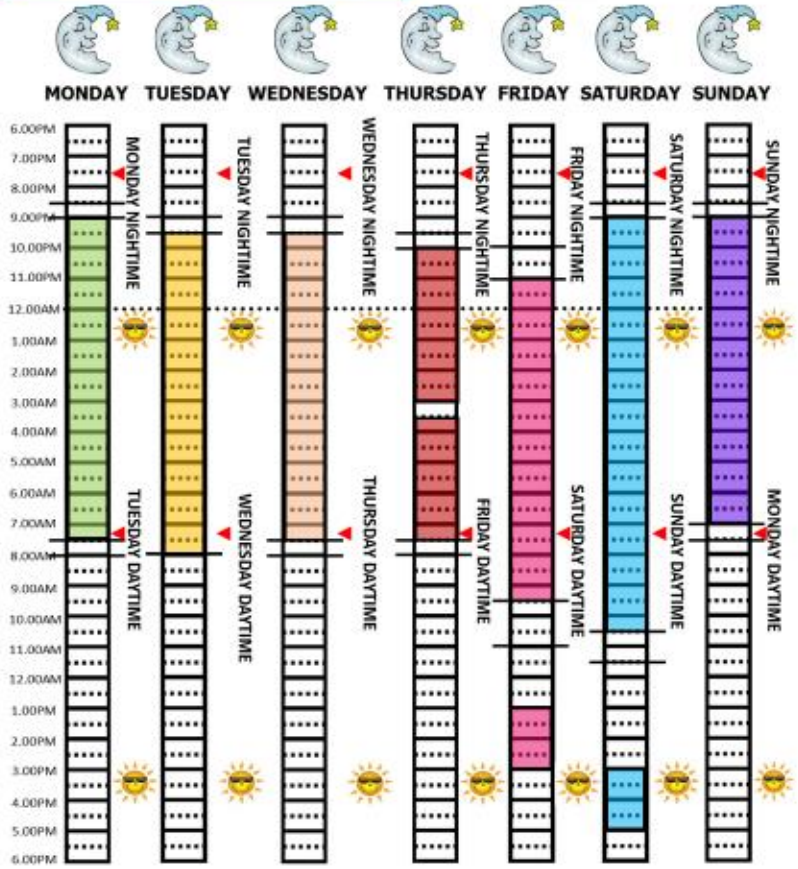
- Make sure you colour in the right day, and don't forget to ask an adult for what the date is. This stops me getting confused!
- Make sure you draw on your chart. If you wake up and get out of bed at the same time, don't worry! Draw one line and let Mum/Dad know that's what you've done. They can then write it down in the 'notes' section.
- Make sure you mark in your diary if you wake up during the night.
- If you do wake up during the night that will mean that there will be more lines on your diary. That's fine!

If you wake up in the night leave a white gap in between where you woke up and where you went back to sleep:



Have fun! Make it colourful!

so this is the full sleep diary below. Make it as colourful as you want and have fun!



I AM READY TO BE FILLED IN!
WEEK 1: Date:
MONDAY TUESDAY WEDNESDAY THURSDAY FRIDAY SATURDAY SUNDAY
SUNDAY NIGHTTIME
MONDAY NIGHTTIME
TUESDAY NIGHTTIME
WEDNESDAY NIGHTTIME
THURSDAY NIGHTTIME
FRIDAY NIGHTTIME
SATURDAY NIGHTTIME
MONDAY DAYTIME
TUESDAY DAYTIME
WEDNESDAY DAYTIME
THURSDAY DAYTIME
FRIDAY DAYTIME
SATURDAY DAYTIME
SUNDAY DAYTIME
6:00PM
7:00PM
8:00PM
9:00PM
10:00PM
11:00PM
12:00AM
1:00AM
2:00AM
3:00AM
4:00AM
5:00AM
6:00AM
7:00AM
8:00AM
9:00AM
10:00AM
11:00AM
12:00AM
1:00PM
2:00PM
3:00PM
4:00PM
5:00PM
6:00PM

I AM READY TO BE FILLED IN!
WEEK 1: Date:
MONDAY TUESDAY WEDNESDAY THURSDAY FRIDAY SATURDAY SUNDAY
SUNDAY NIGHTTIME
MONDAY NIGHTTIME
TUESDAY NIGHTTIME
WEDNESDAY NIGHTTIME
THURSDAY NIGHTTIME
FRIDAY NIGHTTIME
SATURDAY NIGHTTIME
MONDAY DAYTIME
TUESDAY DAYTIME
WEDNESDAY DAYTIME
THURSDAY DAYTIME
FRIDAY DAYTIME
SATURDAY DAYTIME
SUNDAY DAYTIME
6:00PM
7:00PM
8:00PM
9:00PM
10:00PM
11:00PM
12:00AM
1:00AM
2:00AM
3:00AM
4:00AM
5:00AM
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9:00AM
10:00AM
11:00AM
12:00AM
1:00PM
2:00PM
3:00PM
4:00PM
5:00PM
6:00PM

NAME: ID:

Teenage Sleep Diary

ECHO STUDY

Teenagers need to sleep for longer. You naturally go to bed later and wake up later. You need to! It's a fact! Some teenagers have a lot of trouble sleeping. These troubles could come about when trying to get to sleep or staying asleep.

It's hard for all of us to get out of bed sometimes. A nice lie in at the weekend can help to make up for lost sleep. The trouble is, if you're tired after waking up, it might mean you fall asleep at school or college. Poor sleep will make it hard for you to concentrate.

Your parents said that you and your family were interested in helping me find out a bit more about how you sleep.

This is your **sleep diary!** It is for you to record information about your sleep. It will help you, your family and me to better understand what your sleep is like. Feel free to ask someone to help if you need it.

If you have any questions:

Hayley Moulding
(029 20 688067 or MouldingHA@cardiff.ac.uk).

Thank you for all your help!

The sleep diary will help me know when you go to sleep and wake up. A sleep diary is a place where you write down information about your sleep. Most importantly, I will be able to use this sleep diary and compare it to the actigraphy watch which you wear. Having both of these, the watch and diary, I will get lots and lots of information about your sleep!

The sleep diary is divided into two. There is one page that needs to be filled out in the evening, and the second page to be filled out in the morning. There is also a sheet to write any notes about the nights sleep or anything out of the ordinary .i.e. if you go on holiday, if there is an especially late night, etc…

I ask that this is done for the two weeks that you are wearing the actigraphy watch. In this booklet, **pages 3 and 4** are examples of how to fill out the sleep diary sections. The week3 pages are spares!

This is an example for one week: three weeks were included in each diary during the study.

Page 5

WEEK 1: Evening Date: ☐☐/☐☐/☐☐

EXAMPLE Fill out in the evening about the daytime activities

Date (DD/MM/YY)							
Did you take off the watch today? Why? Times?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Was today a normal day? .i.e. school/work?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Any caffeinated drinks? How many?							
Any alcoholic drinks? How many?							
Did you have a nap today? When?							
Did you exercise today? What?							
Any medication taken today?							

Page 6

WEEK 1: Morning Date: ☐☐/☐☐/☐☐

EXAMPLE Fill out in morning about that morning and previous evening

Date (DD/MM/YY)							
Time that I got into bed (night before)							
Time that I turned the lights off (night before)							
Time I went to sleep (night before)							
Time I woke up in the morning (today)							
Time I got out of bed in the morning (today)							
How would you rate your quality of sleep?	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good
How refreshed his morning do you feel?	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed
Did you wake up on your own? With an alarm, or parent?							
Was this a normal night for you? Comment .i.e. waking up, late night?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

WEEK 1: Date: ☐☐/☐☐/☐☐

Any notes about daytime sleepiness or night time observations…
(please note day, date and time if possible please)

Thank you very much!

NAME: ID:

Parent (on behalf of child) Sleep Diary

ECHO STUDY

This is a sleep diary to record the sleep and wake activities of your child in. Your child has a fun colouring in sleep diary which will help to see their sleeping habits, but I need a bit more information from yourselves please! This following pages will guide you through what I ask to be done over the next two weeks! Thank you.

Teenagers need to sleep for longer. They naturally go to bed later and wake up later. They actually need to! It is a biological requirement and fact! Some teenagers have a lot of trouble sleeping. These troubles could come about when trying to get to sleep or staying asleep.

It's hard for all of us to get out of bed sometimes. A nice lie in at the weekend can help to make up for lost sleep. The trouble is, if you're tired after waking up, it might mean you fall asleep at school or college. Poor sleep will make it hard for teenagers to concentrate at school.

This is the sleep diary. Please read through this, ask questions and if you have any concerns, contact me using the details below.

If you have any questions:

Hayley Moulding
(029 20 688067 or MouldingHA@cardiff.ac.uk).

Thank you for all your help!

The sleep diary will help me know when your child goes to sleep and wake up. A sleep diary is a place where you write down information about sleep. Most importantly, I will be able to use this sleep diary and compare it to the actigraphy watch which your child is currently wearing. Having both of these will ensure I get the maximise information I can about your child's sleep.

The sleep diary is divided into two. There is one page that needs to be filled out in the evening, and the second page to be filled out in the morning. There is also a sheet to write any notes about the nights sleep or anything out of the ordinary .i.e. if you go on holiday, if there is an especially late night, etc…

I ask that this is done for the two weeks that you are wearing the actigraphy watch. In this booklet, **pages 3 and 4** are examples of how to fill out the sleep diary sections. The week3 pages are spares!

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WEEK 1: Evening Date: ☐☐/☐☐/☐☐

EXAMPLE Fill out in the evening about the daytime activities

Date (DD/MM/YY)							
Did you take off the watch today? Why? Times?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Was today a normal day? .i.e. school/work?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Any caffeinated drinks? How many?							
Any alcoholic drinks? How many?							
Did you have a nap today? When?							
Did you exercise today? What?							
Any medication taken today?							

Page 6

WEEK 1: Morning Date: ☐☐/☐☐/☐☐

EXAMPLE Fill out in morning about that morning and previous evening

Date (DD/MM/YY)							
Time that I got into bed (night before)							
Time that I turned the lights off (night before)							
Time I went to sleep (night before)							
Time I woke up in the morning (today)							
Time I got out of bed in the morning (today)							
How would you rate your quality of sleep?	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good	☐ Very poor ☐ Poor ☐ Fair ☐ Good ☐ Very good
How refreshed his morning do you feel?	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed	☐ Not all refreshed ☐ Slightly refreshed ☐ Somewhat refreshed ☐ Refreshed ☐ Very refreshed
Did you wake up on your own? With an alarm, or parent?							
Was this a normal night for you? Comment .i.e. waking up, late night?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

WEEK 1: Date: ☐☐/☐☐/☐☐ 

Any notes about daytime sleepiness or night time observations…
(please note day, date and time if possible please)

Thank you very much!