# Excitatory-inhibitory balance in children with 22q11.2 deletion syndrome

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**2019**

Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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# Acknowledgements

This PhD has been a something of a marathon rather than a sprint and numerous people have been involved along its course from helping me to develop the project proposal, secure funding for the work, gain ethical approval, recruit, collect and analyse the data, as well as write this thesis.

Particular thanks must be given to Hayley Moss, Sam Chawner, Adam Cunningham, Rachael Adams and my other ECHO/DEFINE/IMAGINE colleagues for their help setting up the study, recruiting participants and collecting data; to Peter Hobden, John Evans, Gavin Perry, Loes Koelewijn, Gemma Williams, Sonya Foley, Laura Bloomfield and my other CUBRIC colleagues for their assistance with data collection and analysis; to my friends and colleagues at the DPMCN, particularly Miriam Cooper, Olga Eyre, Kim Kendall, Jude Harrison, Sharifah Agha, Lucy Riglin, Joanna Martin, Katie Lewis and Naomi Warne for their words of wisdom, tea and sympathy; to my supervisors Professor Sir Mike Owen, Professor Marianne van den Bree, Professor David Linden and Professor Krish Singh who have been a constant source of guidance and inspiration; to my mentors, Professor Ian Jones and Professor Jeremy Hall for their advice and encouragement; to the Wellcome Trust for funding my fellowship; and most importantly to all the families who gave up their valuable time to participate in this research.

To my mother Yvonne, thank you for supporting me through many, many years of education and for taking time to proof-read this thesis. To my husband Sam, thank you for your patience and tolerance of the late nights, weekends and holidays spent working on this project and for helping me to get over the finishing line! This thesis is dedicated to the loved ones we lost and welcomed during my PhD, I hope this makes them proud.

# Contributions

I obtained funding for the work presented in this thesis from the Wellcome Trust, through a Clinical Research Training Fellowship. I obtained ethical approval for the project from the South East Wales National Health Service (NHS) Research Ethics Committee.

In preparation for this work, I received training in MEG data acquisition. I collected the majority of the MEG data described in this thesis with the exception of data collected during periods of leave (e.g. maternity leave), when colleagues collected data from a small number of participants on my behalf. I also received training in MRI safety procedures. Acquisition of MRI data was performed by experienced MRI operators at CUBRIC. I assisted with MRI data collection, performed safety screening and MRI familiarisation in the 'mock' scanner. I undertook participant recruitment and coordinated brain imaging visits including arranging travel, accommodation and expenses for participating families.

Neurocognitive data were collected by members of the ECHO field team, either during imaging visits or as part of a home visit. I extracted and cleaned all neurocognitive variables presented in this thesis for analysis. Psychiatric interview data were also primarily collected by members of the ECHO field team. I conducted psychiatric interviews for approximately ten individuals and together with the ECHO field team, I helped to score completed CAPA interviews and provided advice on assigning psychiatric diagnoses based on DSM-IV-TR criteria. I consensus-coded the majority of the ADI-R data and helped with scoring. I extracted and cleaned data on psychiatric diagnoses and derived symptom counts for analysis in the experimental chapters.

I carried out all literature reviews and summarised all background information contained in this thesis. I processed, analysed and summarised all of the data presented in the experimental chapters and interpreted the results. This thesis was written by me with the guidance and support of my supervisors.

# Publications during the PhD

**Joanne L Doherty** and Michael J Owen. "Genomic Insights into the Overlap between Psychiatric Disorders: Implications for Research and Clinical Practice." *Genome Medicine* 6, no. 4 (2014): 29. https://doi.org/10.1186/gm546.

**Joanne L Doherty** and Michael J Owen. "The Research Domain Criteria: Moving the Goalposts to Change the Game." *British Journal of Psychiatry* 204, no. 3 (2014). https://doi.org/10.1192/bjp.bp.113.133330.

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Samuel Chawner, **Joanne L Doherty**, Hayley Moss, Maria Niarchou, James Walters, Michael J Owen, Marianne B Van Den Bree. "Childhood Cognitive Development in 22q11.2 Deletion Syndrome: a Case-Control Study." *British Journal of Psychiatry* 211 no. 4 (2017). https://doi.org/10.1192/bjp.bp.116.195651.

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**Joanne Doherty**, Miriam Cooper and Anita Thapar. "Advances in our understanding of the genetics of childhood neurodevelopmental disorders." *Evidence-Based Mental Health* 21 no. 4 (2018). https://doi.org/10.1136/ebmental-2018-300067.

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Maria Niarchou, Samuel JRA Chawner, **Joanne L Doherty**, Anne M Maillard, Sebastien Jacquemont, Wedy K Chung, LeeAnne Green-Snyder, Raphael A Bernier, Robin P Goin-Kochel, Ellen Hanson, David E Linden Stefanie Linden, Lucy Raymond, David Skuse, Jeremy Hall, Michael J Owen and Marianne Van Den Bree. "Psychiatric disorders in children with 16p11.2 deletion and duplication." *Translational Psychiatry* (2019). https://doi.org/10.1038/s41398-018-0339-8.

# Summary

22q11.2 deletion syndrome (22q11.2DS) is a copy number variant syndrome affecting approximately 1 in 4000 live births. It has a variable phenotype in terms of its physical, cognitive and psychiatric manifestations. People with 22q11.2DS have a range of cognitive difficulties. They also have extremely high rates of psychopathology, particularly attention deficit hyperactivity disorder, autism spectrum disorder, anxiety disorders and psychotic disorders. The mechanisms underlying the risks of cognitive impairment and psychopathology are not well-understood. The balance between excitation and inhibition in the brain may be affected in 22q11.2DS and could underlie its cognitive and psychiatric features.

In this thesis markers of excitatory-inhibitory (E-I) balance were investigated in children with 22q11.2DS (probands) and typically developing children (controls). It was hypothesised that probands would have alterations in E-I balance and that the severity of these alterations would be associated with cognitive and psychiatric features. The phenotypes of children taking part in the brain imaging study was first compared to those of children who did not participate in brain imaging to assess the representativeness of the imaging sample. Resting-state brain networks and visually-induced gamma oscillations were then investigated using magnetoencephalography (MEG) and gamma-amino-butyric acid (GABA) concentrations were investigated using magnetic resonance spectroscopy (MRS). Between-group comparisons were performed and the relationships between markers of E-I balance, psychopathology and cognitive impairment were explored using linear regression.

The phenotypes of children with 22q11.2DS who participated in brain imaging were broadly similar to those who did not participate, suggesting that the imaging sample does not represent a highly-functioning subsample. Compared with controls, probands had alterations in resting-state networks in the delta, alpha and beta bands which were associated with anxiety, social communication problems and cognitive deficits. In the gamma band, there were reductions in the total induced gamma power in probands, which was similarly associated with social communication and cognitive difficulties. There were no alterations in GABA concentrations in 22q11.2DS, suggesting that further work is needed to better understand the mechanisms underlying excitatory-inhibitory imbalance in 22q11.2DS.

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# Glossary of abbreviations

| | |
|---|---|
| **22q11.2DS** | 22q11.2 Deletion Syndrome |
| **3T** | 3 Tesla |
| **7T** | 7 Tesla |
| **AAL** | Automated Anatomical Labelling |
| **aCGH** | Array Comparative Genomic Hybridization |
| **ADHD** | Attention Deficit Hyperactivity Disorder |
| **ADI-R** | Autism Diagnostic Interview – Revised |
| **ADOS** | Autism Diagnostic Observation Schedule |
| **ASD** | Autism Spectrum Disorder |
| **BD** | Bipolar Disorder |
| **BOLD** | Blood Oxygen Level Dependent |
| **CAPA** | Child and Adolescent Psychiatric Assessment |
| **CANTAB** | Cambridge Neuropsychological Test Automated Battery |
| **CNV** | Copy Number Variant |
| **COMT** | Catecholamine Methyl Transferase |
| **Cr** | Creatine |
| **CSF** | Cerebrospinal Fluid |
| **CUBRIC** | Cardiff University's Brain Research Imaging Centre |
| **DC** | Direct Current |
| **DGCR8** | Di George Critical Region 8 |
| **DMN** | Default Mode Network |
| **DNA** | Deoxyribonucleic Acid |
| **DPMCN** | Department of Psychological Medicine and Clinical Neurosciences |
| **DSM-IV-TR** | Diagnostic and Statistical Manual (4$^{th}$ edition) – Text Revision |
| **DTI** | Diffusion Tensor Imaging |
| **ECHO study** | Experiences of people with copy number variants study |
| **EEG** | Electroencephalography |

| | |
|---|---|
| **E-I** | Excitatory-Inhibitory |
| **ENIGMA** | Enhancing Neuro-Imaging Genetics through Meta-Analysis |
| **FISH** | Fluorescent In-Situ Hybridization |
| **fMRI** | Functional Magnetic Resonance Imaging |
| **FSIQ** | Full-Scale Intelligence Quotient |
| **FSPGR** | Fast-Spoiled Gradient echo |
| **GABA** | Gamma-Amino-Butyric Acid |
| **GABA+** | Gamma-Amino-Butyric Acid plus macromolecules |
| **GAD** | Glutamic Acid Decarboxylase |
| **Glx** | Glutamate/glutamine |
| **GMM** | Gaussian Mixture Modelling |
| **IBBC** | International Brain and Behaviour Consortium |
| **ICA** | Independent Component Analysis |
| **ID** | Intellectual Disability |
| **IPSCs** | Induced Pluripotent Stem Cells |
| **IQ** | Intelligence Quotient |
| **IQR** | Inter-Quartile Range |
| **LCMV** | Linearly Constrained Minimum Variance |
| **LCR** | Low Copy Repeat |
| **MDD** | Major Depressive Disorder |
| **MEG** | Magnetoencephalography |
| **MEGA-PRESS** | Mescher-Garwood Point Resolved Spectroscopy |
| **Mi-RNA** | Micro-Ribonucleic Acid |
| **MMN** | Mismatch Negativity |
| **MNI** | Montreal Neurological Institute |
| **MP-RAGE** | Magnetization Prepared Rapid Acquisition Gradient Echo |
| **MRI** | Magnetic Resonance Imaging |
| **MRS** | Magnetic Resonance Spectroscopy |
| **MTS** | Match to Sample |
| **NAA** | N-Acetylaspartate |
| **NGS** | Next Generation Sequencing |
| **NMDA** | N-Methyl-D-Aspartate |

| | |
|---|---|
| **OCD** | Obsessive Compulsive Disorder |
| **ODD** | Oppositional Defiant Disorder |
| **PET** | Positron Emission Tomography |
| **PIQ** | Performance Intelligence Quotient |
| **PRODH** | Proline Dehydrogenase |
| **PV** | Parvalbumin |
| **ROI** | Region of Interest |
| **RTI** | Reaction Time |
| **RVP** | Rapid Visual Processing |
| **SCQ** | Social Communication Questionnaire |
| **SD** | Standard Deviation |
| **SNP** | Single Nucleotide Polymorphism |
| **SOC** | Stockings of Cambridge |
| **SPECT** | Single Photon Emission Computed Tomography |
| **SRS** | Social Responsiveness Scale |
| **SWM** | Spatial Working Memory |
| **SVD** | Singular Value Decomposition |
| **SZ** | Schizophrenia |
| **TBX1** | T-Box 1 |
| **TE** | Echo Time |
| **TMS** | Transcranial Magnetic Stimulation |
| **TR** | Repetition Time |
| **WASI** | Wechsler Abbreviated Scale of Intelligence |
| **WCST** | Wisconsin Card Sorting Test |
| **VBM** | Voxel-Based Morphometry |
| **VIQ** | Verbal Intelligence Quotient |

# 1 Introduction

## 1.1 Copy number variants

### 1.1.1 What are copy number variants?

Copy number variants (CNVs) are submicroscopic deletions or duplications of segments of chromosomes, which constitute a major source of variation between individuals. A high proportion of the genome is subject to copy number variation and this can arise both meiotically (in gametes) or mitotically (in somatic cells). Each CNV may range in size from 1 kilobase (Kb) to several megabases (Mb). Recurrent CNVs occur most often due to non-allelic homologous recombination events in which unmatched regions of chromosomes are mistakenly recombined during meiosis. These events often occur at regions of low-copy repeats (LCRs). LCRs are segmental duplications that occur at several points in the genome and are therefore particularly susceptible to genomic rearrangement.

CNVs can be inherited, or may occur *de novo*. They can be detected using a range of different techniques such as fluorescent in-situ hybridization (FISH), array comparative genomic hybridization (aCGH), virtual karyotyping with single-nucleotide polymorphism (SNP) arrays and more recently with next-generation sequencing (NGS).

CNVs can result in chromosomes having too many or too few dosage-sensitive genes. This may be advantageous, indeed CNVs are thought to be important in human evolution, however, they may also have a negative impact on human development and disease. Of particular interest for the purpose of this thesis is the association between CNVs, neurodevelopmental and psychiatric disorders.

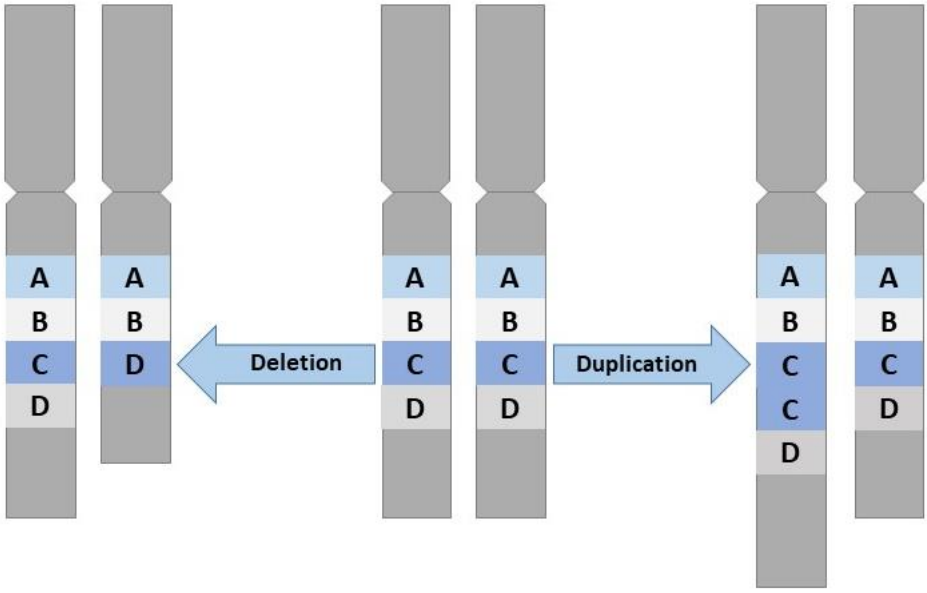


*Figure 1-1 Copy number variant formation*

*The cartoon shows a segmental deletion and duplication, whereby the pair of chromosomes in the centre have two copies of genes A-D, the pair on the left have only one copy of gene C and the pair of chromosomes on the right have three copies of gene C.*

### 1.1.2 What are the associations between copy number variants, psychopathology and cognitive impairment?

It has been shown that the overall burden, size and location of CNVs are associated with a broad spectrum of neurodevelopmental and psychiatric phenotypes including schizophrenia (International Schizophrenia Consortium, 2008; Walsh *et al.*, 2008; Kirov *et al.*, 2009), autism spectrum disorder (ASD, (Pinto *et al.*, 2010; Griswold *et al.*, 2012; Sanders *et al.*, 2015)), attention deficit hyperactivity disorder (ADHD , (Williams *et al.*, 2010; Lionel *et al.*, 2011; Jarick *et al.*, 2014)), cognitive impairment (Stefansson *et al.*, 2014; Kendall *et al.*, 2017), and developmental delay (Cooper *et al.*, 2011). People with neurodevelopmental and psychiatric disorders have a higher burden of CNVs than healthy controls (International Schizophrenia Consortium, 2008; Kirov *et al.*, 2009; Pinto *et al.*, 2010; Williams *et al.*, 2010; Griswold *et al.*, 2012). In particular, they have higher rates of large *de novo* CNVs (Sebat *et al.*, 2007; Xu *et al.*, 2008; Rees *et al.*, 2011; Kirov *et al.*, 2012). Interestingly the presence of CNVs at particular loci is

associated with very high rates of neurodevelopmental and psychiatric phenotypes. For example, CNVs at eleven distinct loci have been robustly associated with schizophrenia risk (Rees *et al.* 2014a), with a further locus at 16p12.1 being recently identified (Rees *et al.*, 2016). Table 1-1 shows the replicated loci associated with schizophrenia with their reported penetrance scores and associated phenotypes (Doherty and Owen 2014).

*Table 1-1 Replicated CNV loci associated with schizophrenia*

| **Locus** | **Copy number change** | **Penetrance for schizophrenia** | **Associations** |
|---|---|---|---|
| 1q21.1 | Deletion/ duplication | 5.2/2.9 | ID, ASD, ADHD |
| 2p16.3 | Deletion | 6.4 | ID, ASD |
| 3q29 | Deletion | 18.0 | ID, ASD |
| 7q11.2 | Duplication | 6.0 | ID, ASD, ADHD, anxiety disorders |
| 15q11.2 | Deletion | 2.0 | ID, ASD, ADHD, OCD |
| 15q11-13 | Duplication | 4.2 | ID, ASD |
| 15q13.3 | Deletion | 4.7 | ID, ASD, ADHD |
| 16p11.2 | Deletion/ duplication | 2.6/8.0 | ID, ASD, ADHD, mood disorders, anxiety disorders |
| 16p13.11 | Duplication | 2.2 | ID, ASD, ADHD |
| 17q12 | Deletion | 4.0 | ID, ASD |
| 22q11.2 | Deletion | 12 | ID, ASD, ADHD, mood disorders, anxiety disorders |

*Abbreviations: ID, intellectual disability; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; OCD, obsessive compulsive disorder. Adapted from Doherty and Owen 2014.*

As can be seen in table 1-1, individual CNVs do not map onto single disorders on a 1:1 basis, rather they seem to confer risk across a spectrum of neurodevelopmental and psychiatric disorders as well as intellectual disability. It is also interesting to note that the penetrance of individual CNVs is extremely

variable, with some CNV carriers exhibiting few or no symptoms, while others are severely impaired.

The causes of this phenotypic variability are not well understood but are crucial to our understanding of neurodevelopmental and psychiatric risk in specific CNV carrying populations and may also give us broader insights into the biological mechanisms underlying risk in the general population. One could hypothesise that genetic factors (such as the nature, quantity, size and location of deleterious genetic variants) and environmental factors (e.g. prematurity, birth trauma, substance use and psychosocial stressors) could act together to alter neurodevelopmental trajectories, with the timing, severity and anatomical location(s) determining the ultimate clinical phenotype. Further investigation integrating genetic findings with cellular, animal, clinical and neuroimaging research will shed further light on whether such a model is plausible.

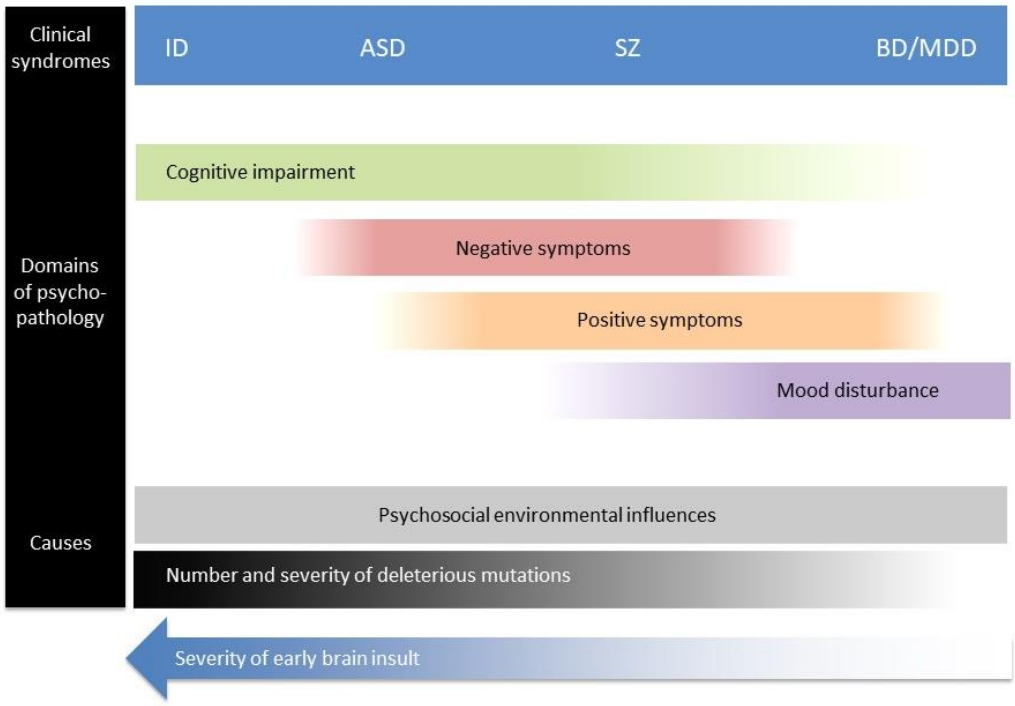


*Figure 1-2 Hypothesised relationship between mutational load, cognitive ability and psychopathology*

*Abbreviations: ID, intellectual disability; ASD, autism spectrum disorder; SZ, schizophrenia; BD/MDD, bipolar disorder/major depressive disorder. Adapted from Doherty and Owen 2014.*

## 1.2 22q11.2 deletion syndrome

### 1.2.1 What is 22q11.2 deletion syndrome?

22q11.2 deletion syndrome (22q11.2DS), also commonly known as velocardiofacial syndrome and Di George syndrome, is one of the most common copy number deletion syndromes, affecting at least 1 in 4000 live births (Botto *et al.*, 2003; Oskarsdottir, 2004; Maisenbacher *et al.*, 2017). It results from a 1.5-3Mb deletion at region q11.2 on chromosome 22. The 22q11.2 region is spanned by four low copy repeats (LCR22 A, B, C and D), making it particularly susceptible to meiotic error during non-allelic homologous recombination (Morrow *et al.*, 2018). The majority of patients diagnosed with 22q11.2DS (90-95%) have *de novo* mutations (McDonald-McGinn *et al.*, 2015), with the remainder inheriting the deletion from a parent.

The typical 3Mb A-D deletion is present in ~ 85% of patients (Edelmann, Pandita and Morrow, 1999; Shaikh *et al.*, 2000) and affects about 45 known protein coding genes, seven miRNA and 10 non-coding genes (Morrow *et al.*, 2018). Nested proximal A-B and A-C deletions are less common but are associated with the major phenotypic features of the A-D deletion. Distal deletions (B-D or C-D) have lower penetrance than the proximal deletions and are more likely to be inherited, presumably due to higher reproductive fitness in distal deletion carriers (McDonald-McGinn *et al.*, 2015).

As with many CNV syndromes, the 22q11.2DS phenotype is extremely variable, involving multiple organ systems. Some of the most common physical manifestations include: structural brain abnormalities, seizures, facial dysmorphology, velopharyngeal insufficiency, parathyroid dysfunction, immune deficiencies, conotruncal cardiac defects, renal and gastrointestinal abnormalities (McDonald-McGinn *et al.*, 2015). The mechanisms underlying the variability in the physical features of 22q11.2DS are, as yet, not well understood**.**

### 1.2.2 What is the relationship between 22q11.2 deletion syndrome, psychopathology and cognitive impairment?

As with the physical manifestations, psychiatric, neurodevelopmental and cognitive outcomes are extremely variable in 22q11.2DS. Data from increasingly large samples of children and adults with 22q11.2DS (including data pooled as part of the 22q11.2DS International Brain and Behaviour Consortium (IBBC)) has revealed that schizophrenia (and related psychotic disorders), attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), anxiety disorders and cognitive impairment are highly prevalent in 22q11.2DS (Swillen *et al.*, 1997; Vorstman *et al.*, 2006; De Smedt *et al.*, 2007, 2009; Antshel *et al.*, 2007a; Antshel *et al.*, 2007b; Niarchou *et al.*, 2014; Schneider *et al.*, 2014).

**Schizophrenia**

Schizophrenia is a severe and enduring mental illness characterized by 'positive' symptoms (such as hallucinations and delusions), 'negative' symptoms (such as flattened affect and avolition) and cognitive impairment. In the 1990s, it was observed that a very high proportion of adults with 22q11.2DS had schizophrenia, and this finding has been consistently replicated (Murphy, Jones and Owen, 1999; Monks *et al.*, 2014; Schneider *et al.*, 2014). The clinical features of schizophrenia in 22q11.2DS do not differ from those seen in the non-deleted population (Bassett *et al.*, 2003; Monks *et al.*, 2014). The current estimate for lifetime prevalence of schizophrenia is approximately 25% in 22q11.2DS compared to about 1% in the general population (Schneider *et al.*, 2014). Furthermore, Rees *et al.* (2014a) found the prevalence of the 22q11.2 deletion to be 10-20 times higher in patients with schizophrenia than the general population. 22q11.2DS is therefore one of the strongest known risk factors for schizophrenia. Interestingly, Rees and colleagues found that the reciprocal duplication of chromosome 22 was less common in patients with schizophrenia than controls, suggesting that it is associated with a lower risk of schizophrenia (Rees *et al.,* 2014b). This further

highlights the importance of studying this genetic locus to better understand resilience as well as risk, and to identify potential novel therapeutic targets.

Since carriers of the deletion are often identified as young children, there has been much interest in 22q11.2DS as a high-risk population in which the clinical, cognitive and neurobiological antecedents of schizophrenia can be studied. Studies of children with 22q11.2DS have found elevated rates of psychotic symptoms even in the absence of a schizophrenia diagnosis (Green *et al.,* 2009; Baker and Skuse 2005; Gothelf *et al.,* 2007a; Lewandowski *et al.,* 2007; Schneider *et al.,* 2014; Chawner *et al.,* 2019). Identification of additional risk factors for psychosis in 22q11.2DS would have clear implications for both research and clinical practice.

**Attention deficit hyperactivity disorder (ADHD)**

ADHD is a childhood-onset neurodevelopmental disorder in which clinically significant levels of inattention, hyperactivity and/or impulsivity are present across more than one setting to a degree that causes significant impairment. It can be divided into three subtypes: inattentive, hyperactive-impulsive or combined according to the symptom dimensions that are present. The background prevalence of ADHD in the general population is 3.4% (Polanczyk *et al.*, 2015). The ADHD rate in 22q11.2DS is considerably higher than that of the general population, indeed ADHD is one of the most common childhood mental disorders in 22q11.2DS, affecting 37% of children in the IBBC sample. The symptoms persist over time with 24% of adolescents and 16% of adults with 22q11.2DS also meeting diagnostic thresholds for ADHD (Schneider *et al.*, 2014).

Unlike schizophrenia, the clinical presentation of ADHD in 22q11.2DS does seem to differ from that of clinically ascertained and population samples (Antshel *et al.,* 2007b; Niarchou *et al.,* 2015). Children with 22q11.2DS have higher rates of the inattentive subtype, higher rates of comorbid anxiety disorders and lower rates of oppositional defiant and conduct disorder symptoms than either clinically ascertained or population samples (Niarchou *et al.*, 2015). These differences are

not accounted for by differences in levels of intellectual disability. Recently an association has been found between ADHD and the development of psychotic symptoms in 22q11.2DS (Niarchou *et al.*, 2018). This finding merits replication but, if confirmed, has important clinical and research implications.

**Autism spectrum disorder**

Autism spectrum disorder is a pervasive neurodevelopmental disorder in which there are deficits in social interaction and communication as well as patterns of restricted and repetitive behaviours. Reported rates of ASD in 22q11.2DS vary greatly between studies from 15-50% (Fine *et al.,* 2005; Vorstman *et al.,* 2006; Antshel *et al.,* 2007a; Ousley *et al.,* 2017) compared with a population prevalence of approximately 1% (Baxter *et al.*, 2015).

Although the high rates of social communication problems are well documented in 22q11.2DS, there has been much debate in the literature as to whether these difficulties meet strict criteria for a diagnosis of ASD or rather reflect prodromal psychotic symptoms, expressive communication problems secondary to velopharyngeal insufficiency, deficits in attention shifting or anxiety symptoms (Ogilvie *et al.*, 2000; Eliez, 2007; Angkustsiri *et al.*, 2014). A recent study addressed this in 22q11.2DS using stringent gold-standard assessment tools - the Autism Diagnostic Interview-Revised [ADI-R; (Lord, Rutter and Le Couteur, 1994)] and the Autism Diagnostic Observation Schedule [ADOS; (Lord *et al.*, 1989)] - as well as a clinician's best-estimate diagnosis. The authors found that 17.9% of participants met criteria for an ASD diagnosis (Ousley *et al.,* 2017), suggesting ASD rates are indeed high in 22q11.2DS. Furthermore, another recent study found no association between ASD symptoms in childhood and later psychotic symptoms in 22q11.2DS, suggesting that social communication problems in 22q11.2DS do not reflect prodromal symptoms of schizophrenia (Fiksinski *et al.*, 2017).

**Anxiety disorders**

Anxiety disorders are highly prevalent in 22q11.2DS, with high rates of generalised anxiety disorder, specific phobia, social phobia, panic disorder,

separation anxiety and obsessive compulsive disorder being reported (Angkustsiri *et al.*, 2012; Niarchou *et al.*, 2014; Schneider *et al.*, 2014; Stephenson *et al.*, 2015). In the pooled IBBC sample, anxiety disorders were prevalent across the age spectrum but were particularly common in childhood and adolescence (Schneider *et al.*, 2014). Many participants had multiple comorbid anxiety disorders. Anxiety disorders have been associated both with mood disorders and schizophrenia in 22q11.2DS (Gothelf *et al.,* 2007a; Schneider *et al.,* 2014; Stephenson *et al.,* 2015; Chawner *et al.,* 2019), with studies finding anxiety symptoms to be predictive for the onset of psychotic symptoms at follow-up (Gothelf *et al.,* 2007a; Chawner *et al.,* 2019). The presence of anxiety symptoms may therefore be a useful prognostic indicator for psychosis risk.

**Cognitive impairment**

Children with 22q11.2DS commonly have developmental delay and special educational needs. In early childhood, language delay is frequently reported and is independent of velopharyngeal problems (Solot *et al.,* 2000). Several studies report that IQ follows a normal distribution in 22q11.2DS but is shifted approximately 30 points to the left of the general population (Swillen *et al.,* 1997; De Smedt *et al.,* 2007; Niarchou *et al.,* 2014). Particular deficits have been found in mathematical skills (De Smedt *et al.,* 2009; Woodin *et al.,* 2001) and language comprehension (Glaser *et al.*, 2002). Cognitive development in 22q11.2DS is variable between individuals with deviant trajectories being reported compared with those of the typically-developing population (Duijff *et al.,* 2012; Vorstman *et al.,* 2015; Swillen and McDonald-McGinn 2015; Chawner *et al.,* 2017). In the IBBC sample, people with 22q11.2DS and a psychotic disorder had lower IQ than those without psychosis and a steeper decline in verbal IQ in childhood (Vorstman *et al.*, 2015). It has been proposed that children with cognitive deterioration may be at heightened risk of developing psychosis, suggesting that regular monitoring of cognitive ability should be performed and cognitive remediation strategies developed (Swillen and McDonald-McGinn 2015; Vorstman *et al.,* 2015). However, clear evidence of cognitive deterioration in 22q11.2DS was not replicated in a large sample of children with 22q11.2DS who were compared to

sibling controls (Chawner *et al.,* 2017), indicating that these suggestions may be premature. Clear associations between cognitive ability and other mental disorders, for example ASD, ADHD or anxiety, have not been robustly demonstrated (Duijff *et al.,* 2012; Niarchou *et al.,* 2014; Chawner *et al.,* 2017). The nature of the relationship between cognitive ability and psychopathology remains to be elucidated due to the lack of large, adequately controlled longitudinal studies with adequate follow-up periods.

### 1.2.3 How does 22q11.2 deletion syndrome confer risk of psychopathology and cognitive impairment?

The mechanisms by which 22q11.2 deletion increases risk of psychopathology and cognitive impairment are still not well understood. The 22q11.2 region contains about 60 genes but as yet no gene or combination of genes has been found to be either necessary or sufficient for the neurodevelopmental, psychiatric or cognitive phenotype. People with the deletion have only one copy of the genes within the deleted region (hemizygosity). It may be that reduced dosage of one or more genes in the region (haploinsufficiency) leads to the clinical manifestations, although other possibilities such as the unmasking of a recessive allele and position effects are also possible.

While there are many interesting candidate genes in the region - including *PRODH, COMT, DGCR8* and *TBX1* - none of these has been convincingly implicated as the sole factor underlying the increased risk of neurodevelopmental, psychiatric or cognitive phenotypes. It therefore remains possible that the increased risk of mental disorders and cognitive impairment is conferred by the impact of the deletion on more than one and possibly several genes.

Another possibility is that sets of functionally related genes (sometimes known as pathways) are particularly impacted by 22q11.2DS and other pathogenic CNVs. A recent pathway analysis across different CNVs has pointed to the role of synaptic genes influencing the balance of cortical excitation and inhibition in psychiatric risk, implicating both glutamatergic and GABAergic neurotransmission

(Pocklington *et al.*, 2015). This is an attractive model, since alterations in excitatory-inhibitory balance have been reported in idiopathic schizophrenia, ADHD and ASD, and therefore may help to explain the overlapping symptoms experienced by people with 22q11.2DS, though which gene or genes in the 22q11.2 region are implicated in these pathways remains unclear.

### 1.2.4 Why does 22q11.2 deletion syndrome have a pleiotropic outcome?

There has been much interest in what factor or factors influence the variable neurodevelopmental, psychiatric and cognitive outcomes seen in 22q11.2DS. As outlined above, the majority of patients with 22q11.2DS (85%) have a 3Mb deletion, whilst approximately 10% have a nested 1.5Mb deletion. It could be that the size of the deletion influences phenotypic outcome. While few studies have identified significant differences in the psychiatric phenotype between those with a 1.5 or a 3Mb deletion, a recent collaborative brain imaging study did find structural brain differences between carriers of the 1.5 and 3Mb deletions (Sun *et al.*, 2018). Furthermore, another recent study using data from the IBBC found that carriers of the A-D deletion had poorer cognitive performance than A-B carriers (Zhao *et al.*, 2018).

Another possibility is that "second hits" from CNVs outside the 22q11.2 region may affect phenotypic expression. The overall burden of rare CNVs (>200Kb) outside the 22q11.2 region has been associated with intellectual disability in 22q11.2DS (Jensen *et al.*, 2018). Additional CNVs have also been associated with schizophrenia risk in 22q11.2DS. Bassett and colleagues used a large dataset from the IBBC and found that although overall CNV burden was not associated with schizophrenia in 22q11.2DS, people with 22q11.2Ds and schizophrenia had more rare duplications overlapping genes from nervous system gene sets (Bassett *et al.*, 2017).

Genomic studies have clearly indicated the polygenic nature of psychiatric and neurodevelopmental disorders. In schizophrenia, for example, about a quarter of the genetic variance is captured by the combined effects of many hundreds of

common SNPs (Lee *et al.*, 2012), and it is now possible to assay this using the polygenic risk score approach. People with schizophrenia and known pathogenic CNVs have been found to have an excess burden of common risk alleles compared with controls (Tansey *et al.*, 2016; Bergen *et al.*, 2018), but interestingly, schizhoprenia cases with risk CNVs had lower polygenic risk scores than cases without risk CNVs suggesting an interaction between polygenic risk scores and pathogenic CNVs. In people carrying risk CNVs, the polygenic risk score is diminished relative to the effect size of the CNV. In people with 22q11.2 deletions, little additional polygenic risk was required in schizophrenia cases (Bergen *et al.*, 2018). Further analysis of polygenic risk in 22q11.2DS is being conducted in the IBBC sample and should yield interesting results.

The effects of environmental factors have not yet been the focus of much research in 22q11.2DS, but studying the interaction between genes and environment in this at-risk group will be crucial to our understanding of pleiotropy and also of environmental risk factors for neurodevelopmental and psychiatric disorders more generally. As larger samples are collected and combined in large international collaborations, some of these questions can start to be addressed.

As one of the strongest known risk factors for mental disorders, 22q11.2DS is a useful model in which to investigate mechanisms underlying risk of psychopathology. As yet the mechanisms underlying increased risk across the diagnostic spectrum are not well understood but are the focus of much research. Furthermore, as there is extensive pleiotropy, 22q11.2DS offers the opportunity to investigate modifiers of risk and to explore the overlaps between traditional categorical diagnoses. Much more data are needed to explore predictors of risk, including clinical symptoms and intermediate phenotypes, for example neuroimaging abnormalities.

## 1.3 Neuroimaging

### 1.3.1 Why use neuroimaging to study 22q11.2 deletion syndrome?

The brain is a relatively inaccessible organ, making it challenging to study the impact of genetic syndromes on its structure and function and to relate this to clinical and cognitive phenotypes. In recent years, there have been tremendous advances in neuroimaging technology, providing a unique window to brain mechanisms underlying risk of neurodevelopmental and psychiatric disorders. Cross-sectional comparisons between people with 22q11.2DS and controls enable differences in brain structure and function to be detected, while longitudinal neuroimaging studies can track these differences over time. These studies may help to identify neural markers that could be used to predict which CNV carriers are most at risk across the spectrum of neurodevelopmental and psychiatric pathologies. This information would be valuable not only to those with 22q11.2DS, but would also provide important insights into disease mechanisms in idiopathic neurodevelopmental and psychiatric disorders, potentially leading to advances in diagnosis, prognosis and treatment.

### 1.3.2 What neuroimaging tools are available to investigate 22q11.2 deletion syndrome?

**Magnetic resonance imaging**

Magnetic resonance imaging (MRI) is a non-invasive neuroimaging technique that can be used to probe both brain structure and function. It principally uses a strong static magnetic field, radiofrequency pulses and magnetic gradients to alter the spins of protons within water molecules in the body. Energy that is released by protons when they return to their original state can be detected by an MRI receiver coil. Using different sequences to exploit different properties within the signal, detailed information about brain morphology, white matter microstructure, neurochemical composition and blood flow can be ascertained.

Structural MRI creates images from the signal generated by the varying relaxation times of protons in different tissues. This signal conveys both spatial and contrast information. Structural MRI can be used to look for discrete abnormalities of brain structure (qualitative studies), to study the volume or density of particular brain regions (region-of-interest (ROI) studies), to measure volume and density of the whole brain (e.g. voxel-based morphometry, VBM (Ashburner and Friston, 2000)) or surface structure such as cortical folding and thickness (e.g. Freesurfer (Dale, Fischl and Sereno, 1999)).

Diffusion MRI uses diffusion-sensitising magnetic field gradients to measure the displacement of water molecules in brain tissue. Displacement that occurs equally in all directions (e.g. in the cerebrospinal fluid (CSF)) is described as isotropic while displacement that occurs preferentially along a particular axis is known as anisotropic. In white matter, the diffusion of water molecules is constrained and occurs preferentially along the axis of white matter bundles. Studying the relative anisotropy of different brain regions or between individuals gives useful information about white matter microstructure (Jones 2008).

Functional MRI (fMRI) relies on the blood oxygen level dependence (BOLD) signal to infer information about brain activity in particular brain regions (Logothetis, 2003). When a region in the brain is active, the ratio of oxygenated to deoxygenated blood increases. Oxygenated and deoxygenated blood have different magnetic properties. Oxygenated blood interferes with the MRI signal less than deoxygenated blood which results in improved signal in areas with increased blood flow.

Magnetic resonance spectroscopy (MRS) allows measurement of endogenous brain metabolites non-invasively. MRS detects radiofrequency signals that arise from hydrogen nuclear spins within metabolites. These signals have specific frequencies depending on the chemical environment of the nuclei. Experiments can be tailored to isolate particular signals from the resulting spectrum in brain

regions of interest, for example to investigate neurotransmitter concentrations (Agarwal and Renshaw, 2012).

**Nuclear imaging**

Nuclear imaging techniques such as single photon emission computed tomography (SPECT) and positron emission tomography (PET) use radioactive tracers attached to molecules of interest, which are injected intravenously. When these tracers decay, gamma rays are released and can be detected by SPECT and PET systems. Nuclear imaging techniques can be used to study brain metabolism (e.g. using [18F] fluorodeoxyglucose) and neurotransmitter systems (e.g. using [11]C-flumazenil which binds to benzodiazepine receptors) relevant to mental disorders and cognitive function (Newberg *et al.*, 2011; Frankle *et al.*, 2015; Bakker *et al.*, 2018).

**Electroencephalography and magnetoencephalography**

Electroencephalography (EEG) is a non-invasive technique that uses highly sensitive electrodes to detect real-time electrical activity in the brain. When neurons fire synchronously, they generate electrical currents that can be measured using electrodes placed on the scalp. The source of this current is populations of closely-aligned and synchronously firing cortical pyramidal neurons (Kirschstein and Köhling, 2009; Cohen, 2017). EEG studies can be performed at rest, during sleep or during experimental tasks. EEG has excellent temporal resolution, however as EEG signals are affected by the conductance of the scalp and skin, source localization and therefore spatial resolution is limited compared to other techniques such as magnetoencephalopgraphy (MEG) and MRI.

MEG also detects neuronal activity in the brain. However, rather than detecting electrical activity directly, superconducting quantum interference devices (SQUIDs) detect the small magnetic fields generated by the synchronous postsynaptic currents from populations of pyramidal neurons, being most sensitive to currents that are tangential to the cortical surface (Hari and Salmelin,

2012). As with EEG, MEG can be used to record brain activity during rest, sleep and experimental tasks. MEG has a significant advantage over EEG in being able to localize the sources of neuronal activity more precisely using techniques such as beamforming because, unlike the electrical signals measured with EEG, the magnetic field signals measured with MEG pass through the skull and scalp without any distortion. MEG is also sensitive to external magnetic fields and therefore MEG equipment has to be housed in a magnetically shielded room. MEG is relatively insensitive to signals from deep sources and sources parallel to the cortical surface.

The rhythmic neuronal activity patterns commonly measured in EEG or MEG experiments are known as neural oscillations. These oscillations can occur spontaneously in resting-state paradigms or in response to a stimulus or task. Stimulus-related responses can either be evoked or induced. Evoked responses are both time- and phase-locked to the stimulus and are thought to arise from bottom-up sensory processing. Induced oscillations on the other hand, are time- but not phase-locked to the stimulus and are thought to reflect higher order processes (David, Kilner and Friston, 2006). Oscillatory responses (spontaneous or in response to stimuli) occur at different frequencies and are typically grouped according to the following characteristic frequency bands; delta (1-4Hz), theta (4-8Hz), alpha (8-12Hz), beta (12-30Hz) and gamma (>30Hz). Low frequency oscillations are thought to reflect long-range synchronisation between different brain regions whereas higher frequencies reflect synchronisation in local networks (Schnitzler and Gross, 2005).

### 1.3.3 What have neuroimaging studies revealed about brain structure and function in 22q11.2 deletion syndrome?

**Structural magnetic resonance imaging studies**

MRI studies in 22q11.2DS have identified qualitative differences between people with 22q11.2DS and controls. These include midline anomalies such as cavum septum pellucidum and cavum vergae (Chow *et al.*, 1999; van Amelsvoort *et al.*,

2001; Shashi *et al.*, 2004), polymicrogyria (Ghariani *et al.*, 2002; Ehara, Maegaki and Takeshita, 2003; Sztriha *et al.*, 2004), pachygyria (Ehara, Maegaki and Takeshita, 2003; Koolen *et al.*, 2004), ventricular enlargement (Chow *et al.*, 1999) and white matter hyperintensities (van Amelsvoort *et al.*, 2001; Chow *et al.*, 2002). These studies demonstrate widespread structural abnormalities in 22q11.2DS and suggest that early cortical development and neuronal migration may be disrupted in the syndrome.

Quantitative neuroimaging studies of 22q11.2DS have employed both region-of-interest (ROI) and whole-brain (e.g. voxel-based morphometry (VBM)) approaches to better characterise brain structure in deletion carriers. Cross-sectional studies report overall reductions in total grey and white matter volume in 22q11.2DS, with differences between 22q11.2DS and controls being most marked in the white matter (Eliez *et al.,* 2001; Kates *et al.,* 2001). A number of studies have found regional volume differences between people with 22q11.2DS and controls. A meta-analysis of these studies reported significant volumetric reductions of the hippocampus and cerebellum in 22q11.2DS, while conversely, the volume of the corpus callosum was increased in 22q11.2DS (Tan *et al.*, 2009). Recent evidence suggests that the effects of 22q11.2 rearrangements on brain volume are dependent on gene dosage. A study comparing people with 22q11.2 deletions, duplications and healthy controls found that 22q11.2 gene dosage varied positively with intracranial, grey and white matter volumes. Subcortical differences were also seen - 22q11.2 duplication carriers had a significantly larger right hippocampus but smaller right caudate and corpus callosum than 22q11.2 deletion carriers (Lin *et al.*, 2017).

Cortical thickness and surface area have been measured in several small studies of 22q11.2DS with somewhat discrepant findings (Bearden *et al.,* 2007; Bearden *et al.,* 2009; Schaer *et al.,* 2009; Jalbrzikowski *et al.,* 2013). A recent large-scale collaborative effort by the ENIGMA 22q11.2 working group (enigma.ini.usc.edu/ongoing/enigma-22q-working-group) aimed to address some of these inconsistencies by pooling data from ten sites to give a sample of 474

people with 22q11.2DS and 315 controls. This study found that people with 22q11.2DS had thicker cortical grey matter overall but focal thinning in temporal and cingulate regions. Surface area was reduced in 22q11.2DS with the biggest reductions seen in those with the 3Mb compared with the 1.5Mb deletion (Sun *et al.,* 2018).

Abnormal patterns of gyrification have also been reported in 22q11.2DS. Reductions in cortical complexity have been found in the frontal and parietal lobes (Schaer *et al.*, 2006; Srivastava, Buonocore and Simon, 2012) and increased complexity in the occipital lobe (Bearden *et al.,* 2009). Interestingly Schaer *et al.* (2009) found an association between a history of congenital heart disease and gyrification of the parieto-temporal-occipital junction suggesting a role for haemodynamic factors in the structural brain abnormalities seen in 22q11.2DS.

Overall, cross-sectional structural imaging findings suggest a deviant developmental trajectory in 22q11.2DS affecting many different brain regions. However, identifying reliable and reproducible imaging biomarkers for neurodevelopmental and psychiatric disorders in 22q11.2DS requires longitudinal studies in large samples scanned before the onset of symptoms and followed-up for a sufficient period of time in order to differentiate between those who go on to develop psychopathology and those who do not. Such designs also have the advantage of controlling for many confounding variables that affect cross-sectional designs such as the effects of psychotropic medication. Longitudinal studies of 22q11.2DS are currently underway and several reports have already been published, albeit in modestly sized samples with relatively short follow-up periods. Longitudinal studies to date have focused on associations between brain development and psychosis rather than other neurodevelopmental outcomes such as ASD and ADHD, which would require the recruitment of infants and young children.

The first longitudinal study of 22q11.2DS used structural neuroimaging data from a small cohort of children with 22q11.2DS (n=29) who were followed-up five years

later (Gothelf *et al.*, 2007b). Reduction in grey matter volume in the left dorsal prefrontal cortex predicted the severity of psychotic symptoms at follow-up. The magnitude of this change was related to *COMT* genotype and to change in verbal IQ (VIQ). Using multivariate pattern analysis, the authors were subsequently able to predict risk of psychotic symptoms with >94% accuracy (Gothelf *et al.*, 2011). A larger study of young people with 22q11.2DS, siblings and community controls found that reduction in temporal lobe grey matter volume and VIQ predicted the presence of positive psychotic symptoms at three-year follow-up (Kates *et al.*, 2011). In the third published longitudinal study in 22q11.2DS, Flahault and colleagues (Flahault *et al.*, 2012) compared hippocampal development between people with 22q11.2DS and controls, again over a three-year period. They did not find any significant differences in hippocampal development between the groups, however the size of the hippocampal head at baseline was associated with the presence of hallucinations at follow-up. Longitudinal changes in cortical complexity in 22q11.2DS have also been studied. Kunwar and colleagues (Kunwar *et al.*, 2012) found that longitudinal change in gyrification of the left occipital region was negatively correlated with positive prodromal symptoms.

Although these studies suggest longitudinal associations between regional brain volumes, gyrification patterns and psychotic symptoms, replication in larger samples and over longer time periods will be necessary in order to determine whether the same regional variations in childhood can reliably predict the development of psychotic disorders in adulthood. If this can be demonstrated, in addition to informing our understanding of the pathophysiology of schizophrenia, serial structural scanning has the potential to provide valuable prognostic information for clinicians and families. Data on the relationships between 22q11.2DS and other neurodevelopmental outcomes are currently lacking but would be interesting to investigate in future studies with infants and young children using suitably adapted brain imaging procedures.

**Diffusion MRI studies**

Studies of white matter microstructure using diffusion tensor imaging techniques have found widespread differences between people with 22q11.2DS and controls including abnormalities of the white matter pathways connecting the frontal and temporal lobes, limbic structures and fronto-occipital connections (Barnea-Goraly *et al.,* 2003; Simon *et al.,* 2005, 2008; Sundram *et al.,* 2010; da Silva Alves *et al.,* 2011; Kikinis *et al.,* 2012; Radoeva *et al.,* 2012; Ottet *et al.,* 2013; Villalon-Reina *et al.,* 2013; Jalbrzikowski *et al.,* 2014; Perlstein *et al.,* 2014; Roalf *et al.,* 2017; Tylee *et al.,* 2017; Nuninga *et al.,* 2017). Furthermore, graph theoretical approaches have been employed, using data from both structural and diffusion MRI, providing preliminary evidence for alterations in the structural organisation of brain networks in 22q11.2DS (Ottet *et al.,* 2013).

White matter abnormalities have been associated with psychopathology and cognitive impairment in several DTI studies of 22q11.2DS (Da Silva Alves *et al.*, 2011; Jalbrzikowski *et al.*, 2014; Kates *et al.*, 2015; Nuninga *et al.*, 2017; Olszewski *et al.*, 2017; Roalf *et al.*, 2017; Tylee *et al.*, 2017). These studies have found associations between the severity of psychotic symptoms and atypical white matter microstructure in a number of regions including the inferior longitudinal fasciculus (da Silva Alves *et al.,* 2011; Jalbrzikowski *et al.,* 2014; Olszewski *et al.,* 2017; Tylee *et al.,* 2017), inferior frontooccipital fasciculus (Olszewski *et al.*, 2017), uncinate fasciculus (Perlstein *et al.*, 2014; Roalf *et al.*, 2017), internal capsule (Perlstein *et al.*, 2014) and cingulum (Kates *et al.*, 2015; Roalf *et al.*, 2017). These studies also found that alterations in matter microstructure were associated with visuospatial awareness (Simon *et al.*, 2008) and cognitive decline in 22q11.2DS (Nuninga *et al.*, 2017).

**Functional MRI studies**

FMRI studies have investigated both task-based and resting-state BOLD responses in people with 22q11.2DS. Task-based fMRI studies have found abnormal activation patterns across a number of task conditions in 22q11.2DS including working memory (Kates *et al.*, 2007; Azuma *et al.*, 2009; Harrell *et al.*, 2017), face

processing (Andersson *et al.*, 2008), reward processing (van Duin *et al.*, 2016), emotion regulation (Coman *et al.*, 2010) and self-referential processing (Schneider *et al.*, 2012). Studies of resting state functional connectivity in 22q11.2DS have found differences between people with 22q11.2DS and controls across several networks including the default-mode, sensorimotor, visuospatial, self-referential and visual networks (Debbané *et al.*, 2012; Scariati *et al.*, 2014; Schreiner *et al.*, 2014; Padula *et al.*, 2015; Mattiaccio *et al.*, 2016). Atypical connectivity in the default-mode network in 22q11.2DS has been associated with thought disturbance (Mattiaccio *et al.*, 2016), executive function (Debbané *et al.*, 2012) and social competence (Schreiner *et al.*, 2014) in 22q11.2DS. The relationship with prodromal psychotic symptoms is less clear with one study reporting an association (Debbané *et al.*, 2012) and another study by the same research group failing to replicate this (Padula *et al.*, 2015). However, overall these studies provide further evidence for alterations in brain networks in 22q11.2DS.

**Magnetic resonance spectroscopy**

To date there have been relatively few neurochemical imaging studies in 22q11.2DS. Da Silva Alves and colleagues (da Silva Alves *et al.,* 2011) used MRS to measure cortical neurometabolite concentrations between 22 adults with 22q11.2DS and 23 healthy controls. While there were no between-group differences in metabolite concentrations between those with 22q11.2DS and controls, the 12 people with 22q11.2DS and a schizophrenia diagnosis had significantly higher concentrations of glutamate/glutamine (Glx) in the hippocampal region compared to the 10 participants with 22q11.2DS and no schizophrenia diagnosis. This is an interesting finding as abnormalities of the glutamatergic system have been implicated in schizophrenia. However, the sample size in this study was small and all those with a schizophrenia diagnosis were taking antipsychotic medication, potentially confounding the results. In another MRS study, Shashi and colleagues (Shashi *et al.*, 2012) studied a sample of 26 children with 22q11.2DS and found that compared to 23 matched controls, their absolute levels of N-acetylaspartate (NAA, a marker of cortical maturation)

were significantly elevated in the dorsolateral prefrontal cortex. Higher NAA levels were associated with poorer global functioning and higher rates of ADHD. These studies provide very preliminary evidence for abnormalities of neural metabolite concentrations in 22q11.2DS but replication in larger samples and across different age ranges is warranted to confirm these findings and to investigate their relationship with neurodevelopmental outcomes.

**Nuclear imaging**

People with 22q11.2DS have reduced dosage of the *COMT* gene, which encodes an enzyme responsible for the degradation of dopamine. Dopaminergic pathways have been implicated in schizophrenia, so it has been hypothesised that disruption to dopaminergic systems may underlie risk of psychosis in 22q11.2DS ( (Boot *et al.*, 2010, 2011; Gothelf *et al.*, 2014). Vingerhoets *et al.* used SPECT with $^{123}$I-labelled iodobenzamide ([$^{123}$I]IBZM) to measure striatal dopamine $D_{2/3}$ receptor binding potential between people at clinically-defined ultra-high risk for psychosis, people with 22q11.2DS and controls. All participants were antipsychotic and psychostimulant naïve. There were no significant between-group differences in dopamine $D_{2/3}$ receptor binding potential suggesting that if dopamine dysregulation does play a role in mediating psychosis risk in 22q11.2DS, the pathology may be presynaptic (Vingerhoets *et al.*, 2018).

**Electrophysiology**

EEG has been used in several studies to investigate neuronal activity in 22q11.2DS. Comparison between resting state networks in patients with schizophrenia, people with 22q11.2DS and healthy controls found abnormalities of salience and resting-state networks in both the schizophrenia and 22q11.2DS groups suggesting that EEG microstates might constitute a marker for schizophrenia (Tomescu *et al.*, 2014). Abnormal responses to auditory and visual stimuli have also been reported in 22q11.2DS (Baker *et al.,* 2005; Zarchi *et al.,* 2013; Larsen *et al.,* 2018a; Larsen *et al.,* 2018b; Rihs *et al.,* 2013; Biria *et al.,* 2018). Several studies have focused on auditory mismatch negativity (MMN) responses in 22q11.2DS. This is a brain response to change detection and abnormalities in

MMN responses have previously been reported in schizophrenia (Catts *et al.*, 1995; Michie, 2001; Umbricht and Krljes, 2005; Näätänen and Kähkönen, 2009). Baker and colleagues found that, compared with controls, people with 22q11.2DS had reduced auditory MMN responses in frontal regions. Two other groups failed to replicate these between-group differences (Zarchi *et al.,* 2013; Larsen *et al.,* 2018b), however, Zarchi *et al.* did find that MMN amplitude was associated with positive and negative psychotic symptom scores. Abnormal auditory steady-state responses have also been found in 22q11.2DS (Larsen *et al.,* 2018b) with a reduction in gamma oscillations being reported in people with the deletion. Finally, visual processing deficits have been found in an EEG study using an illusory contour task (Biria *et al.*, 2018). In this study, activity in the occipital cortex was significantly different between people with 22q11.2DS and controls in both the early and late stages of visual processing. These electrophysiological studies support evidence from structural and functional MRI of cortical network dysfunction in 22q11.2DS.

## 1.4 Excitatory-inhibitory balance

### 1.4.1 What is excitatory-inhibitory balance?

Cortical excitability reflects the balance between excitation and inhibition in brain networks. These networks consist of different types of neurons that each have a role in maintaining this homeostatic balance. These can be broadly divided into excitatory neurons which use glutamate as their primary neurotransmitter and inhibitory neurons which mainly use gamma-aminobutyric acid (GABA). Glutamatergic neurons are the most abundant neuronal type in the neocortex and are predominantly pyramidal neurons that either synapse locally or project to distant targets in the cortex, brainstem or deep subcortical regions (Somogyi *et al.*, 1998). GABAergic interneurons constitute about 20% of cortical neurons and these project locally to regulate neuronal activity. Both types of neurons are further subclassified based on their properties and connections (Markram *et al.*, 2004; Sugino *et al.*, 2006).

The prefrontal and sensory cortices consist of narrow radial arrays of neurons known as minicolumns which are the basic units of signal processing and have been linked to both sensory processing and working memory (Rao and Ballard, 1999; Opris and Casanova, 2014). The activity of pyramidal cells is tightly regulated within and between adjacent minicolumns by the action of inhibitory interneurons. The integration of excitatory and inhibitory activity can control and stabilise network responses. Disruption of synaptic integration in minicolumns could therefore lead to abnormal cortical excitation (Hansel and Sompolinsky, 1996; van Vreeswijk and Sompolinsky, 1996; Tatti *et al.*, 2017).

As well as being linked synaptically, glutamate and GABA are linked metabolically, with glutamate being the metabolic precursor to GABA, which in turn is recycled to synthesise glutamate (Rowley *et al.*, 2012). Alterations in neurotransmitter synthesis and metabolism could therefore also affect excitatory-inhibitory (E-I) balance in favour of either enhanced or reduced excitatory output.

Local cortical synchronisation requires balanced communication between excitatory and inhibitory neuronal populations and there are many possible ways that this balance could be perturbed. For example, abnormalities in excitatory or inhibitory cell structure, migration, receptor density or function, and neurotransmitter synthesis or metabolism could alter the balance between excitation and inhibition. Suboptimal balance between local excitation and inhibition may also disrupt the formation of long-range connections via feedforward mechanisms, which would in turn reduce reciprocal feedback and top-down control of the signal to noise ratio in local cortical circuits (Kessler *et al.,* 2016). This could lead to diverse cognitive psychiatric and neurological manifestations depending on the brain regions affected as well as environmental and contextual factors.

### 1.4.2 How can excitatory-inhibitory balance be investigated?

Animal studies have used optogenetics (a method that uses light to modulate neuronal activity selectively and precisely) to disrupt E-I balance and explore the subsequent effects on behavior and neuronal circuit properties. In the first of these experiments, Sohal *et al.* showed that inhibiting parvalbumin-containing (PV+) inhibitory interneurons suppressed high frequency neural oscillations in the gamma range, while driving these neurons generated gamma frequency activity. Gamma frequency modulation of excitatory activity enhanced signal transmission by increasing signal to noise ratio within cortical circuits (Sohal *et al.*, 2009). In a second experiment, Yizhar *et al.* used optogenetics to elevate E-I balance in the medial prefrontal cortex of freely moving mice and recorded multiunit activity and local field potentials before and after elevation. They found that this manipulation increased high frequency and reduced low frequency oscillatory activity as well as affecting the animals' behavior (Yizhar *et al.*, 2011). These rodent studies measured oscillatory activity using invasive techniques. While such approaches would not be possible in human studies, electrical activity can be measured non-invasively using EEG and MEG, as described above.

Animal studies of neurotransmitter concentrations have also employed invasive techniques, such as microdialysis (Castro *et al.,* 2014). Accessing the glutamatergic and GABAergic neurotransmitter systems non-invasively in humans presents some challenges. While plasma concentrations can be assayed, these do not give insight into cortical concentrations, particularly since these transmitters do not readily cross the blood-brain barrier. PET/SPECT studies require the injection of radioligands that bind to receptors of interest in the brain. While some ligands for GABA and glutamate receptors are available (Delforge *et al.*, 1993; Fu *et al.*, 2018), these tracers typically have short half-lives, meaning that an on-site cyclotron is necessary and experiments may be lengthy and involve multiple injections, limiting their use in non-clinical studies of children. The most non-invasive method available for assaying GABA and glutamate concentrations in children is MRS. However, due to their relatively low concentrations in the brain, large voxels and spectral-editing techniques need to be used to dissociate GABA

and glutamate signals from overlapping and more abundant metabolites (Puts and Edden, 2012).

### 1.4.3 What is the evidence for excitatory-inhibitory imbalance in neurodevelopmental disorders?

It has been proposed that the core features of many neurodevelopmental and psychiatric disorders including schizophrenia, ASD and ADHD can result from disruptions in E-I balance. Evidence for this comes from a number of sources. In schizophrenia for example, genetic studies have converged on the role of the synapse in psychosis risk (Hall *et al.*, 2015) and pathway analysis has implicated both glutamatergic and GABAergic signaling (Pocklington *et al.*, 2015). Postmortem studies have identified a reduction in pyramidal cell dendritic spine density in schizophrenia (Glantz and Lewis, 2000; Sweet *et al.*, 2009) as well as reduced expression of GAD67 in PV+ inhibitory interneurons (Volk *et al.*, 2000; Hashimoto *et al.*, 2003). The amplitude of gamma oscillations has also been found to be reduced in patients with schizophrenia using EEG and MEG (Kwon *et al.*, 1999; Wilson *et al.*, 2008) and alterations in both glutamate and GABA concentrations have been reported in MRS studies (Yoon *et al.*, 2010; Marsman *et al.*, 2014).

In ASD, genetic studies also implicate synaptic genes (Weiss, 2009; Gilman *et al.*, 2011). Postmortem studies in ASD have found increased dendritic spine density on pyramidal cells (Hutsler and Zhang, 2010), lower numbers of PV+ inhibitory interneurons in the prefrontal cortex (Zikopoulos and Barbas, 2013), reduced GAD65 and GAD67 levels (Fatemi *et al.*, 2002; Yip, Soghomonian and Blatt, 2007) and alterations of $GABA_A$ and $GABA_B$ receptors (Collins *et al.*, 2006; Oblak, Gibbs and Blatt, 2010). Electrophysiological studies have reported atypical gamma band responses in people with ASD (Stroganova *et al.,* 2015; Sun *et al.,* 2012) and a SPECT study found reduced accumulation of the $GABA_A$ receptor ligand I-123 iomazenil (Mori *et al.*, 2011). MRS studies in ASD have shown equivocal results for glutamate concentrations between people with ASD and controls (Page *et al.*, 2006; DeVito *et al.*, 2007; Bernardi *et al.*, 2011; Brown *et al.*, 2013; Hassan *et al.*,

2013; van Elst *et al.*, 2014; Horder *et al.*, 2018). However, MRS studies of GABA have reported consistent reductions in ASD (Harada *et al.*, 2011; Gaetz *et al.*, 2014; Rojas *et al.*, 2014).

There has been less evidence for E-I imbalance in people with ADHD. Genetic studies have provided some evidence for the role of glutamatergic pathways in ADHD (Elia *et al.*, 2012). MRS studies have found lower Glx concentrations in the basal ganglia, anterior cingulate cortex and medial prefrontal cortex of adults with ADHD (Perlov *et al.*, 2007; Dramsdahl *et al.*, 2011; Maltezos *et al.*, 2014) but higher Glx concentrations in the frontal lobes of children with ADHD (MacMaster *et al.*, 2003; Courvoisie *et al.*, 2004; Moore *et al.*, 2006). MRS studies of GABA concentrations have found reductions in both children and adults with ADHD (Edden *et al.*, 2012; Bollmann *et al.*, 2015; Schür *et al.*, 2016). Interestingly, patients treated with stimulant medication in childhood had lower baseline levels of GABA than those who were first treated in adulthood, and higher responsivity to stimulant challenge with increased GABA concentrations being found after administration of methylphenidate (Solleveld *et al.*, 2017). There is also preliminary evidence for reduced gamma band activity in adults with ADHD which increased after administration of stimulant medication (Wilson *et al.*, 2012).

### 1.4.4 Is there evidence for excitatory-inhibitory imbalance in 22q11.2 deletion syndrome?

As discussed above, 22q11.2DS is associated with pleiotropic outcomes. In terms of psychopathology, the range of disorders most commonly reported in 22q11.2DS include those which have been associated with E-I imbalance. This, coupled with the finding that 22q11.2DS is associated with a number of seizure-related disorders, including epilepsy, leads one to hypothesise that people with 22q11.2DS may be at increased risk across this spectrum of disorders due to the effects of the 22q11.2 deletion on E-I balance.

There are several lines of evidence that suggest that E-I balance may be perturbed in 22q11.2DS. Firstly, deficiency in *PRODH*, a gene in the 22q11.2 region, has been

shown to affect GABA synthesis and gamma band activity in murine models (Crabtree *et al.*, 2016). Secondly, these models have also found abnormal PV+ inhibitory interneuron migration in 22q11.2DS, a finding that is mirrored in postmortem studies of humans with 22q11.2DS (Kiehl *et al.,* 2009; Meechan *et al.,* 2009; Mori *et al.,* 2011; Meechan *et al.,* 2012; Piskorowski *et al.,* 2016).

Despite this evidence, few studies have directly investigated markers of E-I balance in people with 22q11.2DS. Advances in human electrophysiological techniques such as EEG and MEG offer the opportunity to investigate patterns of neuronal oscillations that are thought to be driven by E-I balance, in particular by the action of the PV+ GABAergic interneurons that have abnormal density, morphology and migration patterns in 22q11.2DS (Meechan *et al.*, 2009, 2012; Piskorowski *et al.*, 2016). As described above, a recent EEG study of cortical responses to auditory stimulation suggests that these oscillatory patterns are indeed atypical in 22q11.2DS (Larsen *et al.,* 2018a) but this has not been replicated under other experimental conditions and in other brain regions. Furthermore, the effects of 22q11.2DS on longer-range brain networks has not yet been explored. Recent advances in neurochemical imaging now enable measurement of both glutamate and GABA *in vivo*. Two previous studies have reported on glutamate/glutamine concentrations in 22q11.2DS but to my knowledge, there have been no published studies of GABA concentrations in 22q11.2DS.

## 1.5 Summary, rationale and objectives

22q11.2DS is a copy number variant syndrome associated with high rates of cognitive impairment and psychopathology across the age spectrum. It has a pleiotropic outcome and, as yet, the mechanisms underlying this pleiotropy are not well understood. A number of different neuroimaging methods have been employed to investigate 22q11.2DS. These have found evidence for abnormal brain structure and function in 22q11.2DS which has been associated with some of the phenotypic outcomes of the disorder. However, as yet, these

methodologies have not given clear mechanistic insight into the neural pathways underlying risk or resilience in 22q11.2DS. One potential mechanism which has not received much attention in brain imaging studies of 22q11.2DS to date is E-I balance. The work presented in this thesis aims to address this gap in our current knowledge by investigating markers of E-I balance in children with 22q11.2DS and investigating how these relate to psychopathology and cognitive impairment.

The overall objectives of this thesis are:

1. To compare the following markers of excitatory-inhibitory balance between children with 22q11.2DS (probands) and children without neurodevelopmental CNVs (controls):

- Resting-state neural oscillatory patterns using MEG
- Visual gamma oscillations using MEG
- Occipital GABA concentrations using MRS

2. To investigate associations between these markers, psychopathology and cognitive function.

# 2 General methodology

An overview of the methods relevant to the subsequent experimental chapters are outlined in this section. Detailed information about the neuroimaging analysis pipelines is described separately in each experimental chapter.

## 2.1 Participants and procedures

Participants were recruited from the ongoing Experiences of people with copy number variants (ECHO) study at Cardiff University (https://www.cardiff.ac.uk/mrc-centre-neuropsychiatric-genetics-genomics/research/themes/developmental-disorders/echo-study-cnv-research). This is a longitudinal study of people with CNVs which commenced in 2010. People over the age of six years old with 22q11.2DS or other neurodevelopmental CNVs were recruited to the ECHO study from clinical genetics services located across the United Kingdom, from charities (including Max Appeal, Unique and the 22Crew) and from self-referral via the ECHO study website and social media pages. Where applicable, an unaffected sibling over the age of six years old was also invited to take part. For families with more than one unaffected child, those closest in age to the affected child(ren) were invited to participate. CNV status was confirmed by medical reports from NHS medical genetics laboratories and, where possible, blood and saliva samples were collected for microarray testing in the laboratory of the Division of Psychological Medicine and Clinical Neurosciences (DPMCN).

The brain imaging study commenced in 2013. Families taking part in the ECHO study with children aged between 10-17 years old were contacted by letter, telephone or email and asked if they would like to receive an information booklet about the research. Once families had received this booklet, further contact was made by telephone or email to ask if they had any questions about the study and if they wished participate. Potential participants and their accompanying parents or carers then underwent rigorous safety screening by telephone to identify potential contraindications for neuroimaging. Any concerns identified during

screening were discussed with radiographers and laboratory managers at Cardiff University's Brain Research Imaging Centre (CUBRIC). When necessary, and with the consent of the participant and their parents or carers, medical information was sought from their responsible clinician (e.g. paediatrician, cardiologist or general practitioner) to determine whether it was safe for the child to take part in the study.

Families who wished to take part in the brain imaging study were invited to visit Cardiff at a time convenient to them. Prior to recruitment into the study, written informed consent was obtained from either parents, carers or participating children depending on the child's age and their capacity to provide consent. Children under the age of 16 years old or those over the age of 16 years old who lacked capacity to consent for themselves were asked to complete an assent form. Recruitment for the study was carried out in accordance with protocols approved by South East Wales National Health Service (NHS) Research Ethics Committee.

Families were given the option to complete the ECHO study's questionnaires, psychiatric and cognitive assessments (see below) during their visit to Cardiff or at another time (e.g. during a separate home visit or by telephone). Typically, families spent 1-2 days in Cardiff but on some occasions (e.g. when there were multiple affected family members), up to five days were required to complete scans, questionnaires and assessments.

## 2.2 Brain imaging

Neuroimaging data were collected at CUBRIC. This facility was upgraded and relocated during the data collection period and while the same MEG system was migrated during this move, the MRI scanner was replaced. Between March 2013 and August 2016 data were collected on a 3T General Electric HDx MRI system (GE Medical Systems, Milwaukee, WI) and from August 2016 to February 2018 a 3T Siemens Prisma MAGNETOM MRI system (Siemens, UK) was used. MRS data were

collected using the GE system only, however, T1 structural MRI data for the co-registration of MEG data were acquired on both MRI systems.

### 2.2.1 MEG session

MEG data were collected using a CTF-275 MEG system enclosed within a shielded room. Participants were given plenty of time to become familiar with the MEG environment and had time to practise each task before data recording commenced. If requested and if no contraindications were present, a parent could accompany their child in the shielded room during recordings for reassurance. Participants were monitored throughout the recordings and experiments were terminated early if participants wished to stop or if they were observed to be uncomfortable or moving excessively. Participants were offered a break between recordings.

Participants and, where applicable, their accompanying parent or carer removed all metallic items prior to entering the shielded room. Children with refractive errors were offered MEG compatible glasses to wear during recordings. Head coils were positioned 1cm above the nasion and 1cm in front of each tragus. Where tolerated, EOG electrodes were placed around the eyes to record eye blinks and eye movements during the recordings. Stimuli were presented on a Mitsubishi Diamond Pro 2070 monitor or PROPixx LCD projector (1024 x 768 pixel and 100Hz frame rate (monitor) or 120Hz frame rate (projector)). Data presented in the thesis will be based on recordings made at rest and during the presentation of a static visual grating.

In the resting-state experiment, participants were instructed to focus on a red fixation point presented in the centre of the screen on a mean luminance background for five minutes. During the visual task, a static vertical square-wave grating pattern with maximum contrast and a spatial frequency of three cycles per degree was presented on a mean luminance background with a red central fixation point. The grating pattern was located in the centre of the display with 8˚ x 8˚ of visual angle and was presented for 1.5-2 seconds followed by a rest period

of 2 seconds in which only the fixation point was presented. Children were instructed to press a response button as soon as the stimulus disappeared. They were given 0.75 seconds to make this response. If no response was detected, a warning message appeared on the screen. The session contained 100 trials and took approximately eight minutes to complete. Data quality and head motion were monitored during the MEG session and recordings were repeated if necessary and with the consent of the participant and their family. I collected the majority of the MEG data presented in this thesis with the assistance of colleagues from the ECHO field team and experienced MEG operators.

### 2.2.2 MRI session

Participants and their families were invited to visit the 'mock' scanner before the MRI session commenced. The 'mock' scanning suite aims to reproduce the MRI environment but as the scanner does not contain a magnet, it is a safe even for those with contraindications for MRI. During the 'mock' scanning session, children were invited to lie in the scanner and listen to audio recordings of real scanner noises. The aim of this session was to familiarise young people and their families with the MRI environment in order to alleviate anxiety.

For the MRI session, parents were invited to accompany their children into the scanning suite if they wished to do so and if they did not have any contraindications for MRI. Children and their accompanying parent or carer (where applicable) were asked to remove any loose metal items and change into alternative clothing if necessary (e.g. surgical scrubs). Participants were able to watch a movie of their choice during the session. Children with refractive errors were offered MRI compatible glasses to wear during scanning. MRI data were collected by experienced MRI operators at CUBRIC. I was present throughout the majority of the scanning sessions, screening participants, demonstrating the mock scanner and chaperoning the family.

T1 structural images were acquired with a 3D fast spoiled gradient echo (FSPGR) sequence on the General Electric system (TR=7.8ms, TE=3.0ms, voxel size = 1mm

isotropic) and a 3D magnetization prepared rapid acquisition gradient echo (MP-RAGE) sequence on the Siemens scanner (TR=2.3ms , TE=3.06ms voxel size = 1mm isotropic). MRS data were collected using a Mescher-Garwood point resolved spectroscopy [MEGA-PRESS; (Mescher *et al.*, 1998)] sequence (TR=2000ms, TE=68ms) with a 3cm x 3cm x 3cm voxel placed in the midline of the occipital lobe. Gaussian editing pulses (duration = 16ms) were placed at either 1.9ppm (ON) or 7.5ppm (OFF) to produce GABA edited spectra. Data were visually inspected after acquisition and the sequence was repeated if data quality was poor and the participant consented to repeating the scan.

## 2.3 Psychiatric assessment

Psychiatric assessments were conducted by trained researchers from the ECHO field team under the supervision of experienced psychologists and psychiatrists by means of semi-structured interviews with primary carers using the Child and Adolescent Psychiatric Assessment [CAPA; (Angold *et al.*, 1995)] and the Autism Diagnostic Interview-Revised [ADI-R; (Lord, Rutter and Le Couteur, 1994)]. In addition, psychotic experiences were assessed through child-report using the psychosis section of the Child CAPA. Interviews were audio-taped for monitoring purposes. As a clinical member of the study team, I was conducted a small proportion of these assessments, double coded interviews and reviewed diagnoses, particularly when there was diagnostic uncertainty.

The CAPA is a semi-structured interview which assesses the presence or absence of a broad range of psychiatric symptoms in the three months preceding the interview. The interview does not cover symptoms of ASD so these were assessed separately using the ADI-R. The ADI-R assessed ASD symptomatology across three domains: reciprocal social interaction, communication and language, and restricted and repetitive stereotyped interests and behavior. Unlike the CAPA which focusses on the last three months, the ADI-R focusses on both current behavior and developmental history.

Diagnostic criteria were applied to the CAPA interview scores using the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition - Text Revision [DSM-IV-TR; (American Psychiatric Association, 2000)] to establish diagnoses. Diagnostic algorithms were applied to the ADI-R scores for ASD diagnosis.

The DSM-IV-TR has mutual exclusivity rules, for example under these criteria ASD and ADHD cannot be co-diagnosed. However, it is now recognised that these disorders are highly comorbid in people with 22q11.2DS (Niarchou *et al.*, 2014) and in the general population (Leitner, 2014), so for the purposes of this study, mutual exclusivity rules were not applied. Psychotic experiences (e.g. hallucinatory experiences or abnormal beliefs) were recorded as being present if they were reported by either the parent or child. Total symptom scores were generated by counting the number of symptoms present for the most prevalent DSM-IV-TR diagnoses identified by the CAPA for use in the regression analyses. ADI-R algorithm items were used to derive ASD symptom scores.

## 2.4 Cognitive assessment

Cognitive assessments were performed by trained researchers from the ECHO field team. I received training in the administration and scoring of these measures and conducted a small number of assessments during the study period.

Global cognitive ability was assessed using the Wechsler Abbreviated Scale of Intelligence [WASI; (Wechsler, 1999)]. This comprises four subtests: vocabulary, similarities, block design and matrix reasoning. The vocabulary subtest requires the participant to define pictures or words and the similarities subtest assesses the participant's ability to express the similarity between objects and concepts. Verbal IQ (VIQ) is derived from these subtests. In the block design subtest, the participant has to replicate a geometric pattern using coloured cubes within a predetermined time limit. Matrix reasoning involves completing patterns by choosing between five possible options. These subtests measure performance IQ (PIQ). Full scale IQ (FSIQ) is calculated from the scores on all four subtests. Ratings

were made by two independent researchers who were blind to deletion status. Raw scores were converted to age-adjusted t-scores for each of the four subtests according to normative sample tables in the WASI manual. Final FSIQ, VIQ and PIQ scores were calculated by transforming the additive total of the age-adjusted t-scores from the four subtests. The resulting score distributions had a mean of 100 and a standard deviation of 15.

The Wisconsin Card Sorting Test [WCST; (Heaton *et al.*, 1993)] was used to assess executive function. In this task, participants match response cards to stimulus cards without being explicitly told the matching rule. After ten correct responses, the rule is changed. Failure to respond to this change is recorded as a perseverative error. The total number of perseverative errors is used as a measure of set-shifting ability. Raw scores were transformed to age-adjusted standardised scores according to WCST normative sample tables. The resulting distibutions had a mean of 100 and a standard deviation of 15.

The Cambridge Neuropsychological Test Automated Battery (CANTAB; Cambridge Cognition Limited, UK, 2006) is a computerised touch screen platform with which tasks assessing different cognitive domains can be administered. The Spatial Working Memory (SWM) task measures spatial working memory aspects of executive function. This task requires the participant to locate a blue token hidden in one of several coloured boxes. Once a token has been found in a particular box, it will not appear in that location during the next round. The outcome measure is the number of times the participant revisits a box in which a token has been previously found. The Stockings of Cambridge (SOC) task measures the spatial planning aspect of executive function. In this task the participant is shown two displays each with three coloured balls which are stacked in suspended stockings. They must use the balls in the lower panel to copy the pattern in the upper panel. The outcome measure is the number of times they complete the problem in the minimum number of moves possible. The 5-choice Reaction Time (RTI) task is a measure of processing speed in which participants hold down a press pad until a yellow spot appears in one of five locations on the screen. When they see the

spot, they release the pad and tap it. The time taken to release the pad is recorded as the reaction time. The Match to Sample (MTS) task measures visual attention. A patterned stimulus appears in the centre of the screen. Similar patterns are then displayed in boxes around the edge of the screen. The participant must identify the matching pattern. The number of correct responses is recorded as their score. The Rapid Visual Processing (RVP) task is a measure of sustained attention. Digits between 2 to 9 are presented on the screen. Participants are instructed to respond when the digits 3, 5 and 7 appear in a row. The outcome measure is sensitivity to the target sequence. With the exception of the MTS data, all CANTAB scores were transformed to age-adjusted scores according to CANTAB normative sample tables. CANTAB standardised scores were calculated as a z-score with a mean of zero and a standard deviation of one. As no normative data are available for the MTS data, raw scores were used for this task.

For all cognitive tasks used in the results chapters that follow, higher scores indicate better cognitive performance.

## 2.5 Questionnaires

Questionnaires were given to parents or carers of participating children during their brain imaging visit or were sent separately by post. The questionnaire pack included questions about family background (e.g. maternal education and household income), ethnicity and general health. The questionnaire packs also included the Social Communication Questionnaire [SCQ; (Rutter, Bailey and Lord, 2003)], a 40-item questionnaire which is derived from the ADI-R and is designed to be completed by parents and carers to identify behaviours that may indicate an ASD diagnosis.

## 2.6 Data analysis

### 2.6.1 Data cleaning and preprocessing

After data collection, MEG data were downsampled to 600Hz, stimulus markers were added and data were epoched. Epoched data were then imported into the

data analysis package DataEditor for preprocessing. Each dataset was recorded in 3rd order mode to reduce noise artefact. In addition, data were filtered by removing the direct current (DC) offset. Each trial was manually inspected for artefacts. Trials containing artefacts were removed from the dataset by labelling these trials as 'bad'.

Structural MRI data were downloaded from CUBRIC servers and imported into the package MRIViewer. Structural MRI and MEG data were co-registered by marking the locations of the nasion and bilateral preauricular fiducials on the participant's MRI scan. Where a participant was unable to have an MRI scan (e.g. due to contraindications) or where the MRI data quality was too poor for co-registration, a suitable alternative MRI scan was identified by matching the distances between the participant's fiducial locations with those of another participant. MEG data were subsequently analysed using in-house analysis pipelines based around the FieldTrip package (Oostenveld *et al.*, 2011). These will be described in detail in Chapters 4 and 5.

MEGA-PRESS data were downloaded from CUBRIC servers. Voxel locations were checked using the package FSLView (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FslView) to confirm their location in the occipital cortex. MRS data were subsequently analysed using the Gannet toolkit (version 2.0, www.gabamrs.com) in MATLAB (version R2015a, MathWorks). The MRS analysis pipeline is described in detail in Chapter 6.

Demographic, psychiatric and cognitive data were coded and inputted into databases created and maintained by the ECHO field team. Data relevant to each chapter were cleaned, scored and exported into R studio (version 1.1383 for Mac, www.rstudio.com) for statistical analysis together with variables extracted from the neuroimaging analysis pipelines.

### 2.6.2 Statistical analyses

The data distributions for each variable of interest were checked using Shapiro-Wilk's test of normality. Between-group differences for normally-distributed data (e.g. age) were analysed using parametric statistical tests e.g. t-tests. Non-normally-distributed data (e.g. psychiatric diagnoses and WCST scores) were analysed using non-parametric statistical tests e.g. chi-squared tests or Fisher's exact tests (for categorical variables) and Mann-Whitney U tests (for continuous data). Linear regression was used to explore the relationships between brain imaging measures, cognitive function and psychopathology as well as to examine the effects of covariates and potential confounders. Brain imaging variables were included as dependent variables in the regression models while cognitive and psychiatric variables were included in the models as independent variables. Dependent variables that were not normally distributed were transformed using Tukey's Ladder of Powers and transformed into z-scores with a mean of zero and a standard deviation of one before inclusion in the regression models. The effects of covariates on the relationships between dependent and independent variables were assessed in a hierarchical manner. The variables that were included as covariates were age, gender and handedness.

# 3 Sample phenotype

## 3.1 Summary

22q11.2DS is associated with high rates of psychopathology and cognitive impairment but the phenotype is highly variable. Many of the phenotypic features of 22q11.2DS pose barriers to participation in research, particularly neuroimaging. A history of surgery involving implanted metal, dental prostheses, learning difficulties, anxiety, hyperactivity and hypersensitivity to noise are some of the issues that are common in children 22q11.2DS and which can be challenging for neuroimaging research.

The aim of this chapter was to characterise the sample of children with 22q11.2DS (probands) who participated in the brain imaging study study (n=39) and to compare their demographic, psychiatric and cognitive profile to controls (n=26) and to probands taking part in research at Cardiff University (the ECHO study) but who did not participate in brain imaging (n=32).

In line with existing literature in 22q11.2DS, probands had poorer cognitive ability than controls and higher rates of psychopathology. Anxiety disorders, ADHD and ASD were the most common diagnoses in the proband group. Probands who participated in the brain imaging study had higher IQ scores and better sustained attention scores than probands who did not participate. There were no statistically significant differences in the burden of psychopathology between proband groups. Mothers of participating probands had higher educational attainment than mothers of those who did not. These findings suggest that the cohort of children participating in the brain imaging studies described in Chapters 4, 5 and 6 of this thesis are broadly representative of the wider ECHO cohort and do not represent an atypical subgroup of children with 22q11.2DS.

## 3.2 Introduction

22q11.2DS is associated with a variable phenotype in terms of psychopathology, cognition and physical health. There are high rates of psychopathology across the lifespan, with the most common presentations in the age range of interest for the present study (10-17 years old) being ADHD, ASD and anxiety disorders (Niarchou *et al.*, 2014; Schneider *et al.*, 2014). These are conditions that present particular challenges in neuroimaging research as they can potentially affect a participant's ability to tolerate the scanning environment and remain adequately still for good quality data to be obtained.

The IQ distribution in 22q11.2DS has been found to follow a normal distribution that is shifted approximately 30 points to the left of the typically developing population (Niarchou *et al.,* 2014; Chawner *et al.,* 2017). This means that some children with 22q11.2DS may have difficulties in comprehending instructions, communicating responses and performing tasks, which could also limit their ability to participate in brain imaging, particularly task-related functional imaging studies.

Children with 22q11.2DS also have high rates of physical health problems that may prevent participation in MRI and MEG studies. For example, congenital cardiac defects are highly prevalent and are often the initial indication for referral to clinical genetics services. Surgical intervention for such defects and other 22q11.2-related physical health problems (such as scoliosis) can involve devices and implants that are either not safe (or are not known to be safe) in 3T MRI environments, or could cause large artefacts during MRI scanning or MEG recordings.

## 3.3 Rationale, aims and hypotheses

The primary aim of this chapter is to characterise the sample of children who were recruited to the brain imaging investigations that are described in the subsequent results chapters. In line with existing literature, it is hypothesised that children

with 22q11.2DS have higher rates of psychopathology and lower cognitive ability than children without neurodevelopmental CNVs. A secondary aim of this chapter is to compare the phenotype of children taking part in neuroimaging with those who either declined to participate or who were unable to participate due to contraindications, in order to determine whether the imaging subsample is representative of the wider 22q11.2DS ECHO cohort. Due to the practical challenges presented by brain imaging studies, it is hypothesised that those taking part in brain imaging have a milder phenotype with lower rates of psychiatric disorders and better cognitive function than children who did not take part in brain imaging.

## 3.4 Methods

### 3.4.1 Recruitment

Participants were recruited from the Experiences of people with copy number variants (ECHO) cohort at Cardiff University. As described in Chapter 2, this is a longitudinal follow-up study of people over the age of six years old with neurodevelopmental CNVs and their unaffected siblings. Children between the ages of 10-17 years old with a confirmed 22q11.2 deletion (probands) or who were siblings of a child with a neurodevelopmental CNV (controls) were invited to take part in the imaging study. When the neuroimaging study commenced in 2013, there were 61 families in the ECHO cohort who had a child with 22q11.2DS aged between 10-17 years old and who had consented to be contacted about future research. Of these, 35 expressed interest in participating and 26 did not wish to participate. The most common reasons for families not wishing to take part were distance to the imaging centre, difficulty taking time off school and work to attend imaging appointments, and concerns that their child would not cope in the scanner environment. Ten additional eligible families were recruited to the ECHO study during the course of this investigation, increasing the potential sample size to 45 probands. 27 families with an unaffected sibling aged between 10-17 years old expressed interest in participating. 22 of these were siblings of children with 22q11.2DS and five were siblings of children with other

neurodevelopmental CNVs (three siblings of children with 16p11.2 deletions, one sibling of a child with a neurexin deletion and one sibling of a child with a 22q11.2 duplication).

Before taking part in the study, potential participants underwent rigorous screening over the telephone to identify potential contraindications to their participation in the MEG session, the MRI session or both. Where necessary, screening was followed-up by correspondence with medical professionals involved in the child's care to determine whether it was safe for the child to participate in the MEG session, MRI session or both. Following screening, a further 6 probands were excluded from the whole study, one was excluded from the MEG session only and 15 were excluded from the MRI session only. No controls were excluded during the screening process.

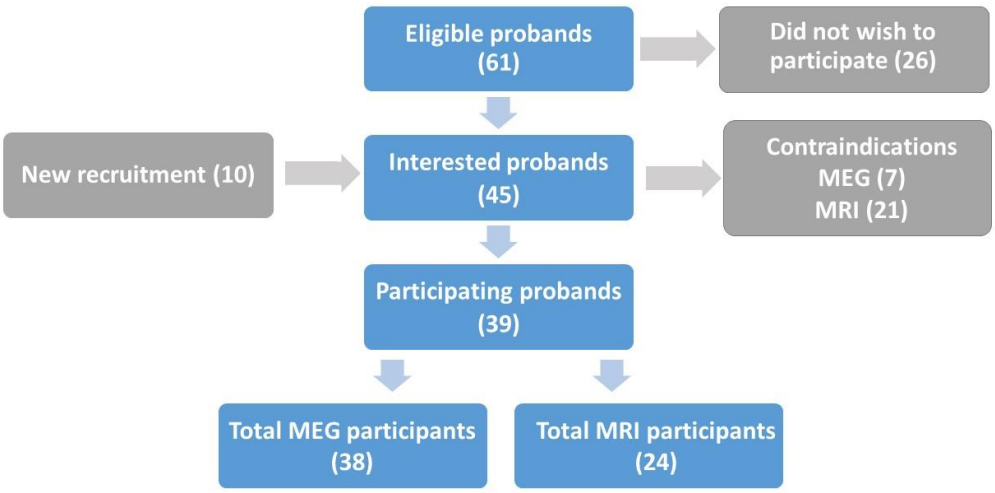


*Figure 3-1 Flowchart of proband recruitment.*

CNV status was confirmed for all participants in the study. 36 probands were tested by microarray in the laboratory of the Division of Psychological Medicine and Clinical Neurosciences (DPMCN) and of these, 34 had the 3Mb deletion (A-D) and two had the nested 1.5Mb deletion (A-B). Three children did not have in-house genetic testing however, all had medical records from clinical genetics

services confirming their diagnosis of 22q11.2DS (either by fluorescent in-situ hybridisation (FISH) or microarray). Two probands had an inherited CNV, other probands had *de novo* deletions. All controls had microarray testing at the DPMCN. One child was found to have a neurodevelopmental CNV (16p11.2 deletion) and was excluded from control sample.

In total 39 probands and 26 sibling controls participated in the study. This sample comprised 14 sibling pairs, other participants were unrelated. 38 probands and 26 controls took part in the MEG session and 24 probands and 26 controls took part in the MRI session. Recruitment for the study was carried out in accordance with protocols approved by South East Wales National Health Service (NHS) Research Ethics Committee.

### 3.4.2 Demographic information

Demographic information about participating families was collected by means of a parent questionnaire and telephone screening. Data were collected on participant age, gender, handedness, ethnicity, maternal education and household income. Maternal education was categorised according to the highest qualification obtained by the participating child's mother. 'Low' maternal education indicates that a GCSE or equivalent qualification was the highest obtained, the 'middle' category indicates that A-levels (or equivalent) or a vocational qualification was the highest obtained and 'high' maternal education indicates that a university degree or higher postgraduate qualification was obtained.

### 3.4.3 Psychopathology and medication use

Detailed information about proband and sibling psychopathology was collected using the Child and Adolescent Psychiatric Assessment [CAPA; (Angold *et al.*, 1995)]. This semi-structured interview was conducted with the primary caregiver and provided detailed information about the major psychiatric disorders coded in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition – Text Revision [DSM-IV-TR; (American Psychiatric Association, 2000)] except autism

spectrum disorders (ASD). The psychosis sub-section of the Child CAPA was also used to assess for the presence of psychotic symptoms. These were coded as being present if they were reported by either the child or their parent. ASD symptomatology was assessed using the Autism Diagnostic Interview-Revised [ADI-R; (Lord, Rutter and Le Couteur, 1994)] in probands and the Social Communication Questionnaire [SCQ; (Rutter, Bailey and Lord, 2003)] in both probands and siblings. CAPA interviews were conducted in Cardiff during imaging visits or separately during a home visit or by telephone. Due to time constraints during imaging and home visits, the majority of ADI-R interviews were conducted over the telephone. Information about past medical history and medication use was obtained from parent questionnaires, telephone screening and parent interviews.

### 3.4.4 Cognitive ability

IQ estimates were derived from performance on the Wechsler Abbreviated Scale of Intelligence [WASI; (Wechsler, 1999)], including verbal (VIQ), performance (PIQ) and full-scale IQ (FSIQ). Other domains of cognitive functioning were assessed using the Cambridge Neuropsychiatric Test Automated Battery (CANTAB; Cambridge Cognition Limited, UK, 2006) and the Wisconsin Card Sorting Task [WCST; (Heaton *et al.*, 1993)]. The CANTAB metrics used for between-group analyses were: number of correct items in the Match to Sample (MTS) task, a measure of visual attention; reaction time in milliseconds in the 5-choice Reaction Time (RTI) task, a measure of processing speed; ability to discriminate target stimuli from distractors in the Rapid Visual Processing (RVP) task, which measures sustained attention; problems solved in minimum moves in the Stockings of Cambridge (SOC) task, which measures spatial planning; and number of errors in the Spatial Working Memory (SWM) task. For the WCST, the number of perseverative errors was analysed as this reflects set-shifting ability. IQ and WCST scores were transformed to age-adjusted standardised scores with a mean of 100 and a standard deviation of 15. With the exception of the MTS task, scores on each of the CANTAB subtests were age-adjusted and standardised to z-scores with a mean of zero and a standard deviation of one. As no CANTAB norms were

available for the MTS task, raw scores were used. For all tasks, higher scores represent better performance. As with the psychiatric interviews, cognitive assessments were carried out during the imaging visits or separately at participants' homes.

### 3.4.5 Statistical analysis

Demographic, psychiatric and cognitive data were coded and inputted into dedicated databases maintained by the ECHO study team. From these databases, data were extracted for participants who were invited to take part in the brain imaging study. Data were then cleaned, scored and imported into R Studio (version 1.1383 for Mac, www.rstudio.com) for statistical analysis.

Group comparisons were conducted between probands and controls who took part in brain imaging and between probands who took part in brain imaging and those who did not take part in brain imaging. Group differences in categorical variables such as ethnicity, maternal education, family income, gender, and psychiatric diagnoses were investigated using chi-squared tests or Fisher's exact tests when expected cell counts were low. Between-group differences in continuous variables e.g. age and cognitive scores were compared with t-tests or Mann-Whitney U-tests as appropriate with respect to normality. P values were not corrected for multiple comparisons.

## 3.5 Results

### 3.5.1 Demographics

Probands who took part in brain imaging had a mean age of 13.3 years (SD=1.9 years) which was not significantly different from the mean age of those who did not participate (13.1 years, SD=2.0 years, t=0.45, p=0.65). Controls taking part in brain imaging were approximately one year older than participating probands with a mean age of 14.4 years (SD=1.8 years, t=-2.20, p=0.03). There were no significant differences between the gender distributions of participating and non-participating probands (p=0.86) or between participating probands and controls

(p=0.48). Table 3-1 shows the data on ethnicity, maternal education and family income for probands and controls, demonstrating that there were no significant differences between groups.

*Table 3-1 Family background of participating probands and controls*

| | **Probands** | **Controls** | **P** |
|---|---|---|---|
| **Ethnicity** | | | **>0.99** |
| European | 82.1% | 84.6% | |
| Other | 12.8% | 11.5% | |
| Unknown | 5.1% | 3.9% | |
| | | | |
| **Maternal education** | | | **0.21** |
| None | 15.4% | 3.8% | |
| Low | 10.3% | 15.4% | |
| Middle | 30.8% | 15.4% | |
| High | 41.0% | 57.7% | |
| Unknown | 2.6% | 7.7% | |
| | | | |
| **Income** | | | **0.70** |
| <19,999 | 25.6% | 15.4% | |
| 20,000-39,999 | 20.5% | 15.4% | |
| 40,000-59999 | 17.9% | 26.9% | |
| >60,000 | 33.3% | 30.8% | |
| Unknown | 2.6% | 11.5% | |

Table 3-2 shows the same data comparing probands who participated in the brain imaging study with those who did not.

*Table 3-2 Family background of participating and non-participating probands*

| | **Participating probands** | **Non-participating probands** | **P** |
|---|---|---|---|
| **Ethnicity** | | | **0.31** |
| European | 82.1% | 96.9% | |
| Other | 12.8% | 3.1% | |
| Unknown | 5.1% | 0.0% | |
| | | | |
| **Maternal education** | | | **0.01*** |
| None | 15.4% | 3.1% | |
| Low | 10.3% | 37.5% | |
| Middle | 30.8% | 34.4% | |
| High | 41.0% | 18.8% | |
| Unknown | 2.6% | 6.2% | |
| | | | |
| **Income (%)** | | | **0.65** |
| <19,999 | 25.6% | 28.1% | |
| 20,000-39,999 | 20.5% | 25.0% | |
| 40,000-59999 | 18.0% | 21.9% | |
| >60,000 | 33.3% | 18.7% | |
| Unknown | 2.6% | 6.3% | |

These data show that while there were no statistically significant between-group differences in ethnicity or household income, there was a significant difference in

maternal education, with higher qualifications being obtained by mothers of children who participated in neuroimaging than mothers of those who did not.

### 3.5.2 Psychopathology and medication use

Probands and controls who took part in the brain imaging study were first compared. DSM-IV-TR categorical diagnoses were assigned based on scores from the CAPA with the exception of ASD diagnoses, which were derived from ADI-R scores (probands only) and SCQ scores (probands and controls). CAPA interview data were available for 37 probands and 21 controls, ADI-R data for 26 probands and SCQ data for 29 probands and 20 controls.

As shown in table 3-3, the burden of psychopathology in the sample was very high, however at the level of individual diagnoses, between-group differences were not statistically significant with the exception of putative autism spectrum disorder (ASD) derived by the SCQ (p=0.04). 17 of the 25 probands with complete CAPA and ADI-R data (68.0%) had at least one DSM-IV-TR diagnosis and comorbidity was very common with 12 of the 25 (48.0%) having more than one disorder. ASD was the most common diagnosis (50.0%) of which 30.8% of the sample had narrowly-defined autism. Anxiety disorders were highly prevalent (29.7%) as was attention deficit hyperactivity disorder (ADHD, 16.2%). Furthermore, psychotic experiences were described by 16.2% of probands, although none of these children met the criteria for a psychotic disorder. Rates of psychiatric disorders were much lower in controls with only one child meeting the criteria for a DSM-IV-TR disorder (social phobia). One child described psychotic symptoms but these did not reach the threshold for diagnosis of a psychotic disorder.

Although ADI-R data were not available for controls, SCQ data were collected for both probands and controls. Using these data, 27.6% children with 22q11.2DS screened positive for possible ASD using an SCQ cut-off score of 15 while no siblings scored above this cut-off. There was a marked discrepancy between the rates of ASD derived from the ADI-R (50.0%) and SCQ (27.6%). 23 probands had data available for both measures. Of these, ten had an ADI-R derived ASD

diagnosis and six had an SCQ-derived diagnosis (SCQ score ≥15). Five of the six with a positive SCQ screen also received an ASD diagnosis by the ADI-R criteria. The child who did not had a borderline SCQ score of 15. The correlation between ADI-R score and SCQ score was 0.57 ($t=3.14$, $p=5.16x10^{-3}$).

*Table 3-3 Rates of psychiatric and neurodevelopmental disorders in participating probands and controls*

| | **Probands** | **Controls** | **Odds ratio** | **P** |
|---|---|---|---|---|
| **CAPA** | N=37 | N=21 | | |
| Any CAPA diagnosis | 48.6% | 4.8% | 10.0 | <0.01* |
| Any anxiety disorder | 29.7% | 4.8% | 6.10 | 0.09 |
| Social anxiety | 18.9% | 4.8% | 3.90 | 0.25 |
| Agoraphobia | 10.8% | 0.0% | NA | 0.29 |
| Specific phobia | 10.8% | 0.0% | NA | 0.29 |
| Separation anxiety | 8.1% | 0.0% | NA | 0.54 |
| GAD | 8.1% | 0.0% | NA | 0.54 |
| Selective mutism | 2.7% | 0.0% | NA | >0.99 |
| OCD | 2.7% | 0.0% | NA | >0.99 |
| MDD | 2.7% | 0.0% | NA | >0.99 |
| ADHD | 16.2% | 0.0% | NA | 0.17 |
| ODD | 10.8% | 0.0% | NA | 0.29 |
| Tic disorder | 2.7% | 0.0% | NA | >0.99 |
| Psychotic experiences | 16.2% | 4.8% | 3.37 | 0.41 |
| **ADI-R** | N=26 | N=0 | | |
| ASD | 50% | NA | NA | NA |
| **SCQ** | N=29 | N=20 | | |
| SCQ score ≥15 | 27.6% | 0.0% | NA | 0.04* |

*Abbreviations: CAPA, Child and Adolescent Psychiatric Assessment; GAD, generalised anxiety disorder; OCD, obsessive compulsive disorder; MDD, major depressive disorder; ADHD, attention deficit hyperactivity disorder; ODD, oppositional defiant disorder; ADI-R, Autism Diagnostic Interview – Revised; ASD, autism spectrum disorder; SCQ, Social Communication Questionnaire. P values are uncorrected.*

One proband had a past medical history of epilepsy but had not had any recent seizures and was not taking anticonvulsant medication. Few children were taking any regular medication at the time of the scans. Four probands (11.4%) were taking melatonin for sleep problems. One of these children was also taking aripiprazole 5mg once daily for challenging behaviour. No controls were taking any medication for neurological, psychiatric or sleep disorders.

To determine whether probands taking part in the brain imaging study had a different neurodevelopmental and psychiatric phenotype than those who did not participate in brain imaging but did undergo phenotypic assessment as part of the longitudinal ECHO cohort, rates of DSM-IV-TR disorders were compared between groups.

As shown in table 3-4, the burden of psychopathology was remarkably similar between probands who participated and who did not participate in brain imaging. Although not statistically significant, the rates of CAPA-derived diagnoses were higher overall in the imaging subsample. The rates of ASD diagnoses derived from the SCQ were identical in both groups, however fewer of the non-participating probands had an ADI-R derived ASD diagnosis and the correlation between ADI-R and SCQ scores was higher than for children who participated in brain imaging ($r=0.86$, $t=8.23$, $p=2.61 \times 10^{-8}$). Three of the children who did not participate in brain imaging were taking melatonin, one child was taking fluoxetine for anxiety and two children were taking anticonvulsant medication for seizures (carbamazepine and lamotrigine).

*Table 3-4 Rates of psychiatric and neurodevelopmental disorders in participating and non-participating probands*

| | Participating proband | Non-participating proband | Odds ratio | P |
|---|---|---|---|---|
| **CAPA** | N =37 | N=32 | | |
| Any diagnosis | 48.6% | 43.8% | 1.11 | 0.83 |
| Any anxiety disorder | 29.7% | 21.9% | 1.35 | 0.61 |
| Social anxiety | 18.9% | 12.5% | 1.50 | 0.75 |
| Agoraphobia | 10.8% | 6.3% | 1.72 | 0.68 |
| Specific phobia | 10.8% | 3.1% | 3.41 | 0.37 |
| Separation anxiety | 8.1% | 3.1% | 1.29 | >0.99 |
| GAD | 8.1% | 12.5% | 0.65 | 0.70 |
| Selective mutism | 2.7% | 3.1% | 0.87 | >0.99 |
| OCD | 2.7% | 0.0% | NA | >0.99 |
| MDD | 2.7% | 3.1% | 0.87 | >0.99 |
| ADHD | 16.2% | 15.6% | 1.04 | >0.99 |
| ODD | 10.8% | 15.6% | 0.70 | 0.73 |
| Tic disorder | 2.7% | 3.1% | 0.87 | >0.99 |
| Psychotic experiences | 16.2% | 9.4% | 1.72 | 0.50 |
| **ADI-R** | N=26 | N=25 | | 0.31 |
| ASD | 50.0% | 36.0% | 1.78 | |
| **SCQ** | N=29 | N=29 | | >0.99 |
| SCQ score ≥15 | 27.6% | 27.6% | 1.00 | |

*Abbreviations: CAPA, Child and Adolescent Psychiatric Assessment; GAD, generalised anxiety disorder; OCD, obsessive compulsive disorder; MDD, major depressive disorder; ADHD, attention deficit hyperactivity disorder; ODD, oppositional defiant disorder; ADI-R, Autism Diagnostic Interview–Revised; ASD, autism spectrum disorder; SCQ, Social Communication Questionnaire. P values are uncorrected.*

### 3.5.3 Cognitive ability

Table 3-5 shows the cognitive performance of probands and controls who took part in brain imaging.

*Table 3-5 Cognitive performance of participating probands and controls*

| | Probands | | | Controls | | | |
|---|---|---|---|---|---|---|---|
| | **N** | **Mean** | **SD** | **N** | **Mean** | **SD** | **P** |
| **WASI** | | | | | | | |
| FSIQ | 38 | 74.4 | 12.2 | 25 | 107.3 | 11.0 | <0.01* |
| VIQ | 38 | 77.6 | 14.9 | 25 | 104.4 | 13.1 | <0.01* |
| PIQ | 38 | 76.6 | 14.4 | 25 | 108.1 | 10.6 | <0.01* |
| **CANTAB** | | | | | | | |
| Visual attention (MTS) | 35 | 43.1 | 8.5 | 24 | 46.3 | 1.8 | 0.02* |
| Processing speed (RTI) | 34 | -0.1 | 1.6 | 24 | 0.6 | 0.7 | 0.02* |
| Sustained attention (RVP) | 32 | -0.7 | 1.2 | 24 | 0.4 | 0.9 | <0.01* |
| Spatial planning (SOC) | 36 | -1.2 | 1.2 | 24 | -0.8 | 1.5 | 0.28 |
| Spatial working memory (SWM) | 36 | -1.1 | 0.9 | 24 | -0.2 | 0.9 | <0.01* |
| **WCST** | | | | | | | |
| Set-shifting ability | 34 | 81.7 | 32.6 | 23 | 99.1 | 46.6 | 0.03* |

*Abbreviations: WASI, Wechsler Abbreviated Scale of Intelligence; FSIQ, full-scale IQ; VIQ, verbal IQ, PIQ, performance IQ; CANTAB, Cambridge Neuropsychological Test Automated Battery; MTS, Match-to-Sample; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing; SOC, Stockings of Cambridge; SWM, Spatial Working Memory; WCST, Wisconsin Card Sorting Test. P values are uncorrected.*

There were statistically significant differences between groups in IQ with a mean FSIQ difference of 32.9 points. There were also significant deficits in visual attention (MTS), processing speed (RTI), sustained attention (RVP), spatial working memory (SWM) and set-shifting ability (WCST). However, no significant differences were found between probands and controls for spatial planning (SOC).

Table 3-6 shows the cognitive performance of probands who took part in brain imaging and those who did not.

*Table 3-6 Cognitive performance of participating and non-participating probands*

| | Participating probands | | | Non-participating probands | | | |
|---|---|---|---|---|---|---|---|
| | **N** | **Mean** | **SD** | **N** | **Mean** | **SD** | **P** |
| **WASI** | | | | | | | |
| FSIQ | 38 | 74.4 | 12.2 | 32 | 68.0 | 11.2 | 0.03* |
| VIQ | 38 | 77.6 | 14.9 | 32 | 69.4 | 10.9 | 0.02* |
| PIQ | 38 | 76.6 | 14.4 | 32 | 71.5 | 11.7 | 0.13 |
| **CANTAB** | | | | | | | |
| Visual attention (MTS) | 35 | 43.1 | 8.5 | 32 | 43.7 | 5.2 | 0.69 |
| Processing speed (RTI) | 34 | -0.1 | 1.6 | 32 | <0.1 | 1.2 | 0.78 |
| Sustained attention (RVP) | 32 | -0.7 | 1.2 | 30 | -2.0 | 3.0 | 0.04* |
| Spatial planning (SOC) | 36 | -1.2 | 1.2 | 31 | -1.2 | 0.9 | 0.94 |
| Spatial working memory (SWM) | 36 | -1.1 | 0.9 | 32 | -1.4 | 1.3 | 0.36 |
| **WCST** | | | | | | | |
| Set-shifting ability | 34 | 81.7 | 32.6 | 32 | 95.2 | 16.5 | 0.23 |

*Abbreviations: WASI, Wechsler Abbreviated Scale of Intelligence; FSIQ, full-scale IQ; VIQ, verbal IQ, PIQ, performance IQ; CANTAB, Cambridge Neuropsychological Test Automated Battery; MTS, Match-to-Sample; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing; SOC, Stockings of Cambridge; SWM, Spatial Working Memory; WCST, Wisconsin Card Sorting Test. P values are uncorrected.*

Children who did not participate in brain imaging had a mean FSIQ which was 6.4 points below children who took part in neuroimaging and a mean VIQ that was 8.2 points lower than participating children. They also had lower sustained attention than participating children. There were no between-group differences in other CANTAB tasks or the WCST.

## 3.6 Discussion

Recruiting a large neuroimaging sample of children with 22q11.2DS and controls presented a number of challenges. Firstly, 22q11.2DS is a relatively rare disorder (Botto *et al.*, 2003; Oskarsdottir, 2004; Maisenbacher *et al.*, 2017) and although the ECHO cohort is one of the largest in the world, there were only 61 probands who fell within the study age range when data collection commenced. Secondly, due to the relative rarity of 22q11.2DS and other neurodevelopmental CNVs, the ECHO study recruits families from across the UK. This posed substantial logistical difficulties for many families who wished to take part but lived a long distance from the imaging centre in Cardiff. This also precluded after-school or evening scanning appointments for the vast majority of participants and meant that families needed to give up a lot of their time to participate in the study, with some staying in Cardiff over one or more nights, which had a subsequent negative impact on participation rates. Furthermore, due to families' school and work commitments, scanning was largely restricted to school holiday periods, reducing the number of families who could be given appointments within the data collection period. Thirdly, children with 22q11.2DS have high rates of congenital cardiac malformations and other physical health problems such as skeletal abnormalities (Kobrynski and Sullivan, 2007; McDonald-McGinn *et al.*, 2015) which can require surgical intervention, often in the early years of their lives. These procedures may involve the insertion of implants or devices (such as prosthetic heart valves, stents or metallic rods) that are either not safe or not known to be safe in a 3T MRI environment. For this reason, many interested families were unable to take part in the MRI session either because of a known MRI incompatible implant or because of lack of available documentation to confirm MRI safety (although in most cases, these children were able to take part in the MEG session). In addition, several potential participants (both probands and controls) had dental prostheses or braces which prevented their participation in either the MEG session, the MRI session or both (depending on the type, size and location of the prosthesis or brace). The frequency of implants and devices in

potential participants limited the neuroimaging sample size, particularly in the proband group.

One of the major concerns when undertaking clinical neuroimaging research is whether the sample is representative of the population of interest (LeWinn *et al.*, 2017). One could hypothesise that those who are interested in taking part in research and who are able to cooperate with brain imaging procedures could represent a high-functioning subgroup of the population of interest. In the design of this study, every attempt was made to ensure that any child who wished to participate could do so, as long as they had no contraindications for brain imaging. Imaging visits were arranged at times convenient to each family and imaging sessions conducted on either the same or different days, according to each child's ability and family's preferences. Travel and accommodation were arranged on the families' behalf and the cost of meals reimbursed so that families were not prevented from participating due to financial constraints. A 'mock' MRI scanner was used to introduce children to the MRI environment in a relaxed and informal manner in order to reduce anxiety. Extra time was booked in the imaging suites so that there was plenty of time for children and their families to become familiar with the equipment, take breaks, and repeat imaging sequences and recordings if data quality was poor, for example due to head motion. Parents or carers were able to accompany their child during scans and recordings for reassurance and children were also able to watch a movie during their MRI scan. These steps aimed to minimise bias towards a higher functioning subsample by facilitating children's involvement, whatever their abilities or difficulties.

The data presented in this chapter are broadly in line with previously published reports of psychopathology and cognition in children with 22q11.2DS both from the ECHO sample (Niarchou *et al.,* 2014; Chawner *et al.,* 2017), and from the International Brain and Behaviour Consortium (IBBC, (Schneider *et al.,* 2014; Swillen and McDonald-Mcginn 2015)). Data from the first wave of the ECHO sample showed that more than half (54%) of the sample had a CAPA-derived DSM-IV-TR diagnosis which is similar to the rate reported in this chapter (48%). This

study finds higher rates of psychotic experiences and lower rates of ADHD in this sample at a mean age of 13.4 years compared to data published from wave one when children had a mean age of 10.2 years (Niarchou *et al.*, 2014). This is consistent with the literature in 22q11.2DS (Schneider *et al.*, 2014) which shows that ADHD symptoms decline and psychotic disorders emerge in late adolescence and early adulthood. While the rates of DSM-IV-TR diagnoses were similar to those reported previously, in contrast to the study by Niarchou *et al.*, there were no statistically significant differences between the rates of specific phobia, ADHD and oppositional defiant disorder between probands and controls. This is most likely due to the smaller sample size recruited for the current brain imaging study than for the previous phenotyping study (80 probands and 39 controls), which will have affected the power to detect such differences. In terms of cognition, an IQ deficit of approximately 30 points was found in children with 22q11.2DS compared with controls, which is consistent with waves 1 and 2 of the ECHO study (Niarchou *et al.*, 2014; Schneider *et al.*, 2014) and other studies of cognition in 22q11.2DS (Swillen *et al.,* 1997; De Smedt *et al.,* 2007). There were also significant deficits in performance in the MTS task (a measure of visual attention), the RTI task (a measure of processing speed), the RVP task (a measure of sustained attention) the SWM task (a measure of the spatial working memory aspect of executive function) and the WCST (a measure of set-shifting ability) which is again consistent with previously published data from the ECHO sample. However, no significant differences were found between probands and controls for the Stockings of Cambridge task, which assesses spatial planning.

Reported rates of ASD in 22q11.2DS are highly variable depending on the ascertainment and assessment methods used (Fine *et al.*, 2005; Vorstman *et al.*, 2006; Kevin M. Antshel *et al.*, 2007; Niklasson *et al.*, 2009; Angkustsiri *et al.*, 2014; Ousley *et al.*, 2017). The rates of probable ASD derived from the SCQ in the present sample are similar to those found in the first wave of the ECHO study (Niarchou *et al.*, 2014) and the IBBC study (Schneider *et al.*, 2014). However, the prevalence of ASD as derived by the ADI-R in the current study is much higher at 50%. Similar prevalence figures have previously been reported in 22q11.2DS using

the ADI-R (Vorstman *et al.*, 2006). The correlation between ADI-R score and SCQ score in the imaging sample was calculated to be 0.57 ($t = 3.13$, $p=5.16x10^{-3}$). This correlation is lower than that of the sample who did not participate in brain imaging and that reported in non-22q11.2DS samples (Bishop and Norbury, 2002; Howlin and Karpf, 2004). Interestingly, the correlation between SCQ and ADI-R score in the imaging sample was similar to the correlation between SCQ score and total anxiety score ($r=0.65$, $t=5.48$, $p=2.38 \times 10^{-6}$), suggesting that the SCQ may be a relatively insensitive and non-specific screening tool for ASD in this sample. Gold-standard assessment of ASD typically involves gathering information from three sources: parent interview (e.g. using the ADI-R), child observation (e.g. using the Autism Diagnostic Observation Schedule (ADOS)), and a clinician's best estimate diagnosis (Ousley *et al.,* 2013). However, until recently, no studies had applied such a rigorous assessment approach in 22q11.2DS. A recent study in 56 people with 22q11.2DS found ASD rates of 37.5% based on the ADI-R alone, 41.1% using the ADOS alone and 17.9% using a combination of ADI-R, ADOS and a clinician's best estimate consensus diagnosis (Ousley *et al.,* 2017). This suggests that people with 22q11.2DS do have elevated rates of ASD, even when strict diagnostic criteria are applied. The discrepancy between the rates based on consensus data and the rates from either ADI-R interviews or ADOS assessments alone highlights the importance of using multiple sources of information in diagnostic decision-making. Although observational data were not collected in the current study, this will be an important consideration for future research.

Comparison between children who took part in brain imaging and those who did not showed that, in line with the hypotheses, participating children had higher FSIQ and VIQ scores than children who did not participate. However, they did not differ in other tests of cognitive function or in the burden of psychopathology experienced. The association between cognitive performance and participation status may be related to parental concern about the ability of children with lower levels of cognitive functioning to cope with the demands of the neuroimaging sessions. There may also be a relationship between cognitive ability and the severity of medical problems in 22q11.2DS (Swillen, Moss, and Duijff 2018).

Children with 22q11.2DS and congenital heart disease have been found to have reductions in cortical gyrification (Schaer, Glaser, *et al.*, 2009) and it is possible that those excluded from imaging due to concerns about medical or surgical contraindications, may be a more cognitively impaired group.

While there were no significant differences in ethnicity or household income between families who participated and those who did not, mothers of children who participated in brain imaging had obtained more high-level qualifications than those who did not. The reasons for these differences are likely to be multifactorial and may reflect differing levels of interest in scientific research among mothers from different educational backgrounds or associations between the cognitive abilities of mothers and their children (Olszewski *et al.*, 2014; Beemer *et al.*, 2016). However, maternal educational attainment does not only reflect maternal IQ, so caution should be used in making any further interpretation of this finding.

While the psychiatric phenotype of the imaging subsample is similar to the wider ECHO cohort (Niarchou *et al.*, 2014) and the IBBC cohort (Schneider *et al.*, 2014), one cannot generalise these data to all individuals with 22q11.2DS in the absence of population-level data. In the United Kingdom, 22q11.2DS is not part of routine screening programmes and therefore genetic testing is only performed when there is clinical suspicion of a genetic disorder e.g. due to developmental delay, psychiatric symptoms or congenital malformations, potentially resulting in ascertainment bias in studies of the 22q11.2 phenotype. Furthermore, families with children who are severely affected may be more likely to take part in research, further biasing studies towards more severe phenotypes. However, it is worth noting that few of the children in the present study were receiving psychiatric care and only one was taking psychotropic medication, suggesting that in most cases referral for genetic testing was not due to the presence of psychopathology.

## 3.7 Strengths and limitations

This study benefited from the existence of a large sample of children with 22q11.2DS and a sample of siblings of children with neurodevelopmental CNVs who were already taking part in a longitudinal study of CNV phenotypes. Steps were taken to ensure maximum participation in the imaging study where this was deemed safe. Due to the ongoing ECHO study, it was possible not only to compare the psychiatric and cognitive phenotypes of participating probands and controls, but also to compare probands who took part in brain imaging with those who did not take part, to assess the representativeness of the sample. Despite the steps taken to maximise the sample size for the imaging study, the relatively rarity of 22q11.2DS, logistical considerations and imaging contraindications resulted in a modest sample size for phenotypic comparison and subsequent neuroimaging data analysis. The study is also limited by the lack of an observational autism assessment such as the ADOS to further characterise social communication difficulties in affected children.

## 3.8 Conclusions

39 children with 22q11.2DS (probands) and 26 sibling controls were recruited to the brain imaging study. Recruitment and data collection were challenging, resulting in a modest sample size for analysis. Probands were on average one year younger than controls but there were no significant differences in ethnicity, maternal education, family income, gender and handedness. As predicted, probands had higher rates of psychopathology than controls and the most common disorders were ASD, ADHD and anxiety disorders. Despite high rates of psychopathology, only one child was taking psychotropic medication. IQ was approximately 30 points lower in probands than in controls and probands also had significant deficits in visual attention, processing speed, sustained attention, spatial working memory and set-shifting ability. In line with the hypotheses, children taking part in the imaging study had higher cognitive ability than those who did not participate, however, there were no differences in the rates of psychiatric disorders. Maternal educational attainment was lower in mothers of

children who did not participate but there were no differences in ethnicity or household income. Overall, the characteristics of the imaging sample were similar to the wider ECHO sample, suggesting that they do not represent an atypical subgroup of children with 22q11.2DS.

# 4 Resting-state connectivity in 22q11.2 deletion syndrome

## 4.1 Summary

Resting-state brain activity reflects excitatory-inhibitory balance in coordinated neural networks and can be studied using different methodologies (e.g. EEG, fMRI and more recently MEG). Previous studies have identified characteristic resting-state networks with altered dynamics in neurodevelopmental disorders including schizophrenia, ADHD and ASD. Preliminary reports using fMRI in 22q11.2DS indicate alterations in the resting-state networks of people with the deletion.

Resting-state MEG offers the opportunity to investigate brain networks with excellent temporal and good spatial resolution. This study aimed to investigate MEG resting-state networks across six frequency bands (delta, theta, alpha, beta, low gamma and high gamma) in children with 22q11.2DS and controls and to relate measures of network connectivity to cognitive function and psychopathology.

38 children with 22q11.2DS (probands) and 26 controls were recruited to the study and MEG data were recorded during a five minute eyes open resting-state paradigm. An amplitude-envelope correlation approach was used to compare the strength of amplitude-amplitude coupling between 90 brain regions across the six frequency bands. Statistical thresholding procedures were used to identify valid connections which were then compared these between groups. Connectivity scores were extracted for the frequency bands showing between-group differences and the relationships between these scores, cognitive function and psychopathology were explored.

Overall, probands had lower amplitude-envelope correlation scores than controls in delta, alpha and beta bands, particularly affecting posterior brain regions. The specific brain regions with the most significant between-group differences in

probands and controls were the visual cortex, insula, precuneus and inferior parietal cortex. In the alpha band, global and right precuneus connectivity scores were associated with anxiety and ASD symptom scores. This study provides preliminary evidence for altered excitatory-inhibitory balance in children with 22q11.2DS which is related to psychopathology.

## 4.2 Introduction

Resting-state neuroimaging studies measure brain activity while participants sit or lie motionless without performing any particular task. In these conditions, the brain shows spontaneous patterns of oscillatory activity across different regions known as resting-state networks (Fox and Raichle, 2007). These oscillations reflect the synchronous firing of populations of neurons and are mediated by excitatory and inhibitory interactions. The networks of brain regions that are active in the resting-state are remarkably similar to those involved when the brain is performing tasks or responding to external stimuli (Smith *et al.*, 2009). Understanding the properties of these intrinsic brain networks can provide insight into the structure and function of cortical circuitry, and how this circuitry is impacted by high-risk genetic syndromes such as 22q11.2DS.

Resting-state networks can be investigated using different imaging modalities such as fMRI, EEG and MEG. Resting-state protocols have practical advantages over task-based studies enabling the investigation of between-group differences in circumstances where participants may find behavioural tasks difficult, e.g. studies of children and those with low cognitive ability. The results of resting-state studies are not therefore biased by participants' ability to perform tasks, removing the need to match study groups on task performance, which can be challenging in small samples. However, in the absence of a task, it can be difficult to control the behaviour and mental activity of participants during resting-state studies and participants may become drowsy or even fall asleep, particularly during eyes-closed paradigms or when recordings are conducted in the supine position (van Diessen *et al.*, 2015).

FMRI uses the Blood Oxygen Level Dependent (BOLD) signal to infer information about neural activity. The BOLD response has been shown to correlate with local field potentials, reflecting the input and local cortical processing in a given brain region (Logothetis *et al.*, 2001). Resting-state fMRI studies measure the temporal correlation of spontaneous BOLD signal between brain regions with the underlying assumption that functional networks will have correlated activity (Biswal *et al.*, 1995; Fox and Raichle, 2007). While there are many analysis methods available, the majority of studies employ either seed-based approaches, independent component analysis (ICA) or graph theory to examine functional connectivity. Seed-based approaches extract fMRI signal from voxels located in a specific region of interest (ROI) and create a map by calculating the correlations between the seed region and all other voxels of the brain (Fox and Raichle, 2007). ICA is a data-driven approach in which all voxels are considered simultaneously and data are separated into spatial maps of four dimensional MRI signal (the dimensions being space and time (Calhoun, Liu, and Adali 2009)). Graph theoretical approaches model the brain as a network consisting of nodes and edges in which nodes represent brain regions and edges the connections between them. Different metrics can then be used to characterise local and global connectivity patterns (Wang, Zuo and He, 2010). One of the major advantages of resting-state fMRI studies is their excellent spatial resolution, however, the temporal resolution is poor and non-neural physiological confounds such as movement, cardiac and respiratory activity can influence apparent associations (Murphy, Birn and Bandettini, 2013).

MEG is a more direct measure of neural activity with excellent temporal resolution, meaning that correlations between activity within and across different brain regions can be determined with millisecond accuracy. This allows researchers to investigate alterations in neural synchronisation patterns (oscillations) across characteristic frequency bands (delta (1-4Hz), theta (4-7Hz), alpha (8-13Hz), beta (13-30Hz) and gamma (30-90Hz)). Low frequency oscillations are thought to reflect long-range synchronisation between different brain regions

while higher frequencies reflect synchronisation in local networks (Schnitzler and Gross, 2005).

Techniques such as beamforming, which estimate the contributions of different brain positions to the measured field, enable the sources of any observed signals to be localised with good spatial resolution, particularly for structures located near the brain surface (Hillebrand and Barnes, 2005). As with resting-state fMRI, there are several analysis methods available for investigating resting-state connectivity using MEG. These methods use the spectral coherence, phase delay or amplitude coupling of oscillation patterns to estimate resting-state connectivity (Stam, Nolte and Daffertshofer, 2007; Brookes, Woolrich and Barnes, 2012; Hipp *et al.*, 2012; Palva and Palva, 2012; Marzetti *et al.*, 2013; Colclough *et al.*, 2016). While there are advantages and disadvantages to each approach, amplitude-envelope correlation has been shown to be one of the most consistent methods for connectivity estimation, being robust, reproducible and repeatable (Colclough *et al.*, 2016). This method measures functional connectivity by estimating the amplitude coupling between different ROIs using linear correlations of the envelopes of the band-pass filtered signals (Brookes, Woolrich and Barnes, 2012).

Atypical functional connectivity within and between brain networks has been reported in resting-state fMRI studies of schizophrenia (Bluhm *et al.,* 2007; Whitfield-Gabrieli *et al.,* 2009; Argyelan *et al.,* 2014; Wang *et al.,* 2017), ASD (Assaf *et al.*, 2010; Anderson *et al.*, 2011; Di Martino *et al.*, 2011; Abrams *et al.*, 2013; Cerliani *et al.*, 2015) and ADHD (Castellanos *et al.*, 2008; Cao *et al.*, 2009; Fair *et al.*, 2010; Tomasi and Volkow, 2012). There have been relatively fewer resting-state MEG studies in these disorders. In schizophrenia, however, atypical connectivity patterns have been shown across a number of frequency bands and brain regions (Kissler *et al.*, 2000; Rutter *et al.*, 2009; Hinkley *et al.*, 2011; Kim *et al.*, 2014; Chen *et al.*, 2016; Houck *et al.*, 2017). While there is a lack of consistency between studies, overall these data show alterations in local and long-range oscillatory dynamics, suggesting that both local and global neural organisation is

altered in schizophrenia (Alamian *et al.*, 2017). MEG studies of ASD have found similarly variable results with hypo- and hyperconnectivity being reported in both short and long-range connections (Cornew *et al.*, 2012; Edgar *et al.*, 2015; Kitzbichler *et al.*, 2015; Datko *et al.*, 2016; Brodski-Guerniero *et al.*, 2018; Lajiness-O'Neill *et al.*, 2018). ADHD has been less extensively investigated but atypical connectivity has been shown across a broad range of frequency bands (Wilson *et al.*, 2012; Franzen *et al.*, 2013; Sudre *et al.*, 2017). These atypical patterns have been found to improve with the administration of stimulant medication (Wilson *et al.*, 2012; Franzen *et al.*, 2013).

Despite the considerable variability in the findings across disorders, one network that is repeatedly implicated in resting-state fMRI and MEG studies across neurodevelopmental disorders is the default-mode network (DMN (Bluhm *et al.*, 2007; Whitfield-Gabrieli *et al.*, 2009; Assaf *et al.*, 2010; Franzen *et al.*, 2013; Kim *et al.*, 2014; Sudre *et al.*, 2017)). This network comprises the precuneus, posterior cingulate, medial prefrontal and parietal cortices. It is known to be active during the resting-state and is deactivated during goal-directed activity (Binder *et al.*, 1999; Raichle *et al.*, 2001). Covariation patterns in DMN functional connectivity are moderately heritable (Glahn *et al.*, 2010) and abnormalities of this network have been found in relatives of people with schizophrenia (Whitfield-Gabrieli *et al.*, 2009; Jang *et al.*, 2011; Liu *et al.*, 2012; van Buuren, Vink and Kahn, 2012) and those at clinical high-risk of schizophrenia (Wang *et al.*, 2016).

Several resting-state fMRI studies in 22q11.2DS have shown altered connectivity in a number of resting-state networks including the DMN (Debbané *et al.*, 2012; Schreiner *et al.*, 2014; Padula *et al.*, 2015; Mattiaccio *et al.*, 2016; Zöller *et al.*, 2017), sensorimotor, visuo-spatial and high-level visual networks (Debbané *et al.*, 2012). Alterations in the DMN have been also been associated with impaired social competence (Schreiner *et al.*, 2014) and psychotic symptoms (Debbané *et al.*, 2012; Padula *et al.*, 2015; Mattiaccio *et al.*, 2016) in 22q11.2DS. There has been one resting-state EEG study in 22q11.2DS. This study found abnormal EEG microstates in adolescents with 22q11.2DS (Tomescu *et al.*, 2014). To my

knowledge there have been no previous resting-state MEG studies of 22q11.2DS meaning that patterns of resting-state neural oscillatory activity have not yet been examined in this high-risk group.

## 4.3 Rationale, aims and hypotheses

The variability in the results of resting-state neuroimaging studies across neurodevelopmental disorders may be due to a number of experimental factors including the sample size, imaging modality (fMRI or MEG), resting-state paradigm (e.g. eyes-open or eyes-closed (Nair *et al.*, 2018)) and analysis approaches used. Furthermore, sample characteristics including participant age (particularly when children and adults are included in the same sample), ascertainment method (e.g. based on clinical diagnosis), medication use (particularly common in clinical and/or adult samples) and control group selection (e.g. community controls from a different socioeconomic background than cases) are potential confounding factors which make it difficult to interpret findings to identify cortical circuit abnormalities relevant to these disorders.

This study seeks to investigate neural circuit abnormalities in children with 22q11.2DS, a syndrome associated with high-risk across the spectrum of neurodevelopmental disorders, by comparing CNV carriers to children without neurodevelopmental CNVs using MEG. In this chapter, eyes-open resting-state connectivity was investigated across the whole cortex for six frequency bands (delta, theta, alpha, beta, low-gamma and high-gamma) using an amplitude-envelope correlation approach. These methods were selected as they are robust, reproducible and repeatable. This study tried to address potential confounding factors by recruiting a group of children, who were ascertained by CNV status rather than by clinical diagnosis, together with a group of unaffected siblings of CNV carriers who were from a similar genetic and socioeconomic background. Detailed phenotypic information was collected so that associations between psychiatric and cognitive variables could be explored.

The primary aim of this chapter is to compare resting-state connectivity patterns between children with 22q11.2DS and children without neurodevelopmental CNVs. A secondary aim is to explore relationships between measures of connectivity, cognitive ability and psychopathology. As children with 22q11.2DS are expected to have altered E-I balance, it is hypothesised that compared to those without neurodevelopmental CNVs, children with 22q11.2DS have reduced resting-state connectivity (as measured by the strength of amplitude-envelope correlations) across multiple frequency bands. It is further hypothesised that the severity of resting-state network dysfunction will be associated with the severity of cognitive impairment and psychopathology.

## 4.4 Methods

### 4.4.1 Participants

Participants were recruited from the Experiences of people with copy number variants (ECHO) cohort at Cardiff University (See Chapters 2 and 3 for a detailed description of recruitment methods and sample characteristics). Children aged between 10-17 years old with a diagnosis of 22q11.2DS (probands) or who had a biological sibling with a neurodevelopmental CNV (controls) were invited to participate. CNV status was confirmed for all participants. Probands were excluded from the study if a 22q11.2 deletion was not confirmed either by in-house testing or by clinical genetics report. All controls had microarray testing at the Division of Psychological Medicine and Clinical Neurosciences (DPMCN) and were excluded if they were found to have a pathological CNV. Exclusion criteria for the MEG session were photosensitive epilepsy, the presence of orthodontic braces and metallic implants or prostheses in the upper half of the body. For the MRI session, participants were excluded if they had contraindications to MRI at 3T (e.g. the presence of a pacemaker or cochlear implant, intracerebral shunt, some types of orthodontic braces or a history of surgery which may have involved metallic implants not certified as MRI safe).

45 children with 22q11.2DS and 27 siblings of children with neurodevelopmental CNVs expressed interest in participating in the study. All potential participants were screened over the telephone for contraindications for MEG and MRI. In cases where participants had potential contraindications, advice was sought from CUBRIC's radiographers and laboratory managers and when necessary the participants' doctors were contacted to ensure that it was safe for them to participate. Seven probands were unable to participate in the MEG session and 21 probands were unable to participate in the MRI session due to contraindications. One control was found to have a 16p11.2 deletion and was therefore excluded from the study. In total, 38 probands and 26 controls had a resting-state MEG recording with 24 probands and 26 controls also having a structural MRI scan for co-registration of their MEG data. For probands who were able to take part in the MEG session but not the MRI session, an alternative strategy was used for co-registration of their MEG data (see below). The controls included 22 siblings of children with 22q11.2DS and four siblings of children with other neurodevelopmental CNVs. Of the siblings of children with 22q11.2DS, 14 were related to participating probands and 12 were unrelated.

Ethical approval for the study was obtained from South East Wales National Health Service (NHS) Research Ethics Committee. Participants over the age of 16 years old with capacity to consent gave written informed consent to participate in the study. Children under the age of 16 years old or those over the age of 16 years old who lacked the capacity to consent for themselves gave written and/or verbal assent to take part and their parents or carers provided consent for their participation.

Participants fulfilling the criteria for participation in the study were invited to visit Cardiff for brain imaging at a time convenient to them. Demographic, psychiatric and cognitive data were collected either during the imaging visit (n=16) or during a home visit (n=48) (mean time gap=0.71 months, SD=6.48 months). The assessments used in the ECHO study have been described in detail in Chapters 2 and 3. Briefly therefore, psychiatric interviews with the child's primary caregiver

were conducted using the Child and Adolescent Psychiatric Assessment [CAPA; (Angold *et al.*, 1995)] to derive DSM-IV-TR (American Psychiatric Association, 2000) diagnoses and symptom counts, and the Autism Diagnostic Interview-Revised [ADI-R; (Lord, Rutter and Le Couteur, 1994)] to derive ASD diagnoses and symptom counts. Cognitive assessments were conducted with participating children using the Wechsler Abbreviated Scale of Intelligence [WASI;(Wechsler, 1999)], the Wisconsin Card Sorting Test [WCST; (Heaton *et al.*, 1993)] and a selection of tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge Cognition Limited, UK, 2006; see Chapters 2 and 3 for a description of the subtests used). Parents or carers were also asked to complete a questionnaire about their child and the wider family. This pack included questions about family background (ethnicity, family income and maternal education) and standardised questionnaires including the Social Communication Questionnaire [SCQ; (Rutter, Bailey, and Lord 2003)] for ASD symptomatology.

### 4.4.2 MEG data acquisition

Children and their accompanying parents or carers were given plenty of time to familiarise themselves with the MEG environment before the session commenced. Parents were invited to accompany their child into the MEG-shielded room if they felt that this would make their child more relaxed and comfortable. Once they were happy to proceed, participants, and where applicable, their accompanying parent or carer removed any metallic clothing and make-up. Electromagnetic coils were placed at three fiducial locations (bilateral preauricular regions and nasion) and their position relative to the MEG sensors was localised at the beginning and end of each recording. Five minute whole-head MEG recordings were acquired at a 1200Hz sample rate using a 275-channel CTF radial gradiometer system. An additional 29 reference channels were recorded for noise cancellation purposes. Primary sensors were analysed as synthetic third-order gradiometers (Vrba and Robinson, 2001). Participants were seated upright in the MEG system during the recordings and were instructed to keep their eyes

open and to attend to a red fixation point presented on a mean luminance background using a Mitsubishi Diamond Pro 2070 monitor (100Hz frame rate) or PROPixx LCD projector (120Hz frame rate). Relative head position at the beginning and end of the recording was used to as a proxy measure of participant head motion.

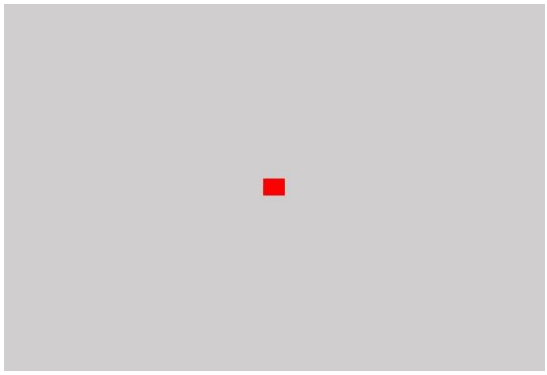

*Figure 4-1* Resting-state stimulus display

### 4.4.3 MRI data acquisition

Individual 1mm isotropic, T1-weighted anatomical MRIs were acquired when possible from participating children for co-registration of MEG data. From March 2013 to August 2016, fast spoiled gradient echo (FSPGR) images (TR=7.8ms, TE=3.0ms, voxel size = 1mm isotropic) were acquired on a 3T General Electric MRI system (GE medical systems, Milwaukee, WI). Due to the upgrade and relocation of CUBRIC facilities during the summer of 2016, from August 2016 to February 2018 magnetisation prepared rapid gradient echo (MP-RAGE) images (TR=2.3ms, TE=3.06ms voxel size = 1mm isotropic) were acquired on a 3T Siemens Magnetom Prisma MRI scanner (Siemens, UK) .

### 4.4.4 MEG analysis pipeline

After the recordings, MEG data were downsampled to 600Hz, band-pass filtered at 1-150Hz and segmented into 2s epochs, generating 150 trials per participant. Data were then visually inspected for artefacts such as motion, muscular

contraction and eye movements. Trials containing such artefacts were removed from the dataset and excluded from further analysis. Datasets were filtered into the following bandwidths: delta (1-4 Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-30Hz), low gamma (30-50Hz) and high gamma (50-90Hz). Participants with head motion greater than 30mm or with greater than half of their trials containing artefacts were excluded from further analysis (n=4). After quality control, data from 35 probands and 25 controls remained and were included in the subsequent analyses.

Co-registration was performed by manually labelling the fiducial points on each participant's MRI using the software package MRIViewer. In cases where it was not possible to acquire MRI data from a child taking part in the MEG study (e.g. due to MRI contraindications), or when MRI data were not of sufficient quality (e.g. due to movement during data acquisition), an appropriate alternative co-registered MRI scan was selected. The most appropriate alternative was identified by comparing the relative distances between the fiducial points for each participant and matching these with another participant's dataset. The resulting head models were visually inspected to ensure goodness of fit.

Amplitude-amplitude coupling between brain regions was analysed by spatial down-sampling to regions of the Automated Anatomical Labelling (AAL) atlas (Tzourio-Mazoyer *et al.*, 2002). 90 cortical and subcortical regions were identified and time-series from these ROIs were extracted and analysed. Source reconstruction was performed for each participant and each frequency band using a linearly-constrained minimum variance (LCMV) beamformer on a 6mm grid using FieldTrip (version 20161011, www.fieldtriptoolbox.org). Single shell source models were registered to the Montreal Neuroimaging Institute's (MNI) standard coordinate space. Virtual sensors were estimated for each of the voxels on a 6 x 6 x 6mm grid. Within each of the 90 ROIs, the virtual sensor with the maximum temporal standard deviation was chosen as the representative time-series for each atlas region. These 90 signals were then orthogonalised using linear regression to suppress any zero-time-lag correlation suggestive of signal

leakage in order to reduce any spurious correlations (Colclough *et al.*, 2015). The amplitude (Hilbert) envelope of source-space neural oscillatory activity for every AAL region per frequency band was then computed from these orthogonalised signals. A median spike removal filter was applied to smooth large deflections in the data before further analysis.

Cross-correlations of the amplitude envelope for each pair of AAL regions and for each frequency band were computed across the entire MEG time-series giving a single correlation coefficient value per pair and frequency band. Amplitude correlations were converted to variance-normalised z-scores by applying a Fisher transform. Each participant's connectivity matrix was then z-scored to have zero mean and unit variance across all connections, in order to correct for variability in data quality between participants (Siems *et al.*, 2016). Connectivity matrices were then constructed for each participant using the z-statistics as edge weights for all the AAL atlas regions, with each connection edge representing the relative strength of amplitude correlations, compared to the mean, for each participant. The averaged, groupwise data and between-group differences were plotted on connectivity matrices and visually compared. The top 5% of connections in probands and siblings were then plotted on circle plots together with plots of group differences.

To avoid analysing noise connections, signals were classified as signal or noise using a statistical thresholding procedure based on Gaussian mixture modelling (GMM, (Plataniotis and Hatzinakos, 2017)) for each frequency band. Using the expectation-maximisation algorithm with two components randomly selected as the initial component means, a Gaussian mixture distribution was fitted to the z-statistics by maximum likelihood. Only connections with greater than 50% probability of being signal were accepted as being valid. To ensure that important between-group differences were not missed, this procedure was carried out separately for probands and controls and connections were labelled as valid if they reached the threshold in either group. The most valid connections were then plotted onto connectivity maps. The signal z-statistic for each region was then

summed horizontally to give the strength of their connectivity with the rest of the brain. Global connectivity was calculated for each group by summing the strength of all signal connections.

The analysis pipeline is summarised in figure 4.2.

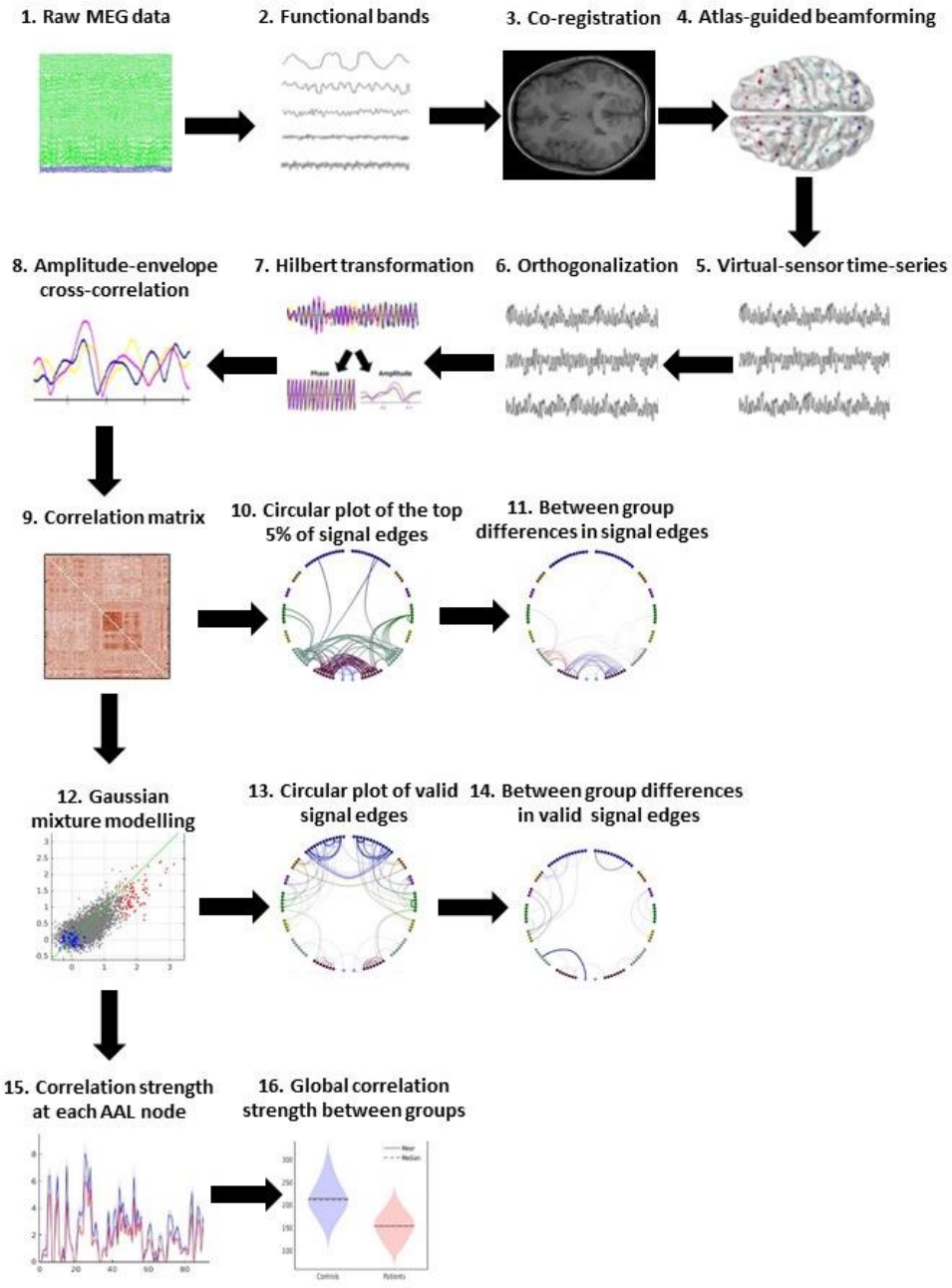


*Figure 4-2 MEG analysis pipeline*

### 4.4.5 Statistical analysis

Network statistics were performed in MATLAB (version R2015a, MathWorks). Descriptive statistics and exploratory analyses of the relationships between cognitive ability, psychopathology and connectivity metrics were conducted in R studio (version 1.1383 for Mac, www.rstudio.com).

**Descriptive data**

Between-group differences in age, gender, handedness, head motion and number of trials were analysed using the appropriate parametric or non-parametric test e.g. t-test, Mann-Whitney U test or chi-squared test. For continuous data, distributions were first checked using the Shapiro-Wilk's test of normality in order to determine the most appropriate statistical test.

**Between-group differences in amplitude-envelope correlations (connectivity)**

Between-group differences at each of the valid connection edges identified in the GMM analysis were tested using unpaired t-tests of the corrected z-scores to identify significant edges at $p<0.05$ (uncorrected). Permutation testing of the maximum t-statistic was then performed to correct for multiple comparisons within each frequency band; case and control labels were swapped randomly to create two artificial matrices which were then compared. This was repeated 2000 times to generate a null distribution and compared with the results previously generated from the real data to determine the probability that the results occurred by chance. This was performed for each of the frequency bands of interest. No correction was applied for testing across the different frequency bands.

**Sensitivity analyses**

Sensitivity analyses were conducted to examine the effects of potential confounding factors such as age and medication use on the between-group differences. This involved excluding individual participants and re-running

analyses to determine whether the exclusion of these participants affected any observed results.

**Relationships between resting-state connectivity, cognitive ability and psychopathology**

Each participant's global connectivity score (corrected z-scores summed across all 90 AAL nodes) was extracted for each of the frequency bands that showed signal connections in the GMM analysis. The AAL node with the most significant difference in connectivity strength (corrected z-score) between probands and controls was then identified for each of these frequency bands and z-scores were extracted for each participant. The relationships between these scores, cognitive ability and psychopathology were explored using linear regression.

As cognitive ability is strongly associated with group status (proband or control), associations between cognitive scores and connectivity scores were explored in each group separately. Full-scale IQ (FSIQ), CANTAB subtest and WCST scores were used as measures of cognitive ability and were included in the regression models as independent variables. Due to the low rates of psychopathology in the control group, the relationships between connectivity scores and psychopathology were computed in the proband group only using symptom counts for the most common disorders as independent variables. As outlined in Chapter 3, the most common psychiatric conditions in the sample were anxiety disorders, ADHD and ASD. Age, gender and handedness were added hierarchically to the regression models to control for these potential confounders. These exploratory analyses were not corrected for multiple testing.

## 4.5 Results

### 4.5.1 Descriptive data

As shown in table 4-1, probands were on average 11 months younger than controls, although this difference was not statistically significant at $p<0.05$ (mean age probands=13.5 years, age range 10.5-17.9 years; mean age controls=14.4

years, age range 10.5-17.5 years, p=0.06). There were no statistically significant differences in the gender or handedness between groups.

*Table 4-1 Age, gender and handedness of participating children*

| | **Probands** | **Controls** | **$t/\chi^2$** | **P** |
|---|---|---|---|---|
| **Age (SD)** | 13.5 (1.9) | 14.4 (1.8) | -1.90 | 0.06 |
| **Gender, % female** | 17(48.6) | 10(40.0) | 0.02 | 0.88 |
| **Handedness, % right** | 27(77.1) | 19(76.0) | <0.01 | 0.95 |

One proband had a past medical history of epilepsy but had not had any recent seizures and was not taking anticonvulsant medication. Few children were taking any regular medication at the time of the scans. Four probands (11.4%) were taking melatonin for sleep problems. One of these children was also taking aripiprazole 5mg once daily for challenging behaviour. No controls were taking medication for neurological, psychiatric or sleep disorders.

### 4.5.2 Resting-state functional connectivity

**Data quality**

In terms of data quality, there were no significant between-group differences in head motion (median=4.9mm (IQR=5.5mm) in probands, median=2.9mm (IQR=4.6mm) in controls, p=0.49) or the number of trials included after quality control (median=130 (IQR=35) in probands, median = 133 (IQR=29) in controls, p=0.17) for the 35 probands and 25 controls who were included in the connectivity analyses.

**Simple visual analysis of differences in the connectivity matrices between probands and controls**

Figure 4-3 shows the connectivity matrices for each of the frequency bands. The resulting matrices are symmetrical with each square representing the corrected

z-statistic of the amplitude-envelope correlation between one AAL brain region and another. The first two columns show the matrices for each group separately (probands and controls), while the column on the right shows the differences in matrix z-scores between the groups. In the within-group matrices, areas of high correlation are represented in red. In the matrices of between-group differences, brain regions in which probands have higher z-scores than controls are coloured red, while regions in which probands have lower z-scores than controls are coloured blue.

These connectivity matrices indicate that across the frequency bands, but most markedly in the alpha and beta bands, there are strong correlations between activity in posterior brain regions (seen as red in the centre of the first two columns, representing occipital and posterior parietal regions). Furthermore, there are differences in the strength of these connections between probands and controls, with probands having lower z-scores than controls in the alpha and beta bands in these brain regions. In the gamma bands, there appear to be strong correlations between activity in frontotemporal regions (seen in the top left of the connectivity matrix) with higher z-scores in probands than in controls.

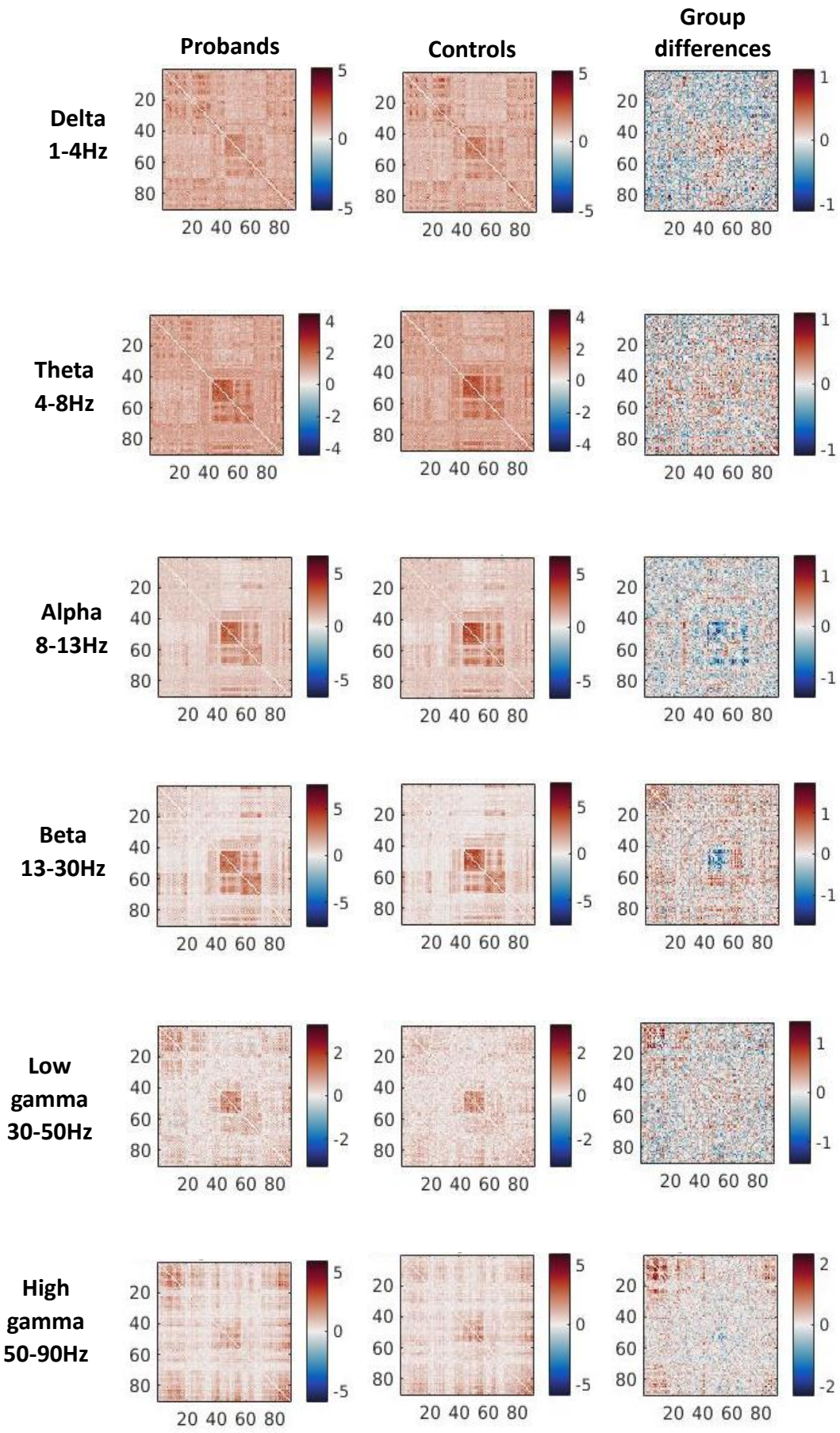


*Figure 4-3 Connectivity matrices for each of the frequency bands of interest*

**Identification of signal connections and comparison between probands and controls**

Figure 4-4 shows an example of the circle plots onto which the top 5% of connections for each group was plotted. Each point on the map represents a different AAL node. The same circle plots were used in the GMM analysis.

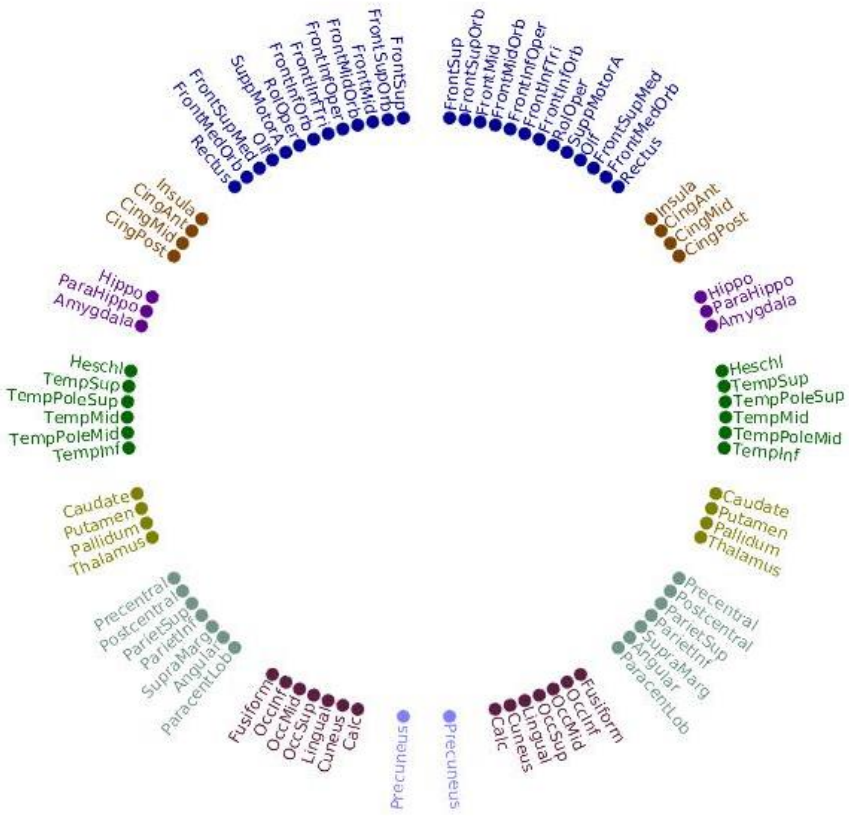


*Figure 4-4 Circle plot of AAL nodes used in the connectivity analyses*

Figure 4-5 shows the connectivity maps across frequency bands with each point representing one of the AAL nodes illustrated in figure 4-4. The first two columns show the top 5% of connections for each group separately, whereas the column on the right shows the differences in connection strength between groups, with red representing higher and blue representing lower amplitude-envelope correlations in probands compared with controls. This figure is a simple visualisation of between-group differences without statistical testing.

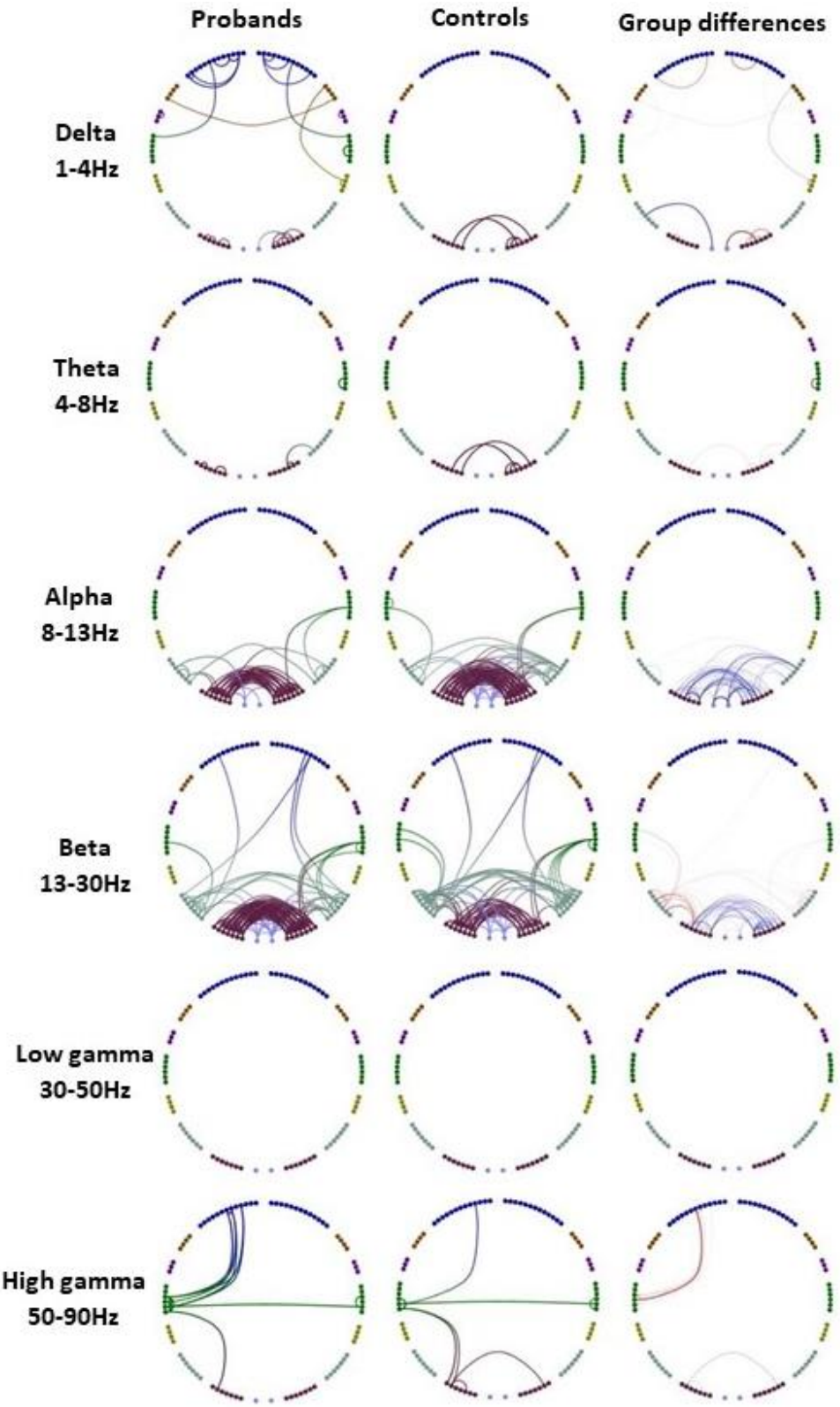


*Figure 4-5 Circle plots of the top 5% of connections for each of the frequency bands of interest*

In the delta band, connections can be seen within and between several brain regions, including frontal, temporal, limbic, parietal, occipital and subcortical nodes. There was increased connection strength between the right precuneus and occipital nodes and reduced strength between the left precuneus and inferior parietal node in probands compared with controls. The theta band shows temporal, parietal and occipital signal connections with stronger connections in the right temporal lobe in probands than controls. In the alpha band, the most prominent connections for both groups are in the occipital, parietal and temporal regions. Comparing these connections between groups, probands can be seen to have a reduction in the strength of connections in the occipital and right parietal regions. In the beta band, occipital, parietal and temporal connections are also present and there are additional signal connections between frontal and parietal nodes. However, between-group differences are restricted to the posterior part of the brain with similar reductions in the strength of occipital connections as those seen in the alpha band as well as increased strength of left parieto-occipital connections. In the high gamma band, frontotemporal, temporo-temporal, temporo-occipital and occipito-occipital signal connections are present with an increase in frontotemporal connectivity in probands compared with controls. No valid signal connections were identified in the low gamma band.

**GMM Analysis of signal connections between probands and siblings**

After GMM analysis, no signal connections were found in the theta, low gamma or high gamma bands. Figures 4-6 to 4-8 therefore show the results of the GMM analysis for delta, alpha and beta bands only.

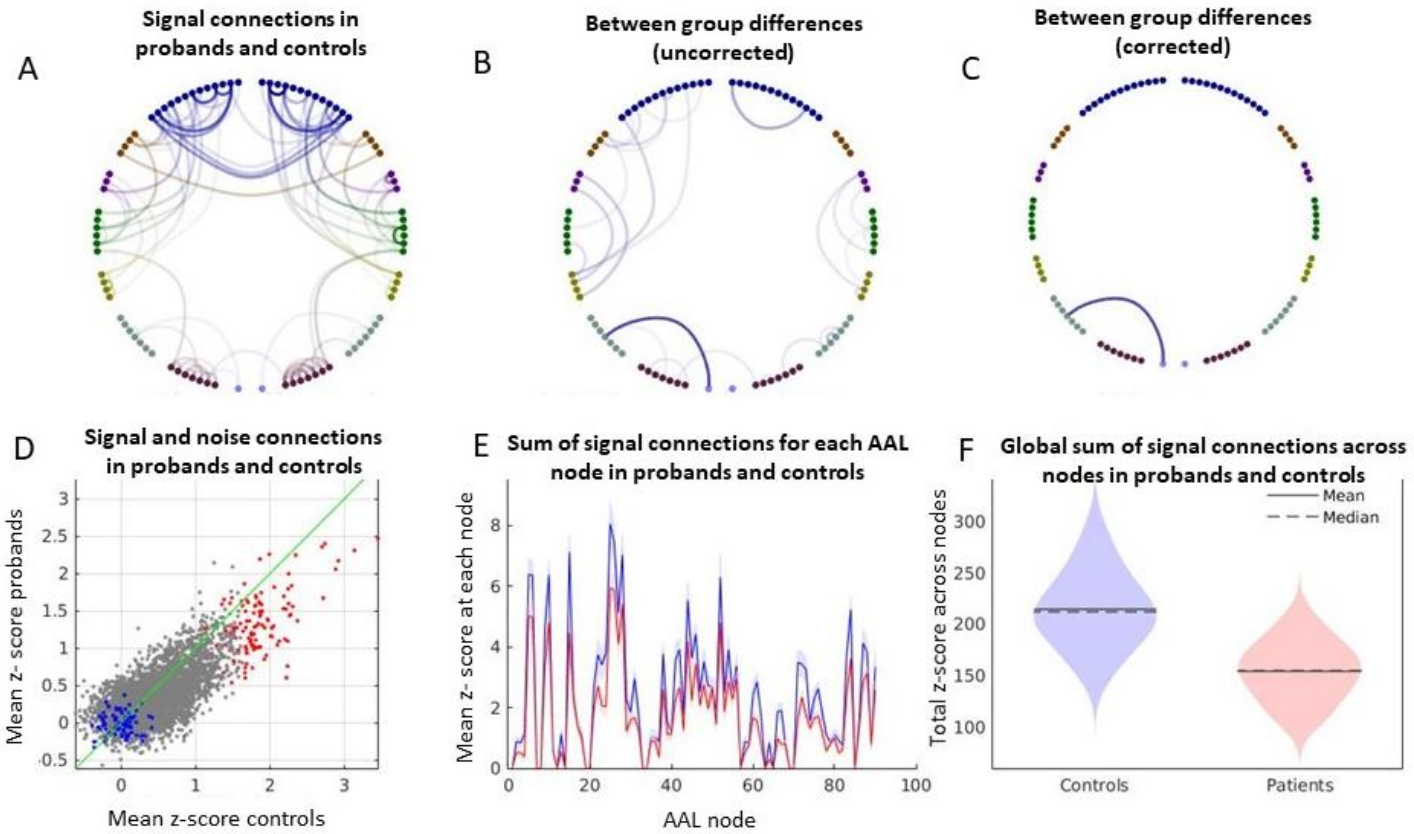


*Figure 4-6 Gaussian mixture modelling results in the delta band.*

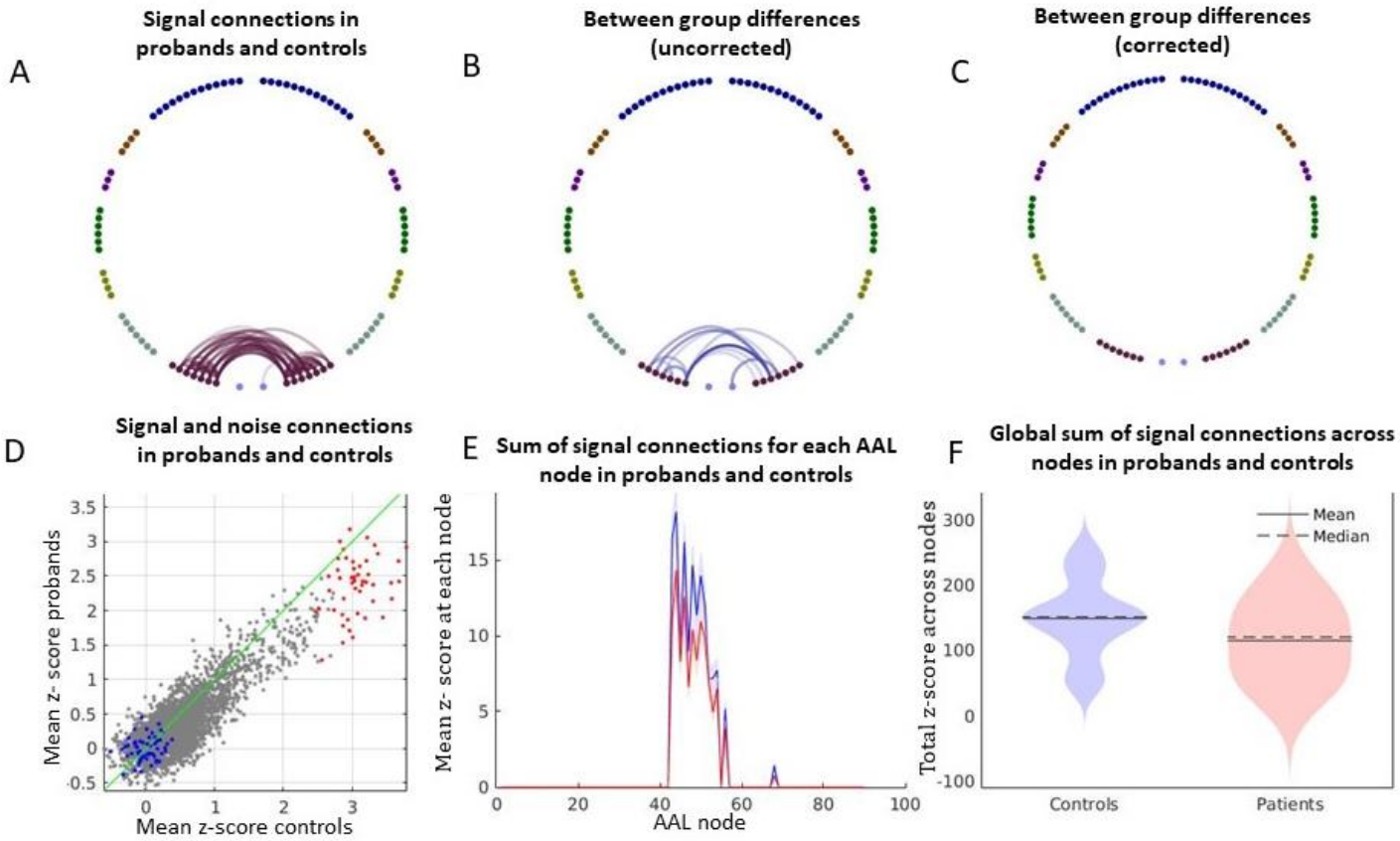


*Figure 4-7 Gaussian mixture modelling results in the alpha band.*

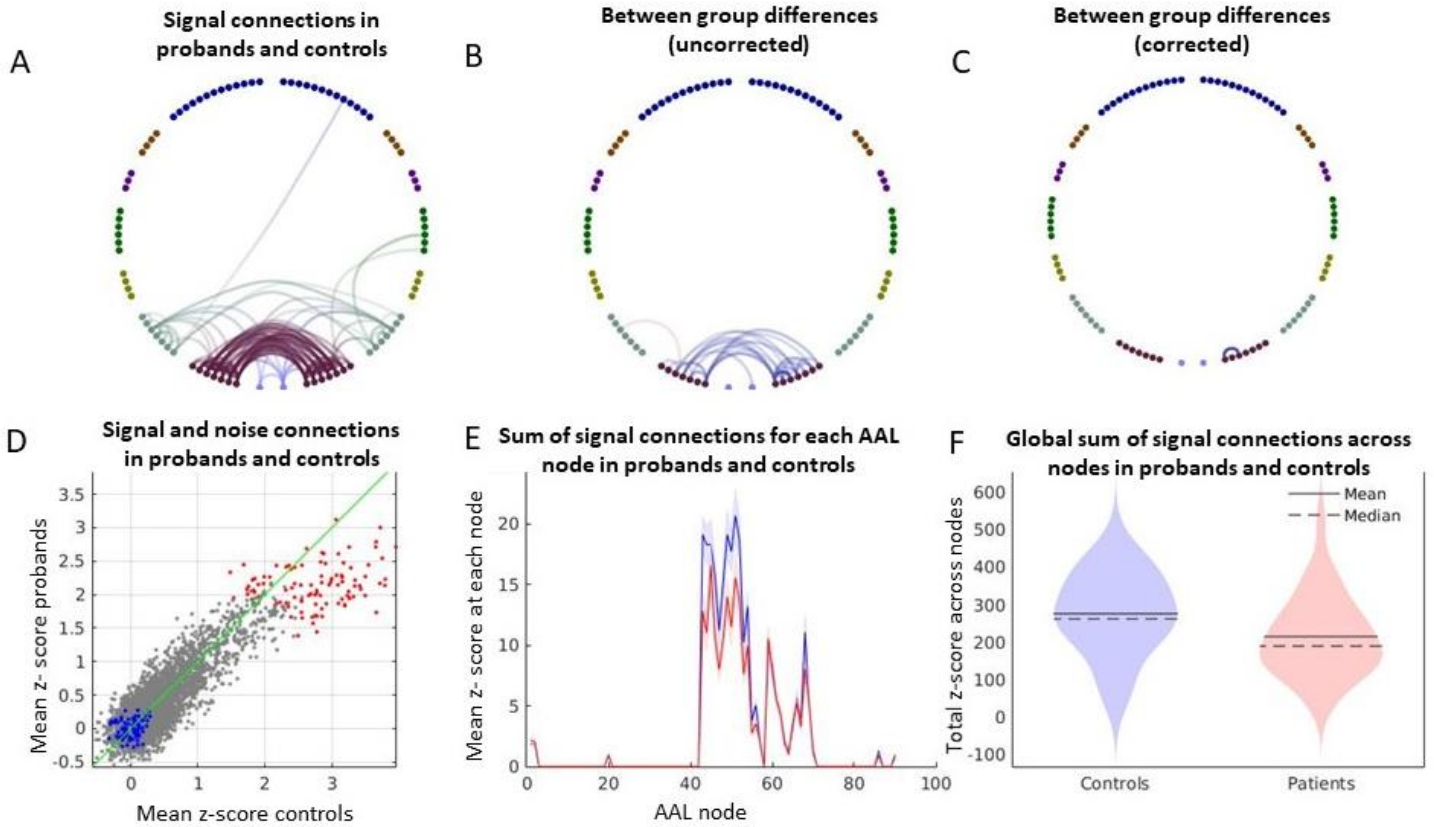


*Figure 4-8 Gaussian mixture modelling results in the beta band.*

In each figure (4-6 to 4-8), panel A shows the valid signal connections in probands and controls identified by GMM, panel B shows the uncorrected difference in the signal connection strength between probands and controls, panel C shows the corrected group differences in signal connection strength, panel D shows the distribution of mean z-scores between probands and controls with blue representing noise and red representing signal connections, panel E shows the sum of all signal connections at each AAL node for probands (red) and siblings (blue), and panel F shows the sum of the z-scores for all signal connections across all 90 AAL nodes in probands (red) and siblings (blue). Circle plots A, B and C in each figure refer to the same brain regions as shown in the circle plot in figure 4-4.

As can be seen in circle plot A in figure 4-6, delta band signal connections were found throughout the brain but were particularly prominent within and between frontal nodes. However, when comparing the differences between probands and controls (circle plot C), only one connection was statistically significantly different at $p<0.05$ (corrected) - the left precuneus to the left inferior parietal cortex. In the alpha band, signal connections were present in the occipital lobe and precuneus (circle plot A) with lower strength of these connections in probands compared to controls (circle plot B). However, randomisation testing of the resulting t-statistic identified no statistically significant connections at $p<0.05$ (circle plot C). The most significant between-group difference was in the connection between the left calcarine region and the right lingual gyrus ($p=0.07$). In the beta band, signal connections were also focussed on posterior brain regions (circle plot A). Probands had reduced amplitude-envelope correlations between occipital nodes with the connections between the right lingual gyrus and right calcarine region remaining statistically significant after randomisation testing (circle plot C, $p=0.01$).

The plots shown in the lower left panels (D) of figures 4-6 to 4-8 show an even distribution of noise connections between probands and controls (blue) across frequency bands suggesting that there are no between-group differences in data

noise. The distribution of signal connections (red) however, does differ between groups with lower signal connection z-scores in probands than controls. The lower middle plots (E) in figures 4-6 to 4-8 show the connectivity strength of each AAL node (calculated as the sum of the z-scores for every signal connection at each node (numbered 1-90)). These also show a reduction in probands (red) compared to controls (blue) across nodes and frequency bands. The nodes with the greatest difference in z-scores between probands and controls were the right precuneus in the alpha band, the right calcarine region in the beta band and the left insula in the delta band. Finally, the violin plots in the lower right panels show the global connectivity scores for each group (F). This score is calculated as the sum of the signal z-scores across all 90 nodes for probands (red) and controls (blue). This shows lower global connectivity in cases and controls, which is statistically significant at $p<0.05$ for delta ($p<0.01$), alpha ($p=0.04$) and beta ($p=0.04$) bands.

**Relationships between connectivity measures, cognitive ability and psychopathology**

In the alpha band, exploratory linear regression analyses found no statistically significant associations between global connectivity scores and age, IQ, WCST performance or CANTAB subtest scores in either proband or control groups. However, in the proband group, total z-scores in the right precuneus were significantly associated with performance in the Stockings of Cambridge (SOC) task of spatial planning and the Rapid Visual Processing (RVP) task of sustained attention. These associations survived correction for age (SOC: estimate=0.25, SE=0.11, $r^2$=0.11, p=0.03; RVP: estimate=0.28, SE=0.12, $r^2$=0.14, p=0.03) but not age and gender (SOC: estimate=0.21, SE=0.13, $r^2$=0.08, p=0.12; RVP: estimate=0.23, SE=0.13, $r^2$=0.26, p=0.08). Furthermore, a statistically significant relationship was found in the proband group between global and right precuneus connectivity scores and CAPA derived total anxiety scores and SCQ scores (see tables 4-2 and 4-3, and figures 4-9, 4-10, 4-11 and 4-12). These associations remained statistically significant even when age, gender and handedness were included in the regression models (global connectivity and total anxiety score, estimate= -3.70, SE=1.44, $r^2$= 0.10, p=0.02; right precuneus connectivity and total

anxiety score, estimate= -0.04, SE=1.39, $r^2$=0.08, p<0.05; global connectivity and SCQ score, estimate= -4.48, SE=1.44, $r^2$=0.27, p<0.01; right precuneus connectivity and SCQ score, estimate= -0.05, SE=0.02, $r^2$=0.18, p=0.02). However, ASD scores derived from the ADI-R and ADHD scores derived from the CAPA were not associated with either global or right precuneus connectivity. There were no statistically significant associations between connectivity scores and age, cognitive or psychiatric variables in either the beta or delta bands.

*Table 4-2 Relationships between alpha band global connectivity, psychopathology and cognitive ability in probands*

| | **Estimate** | **Standard Error** | **$R^2$** | **P** |
|---|---|---|---|---|
| **Age** | 3.18 | 5.87 | <0.01 | 0.59 |
| **ADHD Score** | -2.24 | 2.69 | 0.03 | 0.42 |
| **ADI-R Score** | -0.92 | 0.87 | 0.06 | 0.30 |
| **SCQ Score** | -4.23 | 1.57 | 0.29 | 0.01* |
| **Anxiety Score** | -3.59 | 1.36 | 0.21 | 0.01* |
| **FSIQ** | 0.82 | 0.85 | 0.03 | 0.34 |
| **WCST (set-shifting ability)** | <0.01 | 0.20 | <0.01 | 0.96 |
| **Visual attention (MTS)** | 1.42 | 1.17 | 0.03 | 0.23 |
| **Spatial working memory (SWM)** | 13.85 | 7.74 | 0.06 | 0.08 |
| **Spatial planning (SOC)** | 10.86 | 6.22 | 0.06 | 0.09 |
| **Processing speed (RTI)** | -5.56 | 7.39 | 0.02 | 0.46 |
| **Sustained attention (RVP)** | 10.68 | 9.44 | 0.05 | 0.27 |

*Abbreviations: ADHD, attention deficit hyperactivity disorder; ADI-R, Autism Diagnostic Interview-Revised; SCQ, Social Communication Questionnaire; FSIQ, full-scale IQ; WCST, Wisconsin Card Sorting Test; MTS, Match-to-Sample; SWM, Spatial Working Memory; SOC, Stockings of Cambridge; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing. P values are uncorrected.*

*Table 4-3 Relationship between alpha band connectivity in the right precuneus, psychopathology and cognitive ability in probands*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **Age** | <0.01 | 0.07 | <0.01 | 0.99 |
| **ADHD Score** | <0.01 | 0.03 | <0.01 | 0.79 |
| **ADI Score** | -0.02 | <0.01 | 0.16 | 0.08 |
| **SCQ Score** | -0.06 | 0.02 | 0.29 | <0.01* |
| **Anxiety Score** | -0.04 | 0.02 | 0.16 | 0.03* |
| **FSIQ** | <0.01 | 0.01 | 0.02 | 0.46 |
| **WCST (set-shifting ability)** | <0.01 | <0.01 | 0.02 | 0.52 |
| **Visual attention (MTS)** | <0.01 | 0.01 | 0.01 | 0.53 |
| **Spatial working memory (SWM)** | 0.20 | 0.15 | 0.06 | 0.18 |
| **Spatial planning (SOC)** | 0.24 | 0.11 | 0.16 | 0.03* |
| **Processing speed (RTI)** | -0.02 | 0.09 | <0.10 | 0.81 |
| **Sustained attention (RVP)** | 0.26 | 0.11 | 0.20 | 0.02* |

*Abbreviations: ADHD, attention deficit hyperactivity disorder; ADI-R, Autism Diagnostic Interview-Revised; SCQ, Social Communication Questionnaire; FSIQ, full-Scale IQ; WCST, Wisconsin Card Sorting Test; MTS, Match-to-Sample; SWM, Spatial Working Memory; SOC, Stockings of Cambridge; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing. P values are uncorrected.*

Figures 4-9 to 4-12 show that global alpha connectivity and right precuneus connectivity in the alpha band are lower in probands with higher anxiety scores and social communication problems.

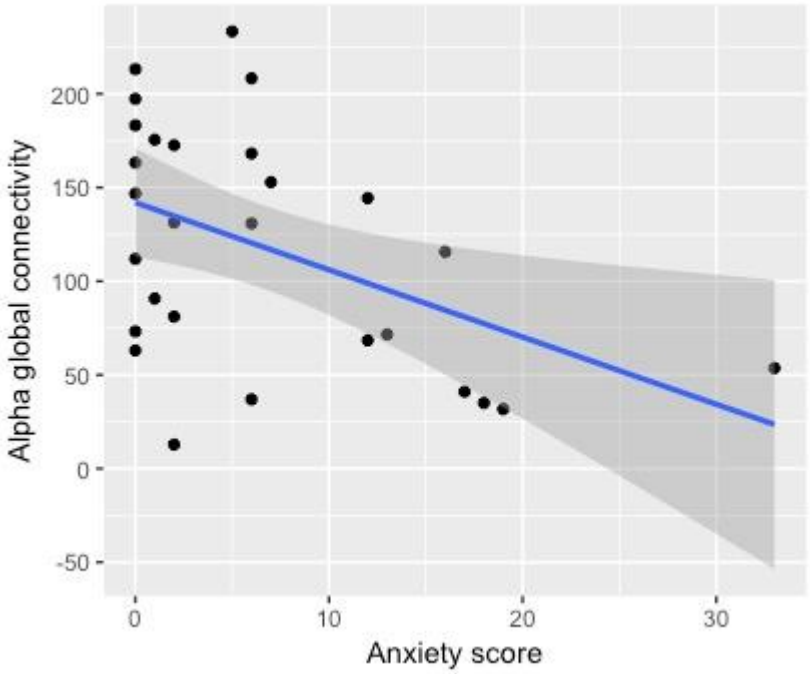


*Figure 4-9 Relationship between alpha global connectivity and total anxiety score in probands*

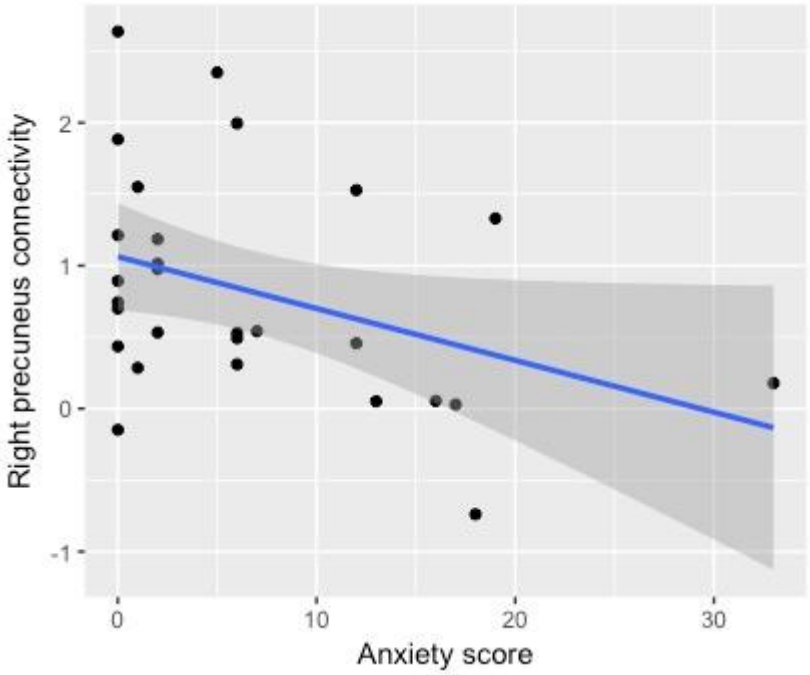


*Figure 4-10 Relationship between right precuneus connectivity and total anxiety score in probands*

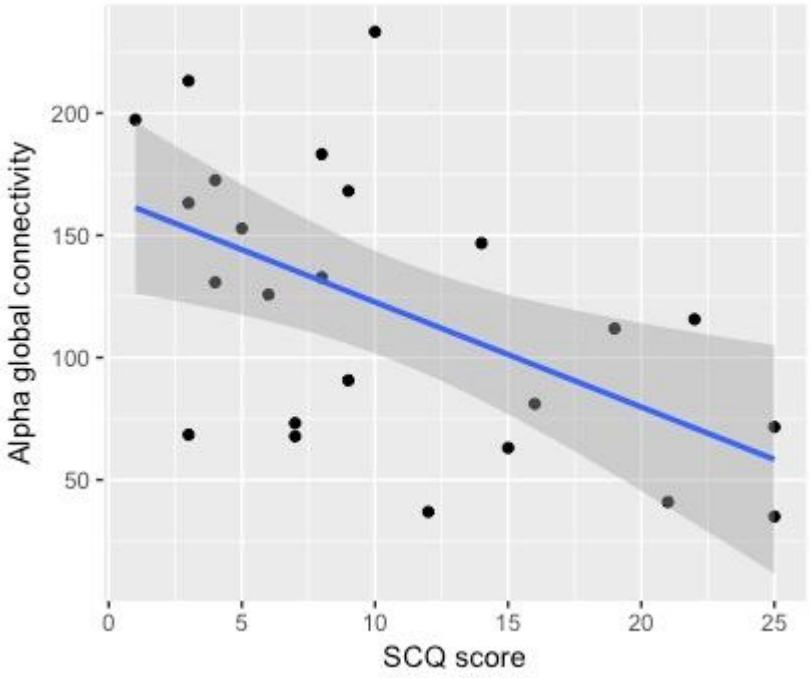


*Figure 4-11 Relationship between alpha global connectivity and Social Communication Questionnaire (SCQ) score in probands*

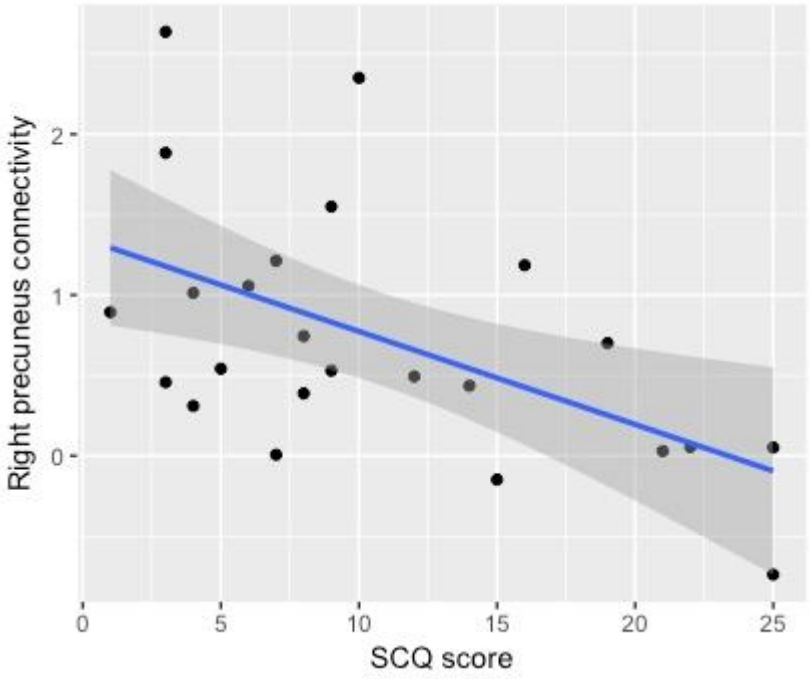


*Figure 4-12 Relationship between right precuneus connectivity and Social Communication Questionnaire (SCQ) score in probands*

**Sensitivity analyses**

Although not statistically significant, there was a mean age difference of 11 months between probands and controls who took part in the study. In the regression analyses reported above, age was not significantly associated with connectivity measures. However, to ensure that age differences between groups were not responsible for the observed differences in connectivity, analyses were re-run on the oldest 25 cases and the 25 controls. Mean age in this analysis was 14.2 years for probands (SD=1.7 years) and 14.4 years (SD=1.8 years) for controls. Analyses were also re-run excluding the child who was taking antipsychotic medication. These analyses confirmed the findings above with reductions in connectivity seen in the same frequency bands and brain regions.

## 4.6 Discussion

In this resting-state MEG study of children with 22q11.2DS, significant differences in global connectivity were found between probands and controls in the alpha, beta and delta bands. Furthermore, in the GMM analysis, probands had a statistically significant reduction in the strength of signal connections between the right lingual gyrus and right calcarine region in the beta band. There were also reductions in connection strength between the right lingual gyrus and the left calcarine region in the alpha band, but this did not survive correction for multiple testing at $p<0.05$. In the delta band, there was a reduction in the strength of the connection between left inferior parietal cortex and precuneus. While the simple analysis of differences suggested an increase in frontotemporal connections in the high gamma band in participants with 22q11.2DS, GMM analysis indicated none of these connections were valid signal. The apparent between-group differences may have been driven by outlier participants or by differences in noise between groups, e.g. from facial muscle and eye motion artefacts (Muthukumaraswamy, 2013). No signal connections were found in the low gamma or theta bands.

The nodes that were most significantly affected by CNV status were the right precuneus (alpha band), the right calcarine region (beta band) and the left insula (delta band). The precuneus and posterior parietal cortex form part of the default mode network (DMN). Atypical connectivity in this network has been found in resting-state fMRI studies of schizophrenia (Whitfield-Gabrieli *et al.*, 2009), ASD (Assaf *et al.*, 2010), ADHD (Franzen *et al.*, 2013; Sudre *et al.*, 2017), and 22q11.2DS (Debbané *et al.*, 2012; Schreiner *et al.*, 2014; Padula *et al.*, 2015; Mattiaccio *et al.*, 2016; Zöller *et al.*, 2017). Abnormal oscillatory dynamics in the DMN have been shown in clinical samples using MEG (Franzen *et al.*, 2013; Kim *et al.*, 2014; Lajiness-O'Neill *et al.*, 2018) but this study provides the first evidence for such alterations in 22q11.2DS.

This study also finds evidence for atypical connectivity in the occipital cortex, suggesting abnormalities of the visual pathways at rest in this high-risk group. People with 22q11.2DS have known deficits in visual perception and processing (Bearden *et al.,* 2001; Simon *et al.,* 2005; Magnée *et al.,* 2011; McCabe *et al.,* 2016) and alterations in visual processing networks have previously been demonstrated in both resting-state and event-related fMRI studies in 22q11.2DS (Andersson *et al.,* 2008; Debbané *et al.,* 2012). Furthermore, a recent EEG study in 22q11.2DS found a reduction in occipital cortical activity in 22q11.2DS compared to controls during an illusory contour task (Biria *et al.*, 2018). Visual processing abnormalities are well-established in schizophrenia (Silverstein *et al.*, 2015) and ASD (Vandenbroucke *et al.*, 2008; Baruth *et al.*, 2010; Vlamings *et al.*, 2010) but the relationship between these deficits in 22q11.2DS and the risk of neurodevelopmental and psychiatric disorders is not yet well-understood.

The altered alpha band oscillatory patterns seen in children with 22q11.2DS were found to be associated with psychopathology and cognitive function in the exploratory analyses. While caution should be exercised in interpreting these results, not least because they have not been corrected for multiple comparisons, the observed associations are nevertheless interesting. Global connectivity and precuneus connectivity were strongly associated with SCQ scores (a measure of

ASD symptomatology) but not with ADI-R scores. Although no association was found with ADI-R scores, fewer participants had complete ADI-R data so the power to detect associations was lower for this measure of ASD symptoms. Atypical alpha band connectivity has been reported in idiopathic ASD where it has been associated with symptom severity as measured by the Social Responsiveness Scale [SRS; (Constantino *et al.*, 2003)], albeit in a different direction to the present findings, with increased alpha power being seen in an eyes-closed resting-state paradigm (Cornew *et al.*, 2012). Global connectivity and precuneus connectivity were also strongly associated with total anxiety scores. To my knowledge, anxiety disorders have not previously been investigated with resting-state MEG. Anxiety disorders are extremely prevalent in 22q11.2DS across the lifespan (Schneider *et al.*, 2014) and have been found to predict conversion to psychosis (Gothelf *et al.*, 2007a; Kates *et al.*, 2015; Tang *et al.*, 2017).

In the present study, children with higher anxiety and SCQ scores had lower global alpha and precuneus connectivity. Due to the low rate of psychotic symptoms in this sample (see Chapter 3), the relationships between alpha band connectivity, anxiety symptoms, ASD symptoms and psychosis could not be explored further. However longitudinal follow-up studies through the risk-period for the onset of psychosis may shed further light on these relationships and the potential utility of alpha band connectivity as a biomarker for psychosis risk. This could have important implications for risk prediction within this already high-risk group.

## 4.7 Strengths and limitations

The investigations presented in this chapter have a number of strengths. A sample of children aged between 10 and 17 years old was recruited thereby reducing the confounding effects of age that can impact studies which include both children and adults. Siblings of children with neurodevelopmental CNVs were recruited as controls to try to match for socioeconomic and environmental factors. Rigorous phenotyping was performed so that relationships between connectivity measures, clinical and cognitive phenotypes could be explored. Although this

phenotyping revealed high rates of psychopathology, few children had received a psychiatric diagnosis or were receiving treatment. Indeed only one child was taking psychotropic medication and excluding this child from the analyses did not affect the observed results.

There are a number of different methods available to investigate functional connectivity using MEG. This chapter sought to identify consistent functional connections and to minimise the effects of noise using amplitude-envelope correlations of beamformer-derived oscillatory source signals. This approach was chosen as it is one of the most robust and repeatable methods available (Colclough *et al.*, 2016). Orthogonalisation was used to suppress any zero-time-lag correlation suggestive of signal leakage. A Gaussian mixture modelling approach was used to identify the correlations most likely to represent valid signal connections. A benefit of this approach is that any observed between-group differences are unlikely to be due to spurious correlations however, this is a conservative approach that can miss weaker correlations that may be relevant to pathology.

There are a number of limitations of the present study. The sample size, while fairly large in the field of 22q11.2DS research, is nevertheless modest for a MEG study and so without replication, the findings should be interpreted cautiously due to the risks of both type 1 and type 2 error (Button *et al.*, 2013). The relatively short duration of the resting-state paradigm was chosen to balance data quantity and quality, however this will have resulted in lower signal to noise ratios than a longer paradigm such as those conducted in adults and typically developing participants (Liuzzi *et al.*, 2017). Unfortunately a longer recording would not have been feasible for the majority of children taking part in this study. The frequency of surgical and other contraindications for MRI scanning meant that it was not possible to co-register MEG data to each participant's own MRI scan. While every attempt was made to closely match the fiducial locations to those of another participant, this is not a perfect substitute for using participants' own data and this may have affected source localisation. Furthermore, continuous head

localisation was not used during data acquisition as this was not standard practice in the CUBRIC MEG laboratory when the study commenced. This meant that detailed analysis of head movement and correction for this was not possible. Head localisation before and after recordings was used as a proxy for head motion, however, this does not give any indication about the amount of movement during the recording. While it is reassuring that this proxy measure did not differ between groups, without continuous monitoring, one cannot be certain that there were no differences between groups in the amount of movement during the task. Finally, there were some psychiatric and cognitive data missing at the time of analysis. This was particularly problematic for the ADI-R data, which were only available for 26 probands (74% of the sample). This will have affected the power to detect relationships between ADI-R scores and connectivity scores.

## 4.8 Conclusions

In conclusion, in this resting-state MEG study of children with 22q11.2DS and controls, there was evidence for a reduction in resting-state connectivity in alpha, beta and delta bands, particularly affecting posterior brain regions. In the alpha band, this reduction was associated with SCQ and total anxiety scores. This finding supports the hypothesis of altered resting-state connectivity in 22q11.2DS which is associated with the severity of psychopathology and provides preliminary evidence for excitatory-inhibitory imbalance in children with 22q11.2DS.

# 5 Visual gamma responses in 22q11.2 deletion syndrome

## 5.1 Summary

High frequency neuronal oscillations in the gamma band are generated by reciprocal connections between excitatory glutamatergic pyramidal cells and inhibitory GABAergic interneurons. These oscillations can be enhanced by simple sensory stimuli such as visual gratings. The amplitude, power and frequency of gamma responses to such stimuli are thought to reflect excitatory-inhibitory balance.

Gamma responses are highly heritable and are altered in schizophrenia, ASD and ADHD. Evidence from postmortem and animal studies suggests that the distribution and function of GABAergic interneurons is perturbed in 22q11.2DS but only one previous study has investigated gamma oscillations, finding reduced power of the EEG auditory steady-state response.

This study aimed to compare visual gamma and evoked responses between children with 22q11.2DS and controls and to explore relationships between these responses, cognitive ability and psychopathology. 36 probands and 26 controls were recruited to the study. MEG recordings were performed while children observed 100 trials of a stationary, vertical square-wave grating, presented at two-second intervals. A beamformer approach was used to identify sources of peak gamma activity in the visual cortex (virtual sensors). Peak gamma amplitude and peak gamma frequency were calculated from time-frequency analysis of the sustained response at these virtual sensors. The sum of broadband gamma power during the sustained response was also computed. The amplitude of visual evoked potentials was investigated by performing time-frequency analysis of the evoked response. Relationships between age, psychopathology, cognitive ability, induced gamma and evoked responses were explored using linear regression.

Probands had a marked reduction in the sum of broadband gamma power between 35-70Hz during the sustained response. In the exploratory analyses, probands' cognitive performance was positively associated with the sum of broadband gamma power, peak gamma amplitude and the amplitude of the evoked response. The sum of broadband gamma power, peak gamma frequency and the amplitude of the evoked response were negatively associated with ASD symptoms. These findings support the hypothesis of altered excitatory-inhibitory balance in 22q11.2DS and provide preliminary evidence for an association with cognitive ability and social communication problems.

## 5.2 Introduction

In Chapter 4, it was shown that low frequency oscillations (in the delta, alpha and beta bands) were altered in 22q11.2DS using a resting-state MEG paradigm. No signal connections were observed in higher frequency bands in the GMM analysis. While low frequency oscillations are thought to predominantly reflect relatively long-distance communication, oscillations at higher frequencies, e.g. the gamma band, are thought to reflect synchronisation in local and distributed circuits (Singer, 1999; von Stein and Sarnthein, 2000; Uhlhaas and Singer, 2010; Donner and Siegel, 2011).

Previous MEG studies have shown that gamma oscillations can be enhanced by simple sensory stimuli. For example, in the visual cortex, gamma oscillations can be induced by high contrast gratings, producing both an early transient gamma spike and a later sustained response (Adjamian *et al.*, 2004; Hoogenboom *et al.*, 2006; Muthukumaraswamy *et al.*, 2009; Swettenham, Muthukumaraswamy and Singh, 2009; Perry *et al.*, 2011). These responses can be modulated by altering stimulus parameters such as contrast (Ray and Maunsell, 2010), orientation (Koelewijn *et al.*, 2011), spatial frequency (Adjamian *et al.*, 2004), motion (Swettenham, Muthukumaraswamy and Singh, 2009) and size (Perry *et al.*, 2013). In contrast to visual evoked potentials, which occur within 100ms after stimulus onset and are time- and phase-locked to it, induced gamma band responses occur

between approximately 300 and 1500ms and are not phase-locked to the stimulus. While evoked responses are considered to reflect bottom-up sensory processing involving driving inputs e.g. from the visual pathways, induced responses are thought to reflect higher order top-down processes such as feature-binding, which involve additional modulatory inputs (Tallon-Baudry *et al.*, 1996; David, Kilner and Friston, 2006).

Gamma oscillations are thought to be generated by reciprocal connections between excitatory pyramidal cells and inhibitory interneurons (Traub, Jefferys and Whittington, 1997; Bartos, Vida and Jonas, 2007; Whittington *et al.*, 2011; Buzsáki and Wang, 2012). Fast-spiking parvalbumin-containing (PV+) interneurons are critical to maintaining balance in these networks. These cells represent approximately 50% of GABAergic interneurons in the cortex and consist of both chandelier and basket cells which form $GABA_A$ synapses on target neurons and also synchronise each other through gap junctions. They are recruited by phasic glutamatergic input from pyramidal cells and provide inhibition to pyramidal cells and thus act as a pacemaker for oscillatory activity (Gonzalez-Burgos, Cho and Lewis, 2015). Optogenetic studies in mice have provided *in vivo* evidence that these interneurons are essential for driving cortical gamma oscillations (Cardin *et al.*, 2009; Sohal *et al.*, 2009).

Computational studies have shown that gamma amplitude, power and frequency are determined by local excitatory-inhibitory (E-I) balance (Brunel and Wang, 2003; Spencer, 2009). Furthermore, pharmaco-MEG studies have found that the gamma responses are affected by drugs that target GABAergic neurotransmission (Saxena *et al.*, 2013; Campbell *et al.*, 2014; Lozano-Soldevilla *et al.*, 2014; Magazzini *et al.*, 2016). Previous studies have shown that while there is considerable variability in peak gamma frequency between individuals, this is reproducible over time (Muthukumaraswamy *et al.*, 2010) suggesting that individuals have a characteristic gamma band response to visual stimuli, albeit one that decreases with age (Gaetz *et al.*, 2012; Robson *et al.*, 2015). As with

oscillations in lower frequency bands, gamma frequency has been found to be highly-heritable, with an estimated heritability of 91% (Vogel, 1970; Young, Lader and Fenton, 1972; van Beijsterveldt and van Baal, 2002; van Pelt, Boomsma and Fries, 2012).

Atypical gamma band responses have been reported in patients with schizophrenia as well as their unaffected relatives during simple perceptual and cognitive tasks (Kwon *et al.*, 1999; Spencer *et al.*, 2003; Spencer, Niznikiewicz, *et al.*, 2008; Spencer, Salisbury, *et al.*, 2008; Haenschel *et al.*, 2009; Tsuchimoto *et al.*, 2011; Hamm *et al.*, 2011; Mulert *et al.*, 2011; Liu *et al.*, 2012; Grützner *et al.*, 2013; Sun *et al.*, 2013; Hirano *et al.*, 2015). While the majority of studies report a reduction in gamma amplitude or synchrony, increased gamma responses in circumscribed brain regions have been associated with positive symptoms (Lee *et al.*, 2003; Spencer *et al.*, 2009). Furthermore, patients with schizophrenia have been found to have lower gamma frequency than controls in response to a visual gestalt stimulus (Spencer *et al.*, 2004). Gamma band abnormalities have also been reported in people with ASD in response to different stimuli (Grice *et al.*, 2001; Brown *et al.*, 2005; Milne *et al.*, 2009; Sun *et al.*, 2012; Wright *et al.*, 2012; Snijders, Milivojevic and Kemner, 2013) and in unaffected relatives of people with ASD (Rojas *et al.*, 2008, 2011). Correlations have been found between gamma responses and clinical symptoms measured by the SRS (Maxwell *et al.*, 2015). The peak frequency of gamma responses in the motor cortex has been shown to be reduced in ASD and to be negatively correlated with ASD severity, measured by the ADOS (An *et al.,* 2018). As with resting-state MEG/EEG studies, there have been few studies of gamma band responses in ADHD. However, a pharmaco-MEG study using an auditory stimulus found reduced gamma band activity in adults with ADHD, which increased after administration of stimulant medication (Wilson *et al.*, 2012)

Post-mortem studies have shown that the distribution and function of GABAergic interneurons is perturbed in patients with 22q11.2DS (Kiehl *et al.*, 2009; Mori *et*

*al.*, 2011). Murine models of 22q11.2DS provide further evidence for abnormalities in GABAergic neurons in this syndrome. These studies have found abnormal density, morphology and migration of PV+ interneurons (Meechan *et al.*, 2009, 2012; Piskorowski *et al.*, 2016) as well as abnormal cortical network activity (Sigurdsson *et al.*, 2010; Amin *et al.*, 2017). Despite this evidence for abnormal inhibitory networks in 22q11.2DS, there has been little research into gamma oscillations in humans with the microdeletion. To date, there has been only one published study of gamma oscillations in 22q11.2DS. This EEG study of auditory steady state responses recruited a mixed sample of children and adults with 22q11.2DS and found reduced gamma power and inter-trial phase coherence in 22q11.2DS compared to healthy non-carriers (Larsen *et al.*, 2018a).

People with 22q11.2DS are known to have deficits in visual processing (Bearden *et al.*, 2001; Simon *et al.*, 2008; Biria *et al.*, 2018). In Chapter 4, abnormal connectivity was found in the occipital cortex in a resting-state MEG paradigm, consistent with a previous fMRI study in 22q11.2DS (Debbané *et al.*, 2012). To my knowledge, there have been no previous studies of induced gamma responses to visual stimuli in 22q11.2DS using MEG, meaning that local excitatory-inhibitory balance in the occipital cortex has not been investigated in people with the syndrome.

## 5.3 Rationale, aims and hypotheses

Evidence from cellular, animal and human studies suggest that excitatory-inhibitory balance is disrupted in neurodevelopmental disorders and that this is reflected by atypical gamma band activity. As outlined in Chapter 4, variability across studies in terms of participant age, clinical status, medication use and control group selection affect the ability to draw firm conclusions about the nature of cortical circuit abnormalities in these disorders. Studying a group of children at high genetic risk for neurodevelopmental disorders offers the opportunity to address some of these biases and confounds.

This study used MEG to compare neural responses to visual stimulation between children with 22q11.2DS (probands) and children without neurodevelopmental CNVs (controls). In this chapter, induced and evoked responses to a static visual grating are investigated. This is an approach that has been shown to produce reliable and repeatable responses in the visual cortices of healthy controls (Muthukumaraswamy *et al.*, 2010). Detailed phenotypic information was collected so that associations between visual responses, cognitive and psychiatric variables could be explored.

The primary aim of this chapter is to compare visually-induced gamma oscillations (peak amplitude, peak frequency and total sum of gamma power between 35-70Hz) between children with 22q11.2DS (probands) and children without neurodevelopmental CNVs (controls). A secondary aim is to compare visual evoked responses between probands and controls. A final aim is to explore relationships between visual responses (induced and evoked), cognitive ability and psychopathology. Previous studies of people with neurodevelopmental disorders find atypical gamma band responses with variable directions of effect. As people with 22q11.2DS have visual processing abnormalities and as the deletion is associated with abnormalities of interneuron distribution and function, I hypothesise that compared to children without neurodevelopmental CNVs, children with 22q11.2DS have reduced evoked and induced gamma band responses. It is further hypothesised that these responses are positively associated with cognitive ability and negatively associated with the severity of psychopathology.

## 5.4 Methods

### 5.4.1 Participants

Participants were recruited from the Experiences of people with copy number variants (ECHO) cohort at Cardiff University (See Chapters 2 and 3 for a detailed description of recruitment methods and sample characteristics). Children with

22q11.2DS and unaffected siblings of children with neurodevelopmental CNVs were invited to take part. Ethical approval for the study was obtained from South East Wales National Health Service (NHS) Research Ethics Committee. Participants over the age of 16 with capacity to consent gave written informed consent to participate in the study. Children under the age of 16 or those over 16 who lacked the capacity to consent for themselves gave written and/or verbal assent to participate and their parents/carers provided consent for their participation.

All potential participants were screened over the telephone for contraindications for MEG and MRI. In cases where participants had potential contraindications, advice was sought from CUBRIC's radiographers and laboratory managers and where necessary the participants' doctors were contacted to ensure that it was safe for them to participate. Inclusion criteria were age 10-17 years old with a diagnosis of 22q11.2DS or a genetically-related sibling of a child with a neurodevelopmental CNV. Exclusion criteria for the MEG were photosensitive epilepsy and the presence of orthodontic braces or metallic prostheses in the upper half of the body. For the MRI, participants were excluded if they had contraindications to MRI at 3T (e.g. presence of a pacemaker or cochlear implant, intracerebral shunt, some types of orthodontic braces or a history of surgery which may have involved metallic implants not certified as MRI safe (or not known to be MRI safe)).

Participants fulfilling the criteria for participation in the study were invited to visit Cardiff for brain imaging at a time convenient to them. Demographic, psychiatric and cognitive data were collected either during the imaging visit (n=16) or during a home visit (n=46, mean time gap=0.69 months, SD=6.56 months). Psychiatric interviews with the child's primary caregiver were conducted using the Child and Adolescent Psychiatric Assessment [CAPA; (Angold *et al.*, 1995)] to derive DSM-IV-TR (American Psychiatric Association, 2000) diagnoses and symptom counts, and the Autism Diagnostic Interview-Revised [ADI-R; (Lord, Rutter and Le Couteur, 1994)] to derive ASD diagnoses and symptom counts. Cognitive assessments were

conducted with participating children using the Wechsler Abbreviated Scale of Intelligence [WASI;(Wechsler, 1999)], the Wisconsin Card Sorting Test [WCST; (Heaton *et al.*, 1993)] and a selection of tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge Cognition Limited, UK, 2006; see Chapter 2 for a description of the subtests used). Parents/carers were also asked to complete a questionnaire about their child and the wider family. This included questions about family background (ethnicity, family income and maternal education) and standardised questionnaires including the Social Communication Questionnaire [SCQ; (Rutter, Bailey and Lord, 2003)] for ASD symptomatology.

36 Children with 22q11.2DS (probands) and 26 non-carrier siblings of children with neurodevelopmental CNVs (controls) took part in this study. The control group included 22 siblings of children with 22q11.2DS and four siblings of children with other pathological CNVs. Of the siblings of children with 22q11.2DS, 15 were related to participating probands and seven were unrelated.

### 5.4.2 MEG data acquisition

MEG recordings were acquired at a 1200Hz sample rate using a 275-channel CTF radial gradiometer system. An additional 29 reference channels were recorded for noise cancellation purposes. Primary sensors were analysed as synthetic third-order gradiometers (Vrba and Robinson, 2001). Children and their accompanying parent/carer were given plenty of time to familiarise themselves with the MEG environment before the session commenced. Parents were invited to accompany their child into the MEG-shielded room if they felt that this would make their child more relaxed and comfortable. Once they were happy to proceed, participants, and where applicable their accompanying parent, removed any metallic clothing and/or make-up. Children with refractive errors affecting their ability to visualise the stimuli were given MEG-compatible glasses to wear during the recordings. Electromagnetic coils were placed at three fiducial locations (bilateral

preauricular regions and nasion) and their position relative to the MEG sensors was localised at the beginning and end of each recording. Participants were seated upright in the MEG system during the recordings. Relative head position at the beginning and end of the recording was used to as a proxy measure of participant head motion.

The stimulus consisted of a stationary, vertical, square-wave grating with maximum contrast, and a spatial frequency of 3 cycles per degree, located in the centre of the display with 8° x 8° of visual angle. Stimuli were presented on a mean luminance background using a Mitsubishi Diamond Pro 2070 monitor or PROPixx LCD projector (1024 x 768 pixel and 100Hz frame rate (monitor) or 120Hz frame rate (projector)). Participants were instructed to attend to a red fixation point in the centre of the screen and to press a response button when the grating disappeared. The grating was presented for 1.5-2 seconds and participants were given 0.75 seconds to respond. If they failed to respond within this time, a warning message was displayed before the next trial. After the response period, there was a rest period of 2 seconds during which only the red fixation square was presented. In total, the session contained 100 trials and lasted approximately eight minutes.

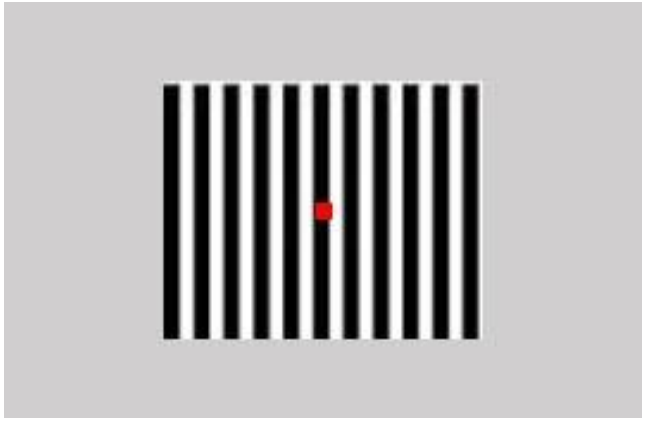

*Figure 5-1 Visual stimulus display*

### 5.4.3 MRI data acquisition

Individual 1mm isotropic, T1-weighted anatomical MRIs were acquired where possible from participating children for co-registration of MEG data. From March 2013 to August 2016, fast spoiled gradient echo (FSPGR) images were acquired on a 3T General Electric MRI system (GE medical systems, Milwaukee, WI) at CUBRIC. Due to the upgrade and relocation of CUBRIC facilities during the summer of 2016, from August 2016 to February 2018 magnetization-prepared rapid acquisition with gradient echo (MP-RAGE) images were acquired on a 3T Siemens Magnetom Prisma scanner (Siemens, UK).

### 5.4.4 MEG analysis pipeline

After the recording, data were epoched into 100 trials (-2 seconds to 2 seconds around stimulus onset) and visually inspected for artefacts such as motion, muscular contraction and eye movements. Trials containing such artefacts were removed from the dataset and were not included in the subsequent analysis. Participants with head motion greater than 30mm or with poor quality data (<50 good trials) were excluded from further analysis. Following quality control, data from 11 probands and four controls were excluded, leaving a sample of 25 probands and 22 controls.

Co-registration was performed by manually labelling the fiducial points on each participant's MRI using the software package MRIViewer. In cases where it was not possible to acquire MRI data from children taking part in the MEG study (for example, due to MRI contraindications or due to difficulty tolerating the MRI environment), or where MRI data were not of sufficient quality (e.g. due to movement during data acquisition), an appropriate alternative co-registered MRI scan was selected. The most appropriate alternative was identified by comparing the relative distances between the fiducial points for each participant and matching these with another participant's data. The resulting head models were visually inspected to ensure goodness of fit.

MEG sensor data were source-localized using FieldTrip (version 20180531, www.fieldtriptoolbox.org). Each participant's MRI was divided into an irregular grid by warping the individual MRI to the Montreal Neurological Institute's (MNI) template brain and then applying the inverse transformation matrix to the regular MNI template grid (5mm isotropic voxel resolution) to allow source estimates at comparable locations across participants. For each grid location inside the brain, the forward model (i.e. the leadfield) was calculated for a single-dipole orientation by singular value decomposition (SVD), using a single-shell volume conduction model (Nolte, 2003). Source power at each location was estimated using a linear constrained minimum variance (LCMV) beamformer (Van Veen *et al.*, 1997), where the weights were computed using a covariance matrix calculated after band-pass filtering the data between 35 and 70Hz. For each participant, the voxel of greatest increase in gamma power was located within the occipital cortex by contrasting the stimulus epochs (0.3 to 1.5 seconds) with baseline (-1.5 to -0.3 seconds). Anatomical masks were created using the Automated Anatomical Labelling (AAL) atlas (Tzourio-Mazoyer *et al.*, 2002) to exclude sources outside the visual cortex. At this peak location, the source-level time-series were reconstructed by multiplying the sensor-level data by the beamformer weights. Trials were represented in the time-frequency domain by calculating the amplitude envelope of signal obtained with the Hilbert transform. Time-frequency plots were visually inspected to identify sustained gamma band responses in these peak locations. The sum of broadband gamma power (35-70Hz), peak amplitude and peak frequency were calculated during the sustained response (0.3-1.5 seconds, (Swettenham, Muthukumaraswamy and Singh, 2009)) and extracted for further analysis. Time-frequency analysis of the evoked response (0.07-0.10 seconds) was also performed for each participant. Individual time-frequency plots were visually inspected and for participants with a clear evoked response, the magnitude of the evoked response amplitude was calculated from the time-series peaks, which were averaged over trials and baselined. The amplitude of the evoked response for each participant was extracted for further analysis.

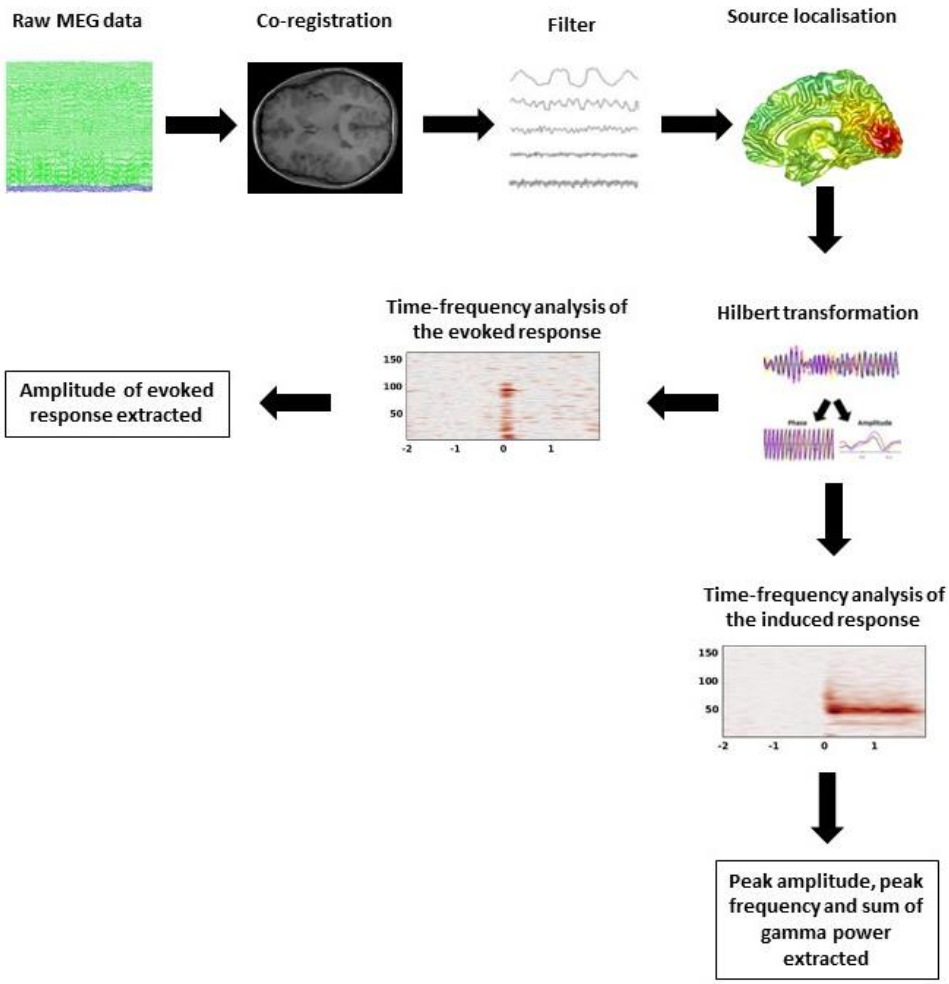


*Figure 5-2 MEG analysis pipeline*

### 5.4.5 Statistical analysis

Statistical analyses were conducted using R Studio (version 1.1383 for Mac (www.rstudio.com). For continuous data, distributions were first checked using Shapiro-Wilk's test for normality, in order to determine the most appropriate statistical test. Between-group differences in age were examined using a t-test while head motion, number of good trials, gamma response variables (sum of gamma power between 35-70Hz, peak gamma amplitude, peak gamma frequency and evoked amplitude) were compared using a Mann-Whitney U test. Gender and handedness proportions were compared using a chi-squared test. P values were not corrected for multiple comparisons.

Exploratory analyses of the relationships between gamma response variables, evoked amplitude, age, cognitive ability and psychopathology were subsequently conducted using linear regression. Shapiro-Wilk's tests showed that the distributions of the dependent variables were not normally distributed (sum of gamma power between 35-70Hz ($p<0.01$), peak gamma amplitude ($p<0.01$), peak gamma frequency ($p=0.04$), evoked amplitude ($P<0.01$)). Peak frequency was negatively skewed (skewness= -0.69), while the sum of gamma power between 35-70Hz, peak gamma amplitude and evoked amplitude were positively skewed (skewness=1.97, 1.58 and 1.30 respectively). Due to their skewness, gamma response variables for each group were first transformed using Tukey's power of ladders, which identified the most appropriate power transformation to make the data fit the normal distribution as closely as possible, before being converted to z-scores with a mean of zero and a standard deviation of one. Associations between gamma responses, evoked amplitude, age and cognitive ability were explored in each group (probands and controls) separately. Full-scale IQ (FSIQ), CANTAB subtest and WCST scores were used as measures of cognitive ability (see Chapters 2 and 3 for a description of the cognitive variables used). Due to low rates of psychopathology in the control group, associations between gamma parameters, evoked amplitude and psychopathology were explored in the proband group only. Symptom counts for the most commonly reported disorders were used to investigate these relationships. As outlined in Chapter 3, the most common disorders in the sample were anxiety, ADHD and ASD. The effects of age, gender and handedness on observed associations were assessed by adding these variables hierarchically to the regression models. These exploratory analyses were not corrected for multiple testing.

## 5.5 Results

### 5.5.1 Descriptive data

A comparison of the descriptive data for the two groups is shown in table 5-1. There were no significant differences in age, gender or handedness between the groups.

*Table 5-1 Age, gender and handedness of probands and controls*

| | **Probands (N=25)** | **Controls (N=22)** | **$t/\chi^2$** | **P** |
|---|---|---|---|---|
| **Age (SD)** | 13.5 (1.8) | 14.3 (1.8) | -1.55 | 0.13 |
| **Gender, female (%)** | 14 (56.0) | 11 (50.0) | -0.27 | 0.91 |
| **Handedness, right (%)** | 20 (80.0) | 16 (72.7) | 0.06 | 0.81 |

None of the children included in the analyses had a history of epilepsy or were taking psychotropic medication at the time of the study.

### 5.5.2 Visual responses

**Data quality**

There were no statistically significant between-group differences in head motion (median=6.7mm (IQR=14.2mm) in probands and 2.2mm (IQR=7.2mm) in controls, p=0.43), or number of clean trials (n=81.0 (IQR=13.0) in probands, n=82.5 (IQR=19.5) in controls, p=0.97) between the 25 probands and 22 controls who passed quality control. Seven probands and three controls had no discernible peak gamma sustained response in the visual cortex and so were excluded from the between-group analyses of peak gamma amplitude and frequency, leaving a sample of 18 probands and 19 controls. 21 probands and 20 controls had broadband sustained responses and clear evoked responses and were therefore included in the analyses of the sum of gamma power between 35-70Hz and the evoked amplitude.

Figure 5-3 shows the source localisation results and time-frequency plots for induced gamma and evoked responses for two representative participants. The plots in the top row are from a participant with good induced gamma and evoked responses. This participant has peak gamma activity in the occipital cortex, a clear transient gamma spike followed by a narrow band sustained response in the time frequency plot on the left, and a clear evoked response in the time-frequency plot on the right. In contrast, the lower plots are from a participant with poor responses. A clear source of gamma activity in the visual cortex is not evident, nor are induced gamma or evoked responses in the time-frequency plots.

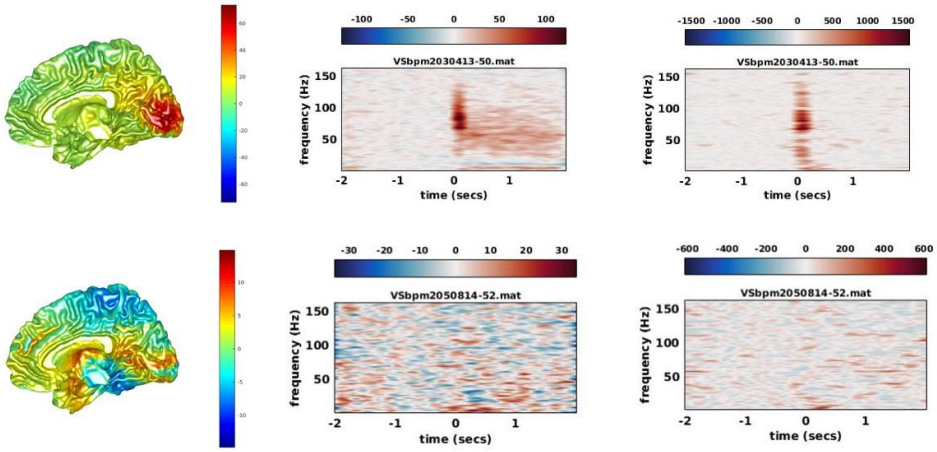


*Figure 5-3 Source localisation results and time-frequency analysis for the induced and evoked responses in two representative participants*

**Average group responses**

Figure 5-4 shows the group average responses between probands and controls. The plots on the left show the combined induced and evoked responses for both groups. This shows a weaker sustained response in the proband group. The plots on the right show the evoked responses which appear to be similar between groups.

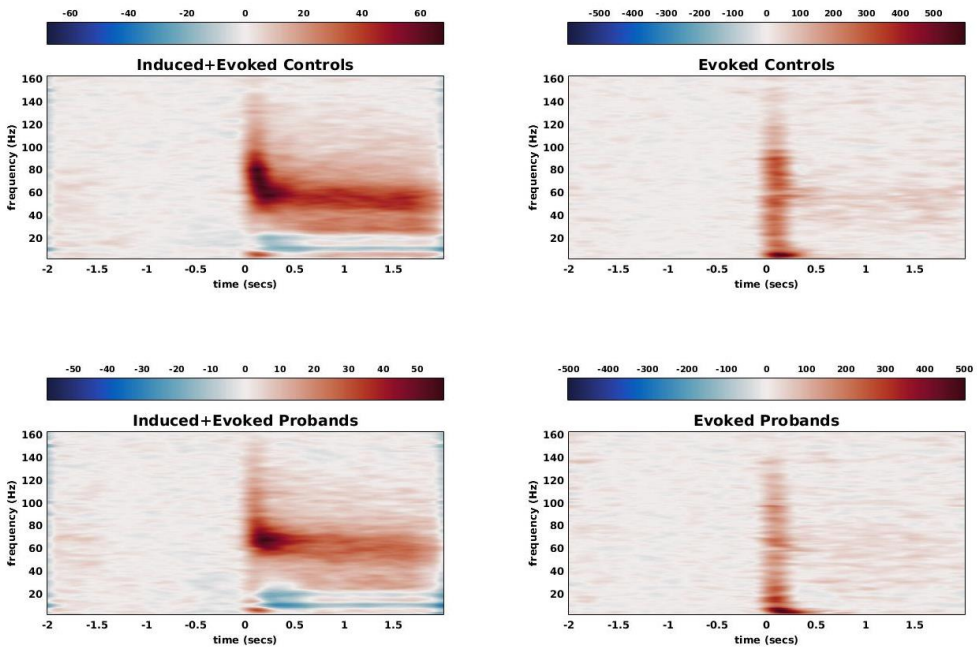


*Figure 5-4 Averaged induced and evoked responses in probands and controls*

**Induced gamma responses**

The sum of induced gamma power between 35-70Hz was compared between 21 probands and 20 controls. As predicted, there was a statistically significant difference in the percentage change from baseline in probands compared with controls. Probands had significantly lower total gamma power than controls (p=0.02). The median sum of gamma power in probands was 1845.8% (IQR=2671.0%) and in controls was 3904.8% (IQR=4707.5%).

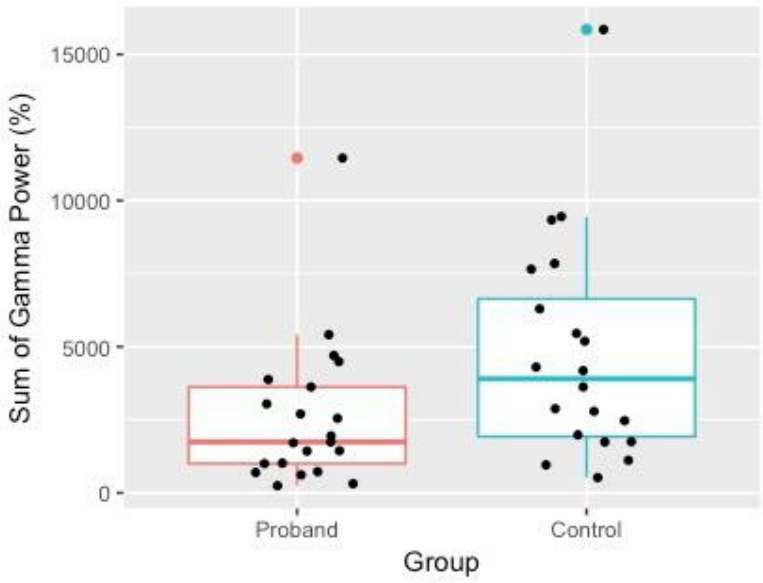


*Figure 5-5 Boxplot showing the sum of gamma power between 35-70Hz in probands and controls*

18 probands and 19 controls had clear peak gamma responses in the occipital cortex. In line with the hypotheses, peak gamma amplitude was lower in probands than controls. The median percentage change in peak gamma amplitude from baseline was 30.3% in probands (IQR=28.1%) and 45.2% in controls (IQR=55.24%), however, this difference was not statistically significant at $p<0.05$ ($p=0.13$).

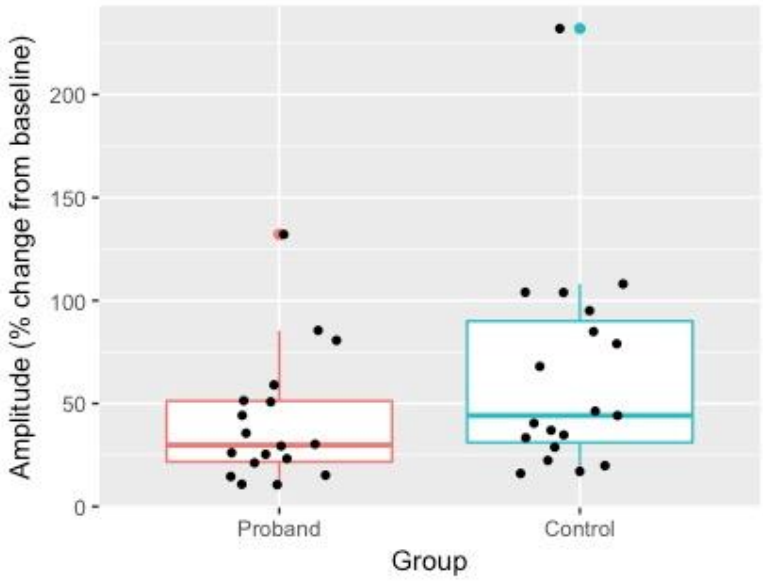


*Figure 5-6 Boxplot showing peak gamma amplitude in probands and controls*

Contrary to expectations, median peak gamma frequency was higher in probands than controls. Probands had a median peak frequency of 65.5Hz (IQR=10.0Hz) while controls had a median peak frequency of 57.5Hz (IQR=7.9Hz). This difference was not statistically significant at $p<0.05$ ($p=0.06$).

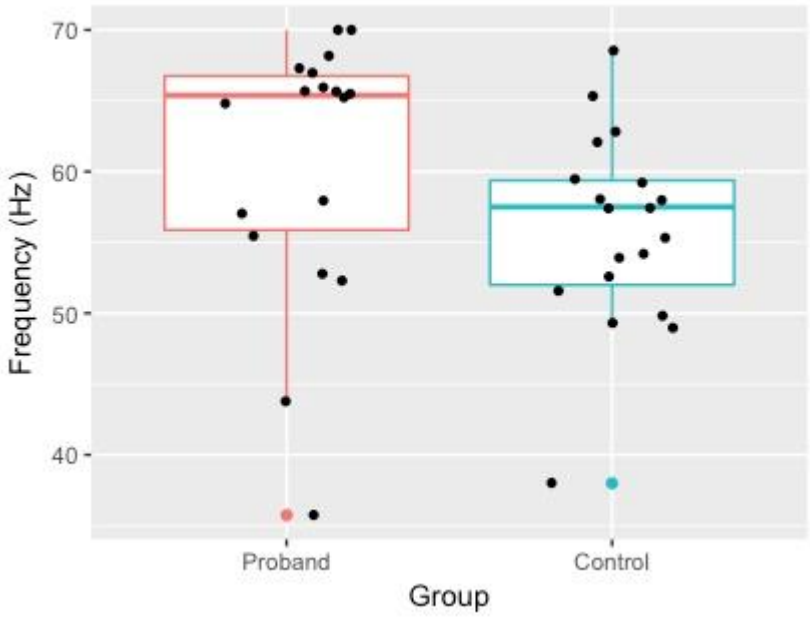


*Figure 5-7 Boxplot showing peak gamma frequency in probands and controls*

**Evoked responses**

21 probands and 20 controls showed clear evoked responses in the visual cortex. The amplitude of the evoked response in probands was 6.4 x $10^{-13}$nAM (IQR=3.3 x $10^{-13}$nAM) and in controls was 8.3 x $10^{-13}$nAm (IQR=5.3 x $10^{-13}$). Contrary to the hypotheses, there was no statistically significant difference in the amplitude of the evoked response between groups ($p=0.34$).

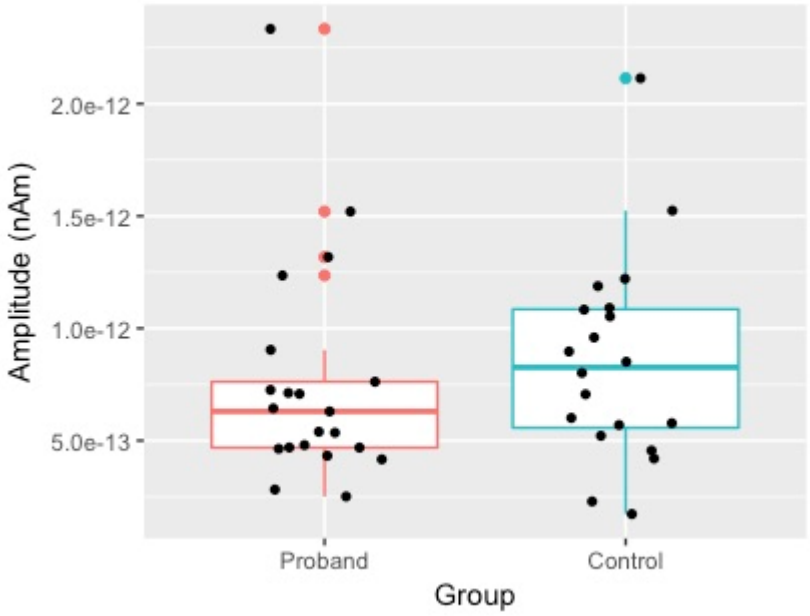


*Figure 5-8 Boxplot showing evoked responses in probands and controls*

**Relationship between visual responses and age**

The scatterplots in figure 5-8 show that across the age range recruited to the study (10-17 years old), the evoked response was remarkably similar between groups. In contrast, for all of the gamma response variables, there were differences between groups across the age spectrum, with lower total broadband gamma power, lower peak gamma amplitude and higher peak gamma frequency in probands than controls. There were no statistically significant associations between evoked or induced gamma response variables and age for either group.

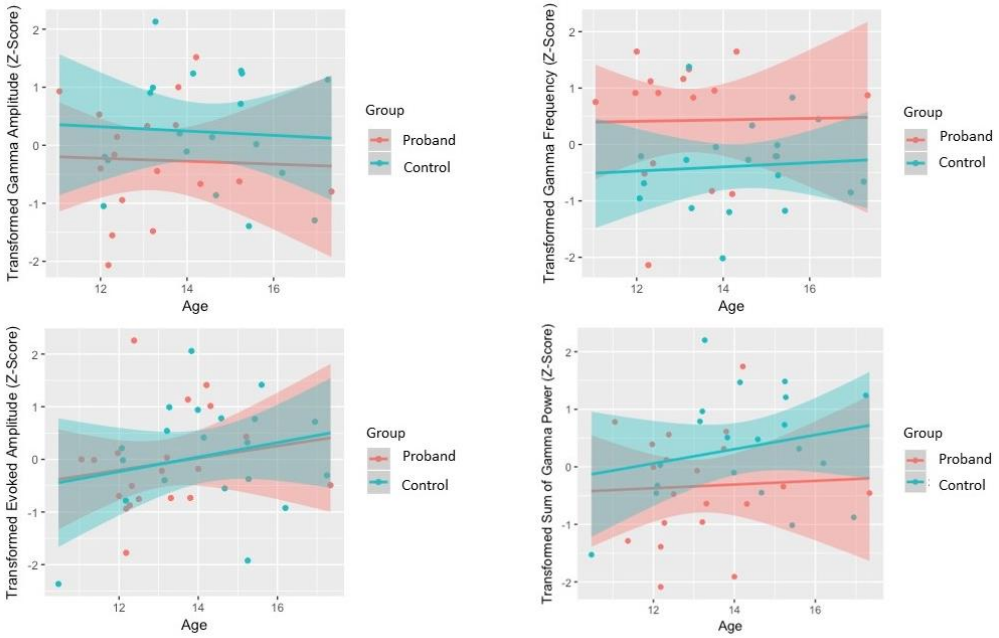


*Figure 5-9 Scatterplots showing the relationship between age and visual response variables for probands (red) and controls (blue)*

**Relationships between gamma responses, psychopathology and cognitive ability**

Table 5-2 shows the relationships between the sum of broadband gamma power, psychopathology and cognitive ability. There were significant associations between SCQ, SWM and MTS scores and the sum of gamma power. The associations between the sum of gamma power and SCQ/SWM scores survived correction for age, gender and handedness (SCQ estimate= -0.11, SE=0.04, $r^2$=0.35, p=0.01 and SWM estimate=0.92, SE=0.37, $r^2$=0.25, p=0.03). The association with MTS survived correction for age (estimate=0.07, SE=0.15, $r^2$=0.13, p<0.05) but not for age and gender (estimate=0.14, SE=0.07, $r^2$=0.13, p>0.05).

*Table 5-2 Relationships between transformed sum of gamma power, psychiatric and cognitive variables in probands*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **ADHD Score** | -0.08 | 0.07 | 0.08 | 0.26 |
| **ADI-R Score** | -0.02 | 0.02 | 0.14 | 0.23 |
| **SCQ Score** | -0.10 | 0.03 | 0.52 | $3.79 \times 10^{-3}$* |
| **Anxiety Score** | -0.08 | 0.04 | 0.22 | 0.06 |
| **FSIQ** | 0.01 | 0.02 | 0.03 | 0.48 |
| **WCST (set-shifting ability)** | $-1.83 \times 10^{-3}$ | $5.87 \times 10^{-3}$ | $5.70 \times 10^{-3}$ | 0.76 |
| **Visual attention (MTS)** | 0.144 | 0.07 | 0.22 | 0.04* |
| **Spatial working memory (SWM)** | 0.72 | 0.25 | 0.33 | $9.65 \times 10^{-3}$* |
| **Spatial planning (SOC)** | 0.26 | 0.19 | 0.10 | 0.18 |
| **Processing speed (RTI)** | -0.08 | 0.13 | 0.02 | 0.57 |
| **Sustained attention (RVP)** | 0.28 | 0.2 | 0.11 | 0.18 |

*Abbreviations: ADHD, attention deficit hyperactivity disorder; ADI-R, Autism Diagnostic Interview-Revised; SCQ, Social Communication Questionnaire; FSIQ, full-scale IQ; WCST, Wisconsin Card Sorting Test; MTS, Match-to-Sample; SWM, Spatial Working Memory; SOC, Stockings of Cambridge; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing. P values are uncorrected.*

Figure 5-9 shows the relationships between SCQ, SWM and MTS scores and the sum of gamma power. In line with predictions, higher SCQ scores (indexing more social communication problems) were associated with lower total gamma power. Also consistent with the hypotheses, poorer cognitive performance (spatial working memory and visual attention) was associated with lower gamma power.

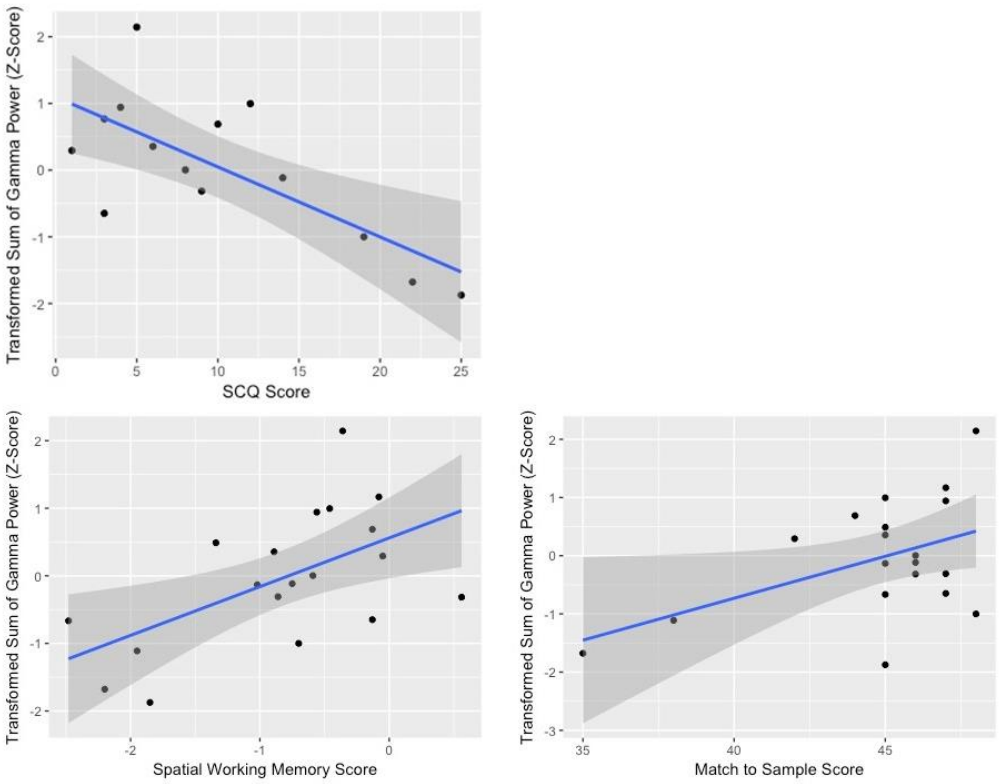


*Figure 5-10 Relationship between transformed sum of gamma power and Social Communication Questionnaire (SCQ), Match to Sample and Spatial Working Memory score in probands*

Table 5-3 shows the results of the exploratory analyses for the relationships between transformed peak gamma amplitude, cognitive and psychiatric variables in the proband group. Similar to the findings for total gamma power, transformed peak gamma amplitude was positively associated with performance on the spatial working memory task. This relationship survived correction for age and gender (estimate=1.07, SE=0.49, $r^2$=0.16, p< 0.05) but not age, gender and handedness (estimate=1.10, SE=0.51, $r^2$=0.10, p=0.06).

*Table 5-3 Relationships between transformed peak gamma amplitude, psychiatric and cognitive variables in probands*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **ADHD Score** | -0.03 | 0.09 | $9.60x10^{-3}$ | 0.74 |
| **ADI-R Score** | $1.22x10^{-3}$ | $2.94 \times 10^{-2}$ | $1.91x10^{-4}$ | 0.97 |
| **SCQ Score** | -0.08 | 0.04 | 0.25 | 0.10 |
| **Anxiety Score** | -0.06 | 0.05 | 0.08 | 0.32 |
| **FSIQ** | $3.44x10^{-3}$ | 0.02 | $1.69x10^{-3}$ | 0.88 |
| **WCST (set-shifting ability)** | $1.84x10^{-3}$ | $5.93x10^{-3}$ | $6.81x10^{-3}$ | 0.76 |
| **Visual attention (MTS)** | 0.08 | 0.18 | 0.01 | 0.67 |
| **Spatial working memory (SWM)** | 0.71 | 0.31 | 0.28 | 0.04* |
| **Spatial planning (SOC)** | 0.08 | 0.24 | $8.13x10^{-3}$ | 0.74 |
| **Processing speed (RTI)** | -0.07 | 0.14 | 0.02 | 0.60 |
| **Sustained attention (RVP)** | 0.14 | 0.23 | 0.03 | 0.55 |

*Abbreviations: ADHD, attention deficit hyperactivity disorder; ADI-R, Autism Diagnostic Interview-Revised; SCQ, Social Communication Questionnaire; FSIQ, full-scale IQ; WCST, Wisconsin Card Sorting Test; MTS, Match-to-Sample; SWM, Spatial Working Memory; SOC, Stockings of Cambridge; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing. P values are uncorrected.*

Figure 5-10 shows the relationship between spatial working memory and transformed peak gamma amplitude. In line with the hypotheses and consistent with findings for total gamma power, worse performance in the SWM task was associated with lower peak gamma amplitude.

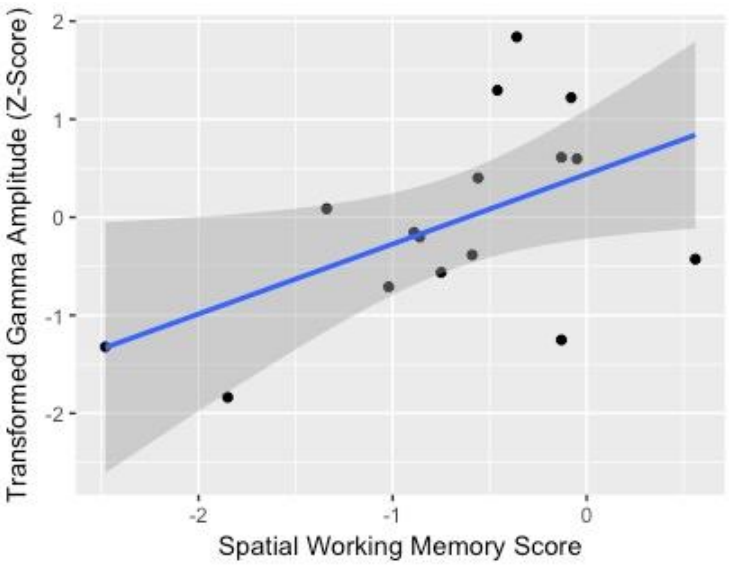


*Figure 5-11 Relationship between transformed peak gamma amplitude and spatial working memory score in probands*

Table 5-4 shows the relationships between transformed peak gamma frequency, psychiatric and cognitive variables in the proband group. Transformed peak gamma frequency was negatively associated with ASD symptoms measured by the ADI-R. This relationship survived correction for age (estimate= -0.07, SE=0.02, $r^2$=0.34, p=0.03) but not age and gender (estimate= -0.05, SE=0.03, $r^2$=0.30, p=0.14).

*Table 5-4 Relationships between transformed peak gamma frequency, psychiatric and cognitive variables in probands*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **ADHD Score** | -0.04 | 0.08 | 0.02 | 0.65 |
| **ADI-R Score** | -0.06 | 0.02 | 0.47 | 0.02* |
| **SCQ Score** | -0.05 | 0.05 | 0.09 | 0.34 |
| **Anxiety Score** | -0.04 | 0.05 | 0.038 | 0.51 |
| **FSIQ** | -0.02 | 0.02 | 0.05 | 0.37 |
| **WCST (set-shifting ability)** | $5.40x10^{-3}$ | $6.32x10^{-3}$ | 0.05 | 0.41 |
| **Visual attention (MTS)** | -0.09 | 0.19 | 0.02 | 0.64 |
| **Spatial working memory (SWM)** | 0.61 | 0.33 | 0.19 | 0.09 |
| **Spatial planning (SOC)** | 0.33 | 0.23 | 0.13 | 0.17 |
| **Processing speed (RTI)** | 0.09 | 0.14 | 0.03 | 0.54 |
| **Sustained attention (RVP)** | 0.18 | 0.24 | 0.04 | 0.46 |

*Abbreviations: ADHD, attention deficit hyperactivity disorder; ADI-R, Autism Diagnostic Interview-Revised; SCQ, Social Communication Questionnaire; FSIQ, full-scale IQ; WCST, Wisconsin Card Sorting Test; MTS, Match-to-Sample; SWM, Spatial Working Memory; SOC, Stockings of Cambridge; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing. P values are uncorrected.*

Figure 5-10 shows the relationship between transformed peak gamma frequency and ADI-R score. As predicted, higher ADI-R scores (indexing more ASD symptoms) were associated with lower peak gamma frequency.

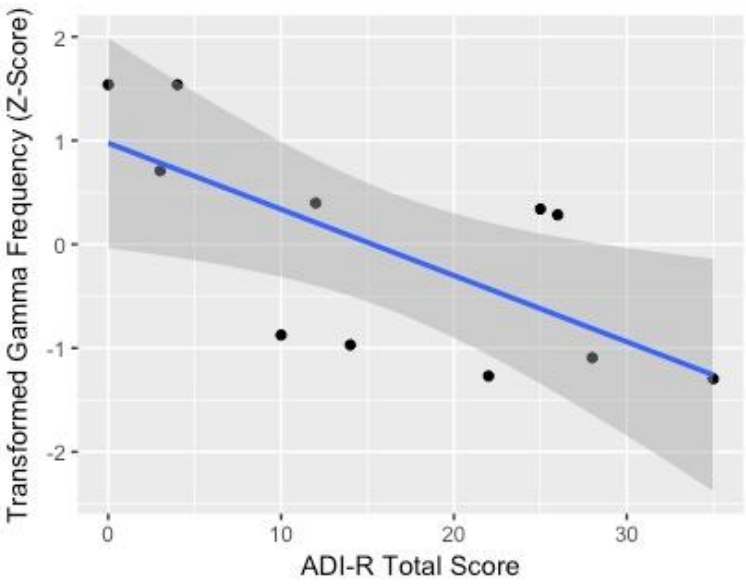


*Figure 5-10 Relationship between transformed gamma frequency and ADI-R score in probands*

Finally, table 5-5 shows the relationships between transformed evoked response amplitude, psychopathology and cognitive ability in probands. Statistically significant relationships were seen between SCQ, FSIQ, WCST and SWM scores and the transformed evoked response amplitude. The relationship with SCQ survived correction for age (estimate= -0.08, SE=0.04, $r^2$=0.21, p=0.04) but not age and gender (estimate= -0.08, SE=0.04, $r^2$=0.14, p=0.09). The relationships with FSIQ, WCST and SWM scores survived correction for age, gender and handedness (FSIQ estimate=0.04, SE=0.02, $r^2$=0.21, p=0.03; WCST estimate= -0.01, SE<0.01, $r^2$ =0.26, p=0.02; SWM estimate=0.85, SE=0.38, $r^2$=0.24, p=0.04).

*Table 5-5 Relationships between transformed evoked response amplitude, psychiatric and cognitive variables in probands*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **ADHD Score** | $-2.15x10^{-3}$ | 0.07 | $6.47x10^{-5}$ | 0.98 |
| **ADI-R Score** | $-6.55x10^{-3}$ | 0.02 | 0.01 | 0.75 |
| **SCQ Score** | -0.08 | 0.03 | 0.31 | 0.04* |
| **Anxiety Score** | -0.06 | 0.04 | 0.13 | 0.15 |
| **FSIQ** | 0.04 | 0.02 | 0.22 | 0.04* |
| **WCST (set-shifting ability)** | -0.01 | $4.28x10^{-3}$ | 0.40 | $3.91x10^{-3}$* |
| **Visual attention (MTS)** | 0.08 | 0.07 | 0.07 | 0.29 |
| **Spatial working memory (SWM)** | 0.78 | 0.24 | 0.38 | $5.11x10^{-3}$* |
| **Spatial planning (SOC)** | 0.23 | 0.19 | 0.08 | 0.25 |
| **Processing speed (RTI)** | -0.07 | 0.14 | 0.02 | 0.61 |
| **Sustained attention (RVP)** | 0.26 | 0.21 | 0.09 | 0.23 |

*Abbreviations: ADHD, attention deficit hyperactivity disorder; ADI-R, Autism Diagnostic Interview-Revised; SCQ, Social Communication Questionnaire; FSIQ, full-scale IQ; WCST, Wisconsin Card Sorting Test; MTS, Match-to-Sample; SWM, Spatial Working Memory; SOC, Stockings of Cambridge; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing. P values are uncorrected.*

Figure 5-11 shows the relationships between transformed evoked response, SCQ and cognitive performance (IQ, WCST and SWM scores). With the exception of WCST scores, all associations are in the expected direction. Higher SCQ scores (indexing social communication problems) were associated with lower amplitude of the evoked response. Lower IQ and SWM scores were also associated with lower evoked responses. However, the association with IQ appears to be driven by an outlier with high IQ. The association was no longer significant when this participant was removed from the dataset (estimate=0.02, SE=0.03, $r^2$=0.02, p=0.46). Contrary to predictions, better performance on the WCST was associated with lower evoked responses.

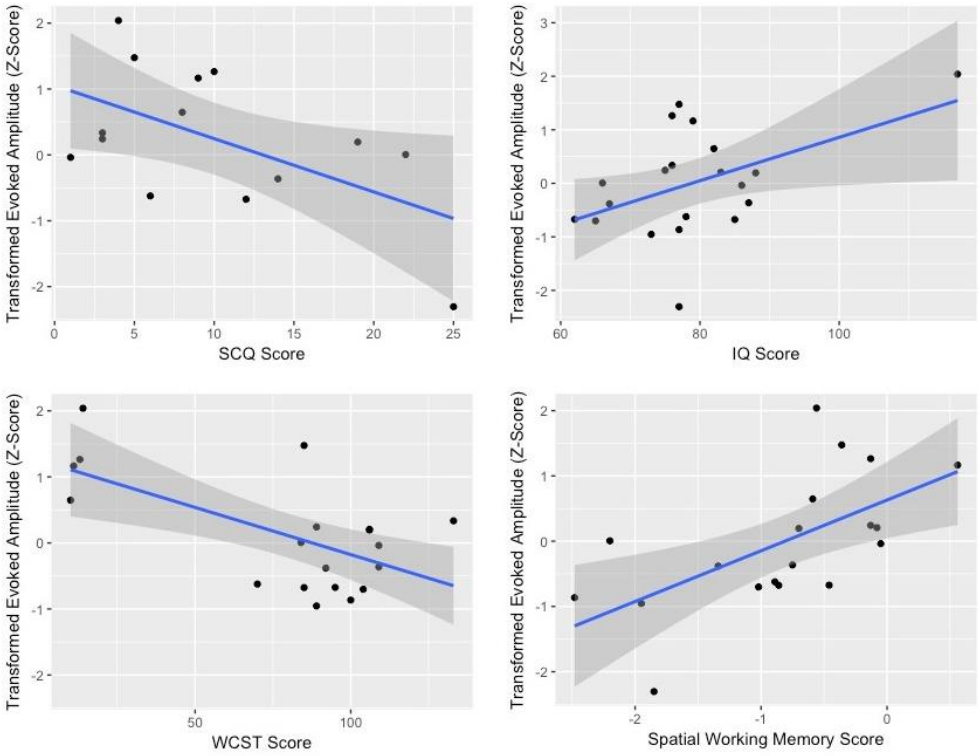


*Figure 5-12 Relationship between transformed evoked amplitude and Social Communication Questionnaire (SCQ), IQ, Wisconsin Card Sorting Test (WCST) and Spatial Working Memory scores in probands*

In the sibling group, the only significant association with cognitive variables was a negative relationship between transformed peak gamma frequency and FSIQ (estimate= -0.05, SE=0.02, $R^2$=0.33, p=0.01). This association survived correction for age, gender and handedness (estimate= -0.05, SE=0.02, $R^2$=0.30, p=0.01).

Figure 5-12 shows the relationship between sibling IQ scores and transformed peak gamma frequency. Lower IQ was associated with higher peak gamma frequency. The association appears to be driven, at least in part, by an outlier with very high IQ. The association between IQ and peak gamma frequency was no longer significant when this individual was removed from the dataset (estimate= -0.03, SE=0.3, $R^2$=0.04, p=0.20).

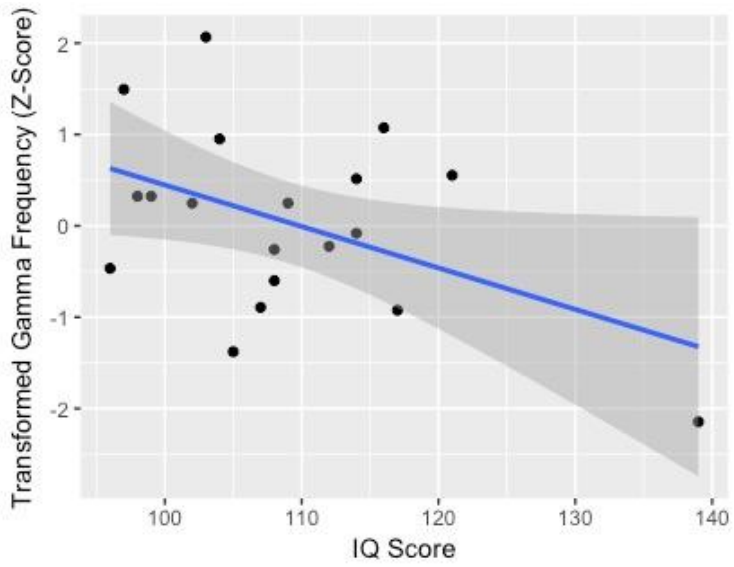


*Figure 5-13 Relationship between transformed gamma frequency and IQ score in siblings*

## 5.6 Discussion

In this investigation of neural responses to visual stimuli in 22q11.2DS, altered induced gamma band responses were found in probands compared to controls but there were no differences in evoked responses. As shown in figure 5-8, these group differences were consistent across the age spectrum studied.

Children with 22q11.2DS had lower total sustained gamma power compared with controls. Although there was no statistically significant difference in peak gamma amplitude between groups at $p<0.05$, there was a trend towards lower gamma amplitude in the proband group. One possible reason for the discrepancy between results for the sum of gamma power and peak gamma amplitude is the lower statistical power to detect group differences in the peak amplitude comparison, due to the smaller sample size available for this analysis. Peak gamma responses show high inter-individual variability and large sample sizes and/or large effect sizes are needed to detect between-group differences (Muthukumaraswamy *et al.*, 2010). Although a relatively large sample of children was recruited, poor data quality and the lack of clear gamma peaks for some individuals led to a large proportion of the proband sample (50%) not being

included in the peak gamma amplitude and frequency comparisons, which limited the ability to detect statistically significant differences. This may also have created bias in the sample as children with more severe phenotypes are more likely to have been excluded. Biria *et al*. (2018) studied visual responses in 45 participants with 22q11.2DS and 31 controls with EEG. The authors also had a high level of data loss in their 22q11.2DS group due poor data quality, leaving a sample of 25 probands and 26 controls in their final analysis. By also investigating the total sum of broadband gamma power between 35-70Hz, it was possible to include three additional probands and one additional control in the analyses, yielding statistically significant between-group differences at $p<0.05$. Reductions in the amplitude and power of induced gamma band responses have been found in a number of neurodevelopmental disorders using EEG and MEG under different task conditions (Sun *et al.*, 2012, 2013; Wilson *et al.*, 2012). The amplitude and power of induced gamma responses are thought to reflect signal to noise ratio in reciprocal excitatory-inhibitory cortical circuits. The observation of a reduction in the total power of induced gamma oscillations provides some evidence of atypical excitatory-inhibitory circuity in 22q11.2DS. In the exploratory analyses, it was found that the severity of social communication problems and impairment in spatial working memory and visual attention were associated with reductions in broadband gamma power (figure 5-9). Similarly, poor spatial working memory performance was associated with reductions in peak gamma amplitude in children with 22q11.2DS but not in controls (figure 5-10). The association between reduced magnitude of induced gamma responses in children with high SCQ scores is in line with the literature on gamma responses in idiopathic ASD (Maxwell *et al.*, 2015).

Although not statistically significant at $p<0.05$, probands had higher peak gamma frequencies than children without neurodevelopmental CNVs. This result was also unexpected; patients with schizophrenia have been found to have reduced peak gamma frequencies in response to a visual task that required perceptual binding (Spencer *et al.*, 2004) and while, to my knowledge, no previous studies have

reported peak visual gamma frequency in ASD, a study investigating gamma responses in the motor cortex found lower gamma frequencies in children with ASD (An *et al.*, 2018). In the exploratory analyses within the proband group, children with higher ADI-R scores (and therefore more features of ASD) tended to have lower peak gamma frequencies than those with lower ADI-R scores. The same relationship was not evident for SCQ scores. In the sibling group, peak frequency was negatively associated with IQ but this finding did not survive after outlier removal. There were no associations with other cognitive variables. The relationship between excitatory-inhibitory balance and peak gamma frequency is not yet fully elucidated. While one study found that peak gamma frequency positively correlated with GABA concentrations (Muthukumaraswamy *et al.*, 2009), other investigators have failed to replicate this finding (Cousijn *et al.*, 2014). Furthermore, drugs that enhance GABAergic neurotransmission have been shown to reduce peak gamma frequency (Lozano-Soldevilla *et al.*, 2014; Schneider *et al.*, 2014; Magazzini *et al.*, 2016). Gamma frequency is determined by the relative balance between N-methyl-D-aspartate (NMDA) glutamate receptor-mediated excitation and $GABA_A$ receptor-related tonic inhibitory processes on the cell membrane of inhibitory neurons. Increased frequency of gamma oscillations may arise due to either increased excitatory influences on inhibitory PV+ interneurons through NMDA receptors or through a reduction of tonic inhibition to the interneurons via $GABA_A$ receptors (Mann and Mody, 2010).

One mechanism by which gamma responses could be affected by 22q11.2DS is via the effects of L-Proline on GABA synthesis. A recent study found that mice deficient in *PRODH*, a gene located in the 22q11.2 region involved in the degradation of L-Proline, have deficits in GABAergic neurotransmission and gamma oscillations (Crabtree *et al.*, 2016). In this study, elevated L-Proline levels inhibited GABA synthesis by GAD67 and impaired the response of GABA synapses during sustained stimulation. This resulted in a reduction in strength of local field potentials in the gamma band. People with 22q11.2DS may therefore have reduced ability to increase inhibitory output when circuit activity is high, resulting in lower amplitude and increased frequency of gamma oscillations during

repeated stimulation. Interestingly, patients with 22q112.DS have been found to have elevations in plasma L-Proline (Goodman *et al.*, 2000) and the severity of hyperprolinaemia has been found to correlate with psychotic symptom severity (Raux *et al.*, 2007). Further evidence that GAD67 affects E-I balance comes from a study in which one allele of the GAD67 gene (*GAD1*) was selectively removed from the PV+ interneurons of juvenile mice (Lazarus, Krishnan and Huang, 2015). This resulted in increased pyramidal cell excitability and increased E-I balance in PV+ interneurons, leading to increased spike frequencies in pyramidal cells in response to current injections.

There were no significant between-group differences in the evoked response. This was unexpected since children with 22q11.2DS have known difficulties with visual processing (Bearden *et al.*, 2001; Simon, Bearden, *et al.*, 2005; Magnée *et al.*, 2011; McCabe *et al.*, 2016), and a previous EEG study in 22q11.2DS using an illusory contour stimulus reported reduced evoked responses in a mixed sample of adults and children with 22q11.2DS (Biria *et al.*, 2018). Interestingly, in their experiment, Biria *et al.* did not find clear evoked response peaks in the 22q11.2DS group in response to the non-contour stimulus and hypothesised that this may have been either due to impairments in local visual processing or to more variability in the responses to non-contour versus contour stimuli in the 22q11.2DS group. The present study also found inter-individual variability in evoked responses between participants (both probands and controls) and a positive association with cognitive function in the proband group. The amplitude of the evoked response was positively associated with spatial working memory performance but negatively associated with set-shifting ability in the WCST (figure 5-11). These preliminary associations warrant further investigation in a larger sample. The amplitude of visual evoked responses has been found to be reduced in patients with schizophrenia (Yeap *et al.*, 2008; González-Hernández *et al.*, 2014) and ASD (Boeschoten *et al.*, 2007; Milne *et al.*, 2009) but this is not consistent between studies with some studies reporting elevated responses in ASD (Takarae *et al.*, 2016). In the exploratory analyses conducted in this chapter, a negative association was found between the amplitude of the evoked response and SCQ

score, with children scoring highly on this measure of social communication problems having lower evoked responses. There was however, no significant relationship with ADI-R score. Due to the low level of reported psychotic symptoms in the present sample, it was not possible to explore the relationships between positive or negative symptoms of schizophrenia and evoked responses. Longitudinal follow-up of the sample through the period of risk for psychosis would provide interesting insights in this regard.

## 5.7 Strengths and limitations

This study has several strengths. It is the first study to use MEG to investigate induced gamma band responses in children with 22q11.2DS. It benefits from the recruitment of a rigorously phenotyped sample of children of a relatively narrow age range. Participants were recruited on the basis of genotype rather than phenotype, minimising some of the biases inherent in clinically ascertained samples. Although there are a number of potential barriers to participation in brain imaging research, the subsample of children participating in this experiment was broadly representative of the whole ECHO 22q11.2DS cohort in terms of demographics and levels of impairment. Despite the high levels of psychopathology, none of the children taking part were on psychotropic medication. Furthermore, the proband and sibling groups were well-matched for age, gender and handedness, facilitating between-group comparisons.

It was clear during data collection that children with 22q11.2DS found this experiment more challenging than the resting-state experiment presented in Chapter 4. Head motion, eye movement and muscle artefact contaminated more trials and led to exclusion of more participants on the basis of data quality. In addition, ten participants (seven probands and three controls) did not generate discrete peak gamma responses, further reducing the sample size available for analysis of peak gamma variables to 18 probands and 19 controls. To maximise the data available for analysis total broadband gamma power during the

sustained response was calculated from all participants who had a clear evoked response to the stimulus. This meant that between-group comparisons of the sustained response could be performed, even in the absence of a clear gamma peak, which resulted in the inclusion of a further three probands and one sibling in the analyses. Despite these additional efforts, the sample size was still small at 21 probands and 20 controls with limited power to detect between-group differences and associations with cognition and psychopathology. The results of the study (particularly the exploratory analyses), should therefore be considered preliminary until replicated in larger samples.

As with the resting-state study presented in Chapter 4, the frequency of surgical and other contraindications for MR scanning meant that it was not possible to co-register MEG data to each participant's own MRI scan. While every attempt was made to closely match the fiducial locations to those of another participant, this is not a perfect substitute for using participants' own data and this may have affected source localisation. Furthermore, continuous head localisation was not used during data acquisition as this was not standard practice in the CUBRIC MEG lab when the study commenced. This however meant that detailed analysis of head movement and correction for this was not possible. Head localisation before and after recordings was used as a proxy for head movement however, this does not give any indication about the amount of movement during the recording. While it is reassuring that this proxy measure did not differ between groups, without continuous monitoring, one cannot be certain that there were no differences between groups in the amount of movement during the task. Gamma amplitude measures may be particularly vulnerable to noise from head movement or distance from the MEG sensors (Magazzini *et al.*, 2016). Finally, there were some psychiatric and cognitive data missing at the time of analysis. This was particularly problematic for the ADI-R data, which were only available for 16 probands (76% of the sample). This will have affected the power to detect relationships between ADI-R scores and gamma responses.

## 5.8 Conclusions

In conclusion, in this study of neural responses to visual stimulation in 22q11.2DS there was evidence for altered induced gamma band responses in affected children but intact evoked responses. Total broadband gamma power was reduced in probands and there was a trend towards a reduction in peak amplitude. In contrast peak frequency was increased in affected children. Associations were found between these variables and both cognitive and social communication problems. The data from this experiment further support the overall hypothesis of altered E-I balance in 22q11.2DS.

# 6 Occipital GABA concentration in 22q11.2 deletion syndrome

## 6.1 Summary

Neurodevelopmental disorders such as schizophrenia, ASD and ADHD have been associated with alterations in gamma-aminobutyric acid (GABA) neurotransmission. Abnormalities of GABAergic interneurons have also been reported in 22q11.2DS. Magnetic resonance spectroscopy (MRS) is a technique that can be used to quantify neural metabolites including GABA non-invasively. To date, there have been no published MRS studies of GABA concentrations in 22q11.2DS.

GABA-edited MRS data were collected from 13 children with 22q11.2DS and 14 controls at 3T using a Mescher-Garwood Point Resolved Spectroscopy (MEGA-PRESS) sequence with a 3cm x 3cm x 3cm voxel in the occipital cortex. GABA concentrations relative to water and creatine were compared between groups and related to psychopathology, cognitive function and gamma responses to visual stimuli.

There were no between-group differences in GABA concentrations and no statistically significant relationships between GABA concentrations and any of the cognitive, clinical or gamma response variables tested. If there are alterations in GABAergic signaling in 22q11.2DS, these are not reflected by MRS derived GABA concentrations at 3T.

## 6.2 Introduction

In Chapter 5, it was shown that the frequency of visually-induced gamma oscillations was higher in children with 22q11.2DS than in controls. Furthermore, children with 22q11.2DS had lower total broadband gamma power than controls. Gamma responses in the proband group were associated with cognitive performance and social communication problems. As discussed in Chapter 5,

gamma band responses reflect cortical excitatory-inhibitory (E-I) balance and are affected by alterations in GABAergic neurotransmission (Mann and Mody, 2010; Campbell *et al.*, 2014; Lozano-Soldevilla *et al.*, 2014; Lazarus, Krishnan and Huang, 2015; Crabtree *et al.*, 2016; Magazzini *et al.*, 2016). The frequency of gamma oscillations has previously been shown to be associated with cortical GABA concentrations in healthy populations (Muthukumaraswamy *et al.*, 2009).

GABA is the major inhibitory neurotransmitter in the brain. Together with the major excitatory neurotransmitter, glutamate, it modulates the E-I balance necessary for normal brain function (Markram *et al.*, 2004). It is synthesised from glutamate by the enzyme glutamate decarboxylase (GAD) which exists in two isoforms, GAD67 and GAD65, encoded by the genes *GAD1* and *GAD2* respectively (Pinal and Tobin, 1998). There are two main types of GABA receptors: $GABA_A$ and $GABA_B$. $GABA_A$ is an ionotropic receptor that mediates fast hyperpolarizing responses via a ligand-gated ion channel. When activated, this channel permits the influx of chloride ions resulting in hyperpolarization of the post-synaptic cell (Luján, Shigemoto and López-Bendito, 2005). $GABA_A$ receptors are located in the synaptic cleft where they are involved in fast, phasic inhibition as well as extrasynaptically, where they have a role in tonic inhibition (Lee and Maguire, 2014). $GABA_B$ receptors are metabotropic transmembrane receptors that are linked to potassium channels via G-proteins. They are responsible for the late component of inhibitory transmission and mediate hyperpolarization of postsynaptic membranes and inhibition of neurotransmitter release from presynaptic terminals (Couve, Moss and Pangalos, 2000).

While GABAeric interneurons are typically considered to be inhibitory, in early development they have an excitatory effect (Sun and Murali, 1999) and are thought to drive the maturation of synaptic networks (Pfeffer *et al.*, 2009). The change in the effect of GABA from depolarizing to hyperpolarizing is known as the "GABA shift". GABA has also been shown to have a trophic effect, controlling the proliferation and migration of neural progenitor cells (Behar *et al.*, 1998; Wang and Kriegstein, 2009) as well as neuronal morphology (Cancedda *et al.*, 2007).

Increased density of GABAergic interstitial white matter neurons with a corresponding reduction in overlying grey matter has been reported in schizophrenia (Yang *et al.*, 2011; Joshi *et al.*, 2013), suggesting abnormal interneuron migration from the white matter to the cortex. Reductions in GAD65 and GAD67 expression have also been reported in schizophrenia (Akbarian and Huang, 2006; Thompson *et al.*, 2009; Curley *et al.*, 2011), as have decreased GABA release and reuptake (Costa *et al.*, 2001; Yu *et al.*, 2013). In ASD, post-mortem neuropathological studies have found reduced expression of GAD65 and GAD67 (Fatemi *et al.*, 2002) as well as of GABA receptors (Oblak, Gibbs and Blatt, 2010), and PET/SPECT studies have shown reduced expression of $GABA_A$ receptors in the brains of people with ASD (Mori *et al.*, 2012; Mendez *et al.*, 2013). In 22q11.2DS, post-mortem neuropathological studies have found evidence for abnormal neuronal migration (Kiehl *et al.*, 2009). Furthermore, studies of murine models of 22q11.2DS have found abnormalities in the proliferation of basal neural progenitors and the migration of parvalbumin-containing (PV+) interneurons (Meechan *et al.*, 2009, 2012). Work in a mouse model of 22q11.2DS also demonstrated a delay in excitatory to inhibitory GABA shift, altered spontaneous network activity, lack of synchronisation and synaptic plasticity defects (Amin *et al.*, 2017). Taken together, these studies suggest abnormal GABAergic function in neurodevelopmental disorders such as schizophrenia and ASD and high-risk genetic syndromes like 22q11.2DS.

Magnetic resonance spectroscopy (MRS) is an MRI technique by which major brain metabolites can be quantified *in vivo*. In the MRI environment, protons precess at a frequency that depends mainly on the external magnetic field. However, this frequency is affected by local magnetic fields produced by electrons. Protons surrounded by high electron densities resonate at lower frequencies than those that are relatively unshielded from the external magnetic field. The chemical structure of a molecule will determine this shift in frequency, known as the “chemical shift”. These chemical shifts can be plotted to enable us to identify and quantify different molecules such as N-acetylaspartate (NAA),

creatine (Cr), choline (Cho), myo-inositol (Myo), glutamate (Glu), glutamine (Gln) and GABA.

Measuring GABA using MRS has been more difficult than some other metabolites because it is present in relatively low concentrations (approximately 1mM compared to approximately 10mM for NAA and 7mM for creatine (Henriksen, 1995; Puts and Edden, 2012). In addition, GABA signals are overlapped by stronger signals from NAA, creatine, glutamate and glutamine. In order to overcome these difficulties, spectral-editing techniques have been developed to separate GABA from other metabolites and quantify it relative to a reference metabolite (typically creatine) or water.

To date, there have been few MRS studies in 22q11.2DS. A previous study in adults with 22q11.2DS found no difference in glutamate concentrations between probands and controls, however they did find elevated glutamate concentrations in the hippocampi of people with 22q11.2DS and schizophrenia compared to those without schizophrenia (da Silva Alves *et al.*, 2011). Although it should be noted that all of the people with a diagnosis of schizophrenia were taking antipsychotic medication and the differences reported may therefore reflect the effects of medication rather than underlying abnormalities in glutamatergic signalling. Furthermore, the authors used water as an internal reference but did not correct for tissue composition. The results could therefore also be explained by differences in the proportions of grey matter, white matter and cerebrospinal fluid (CSF) between groups. Shashi *et al.* (2012) used MRS in children with 22q11.2DS to investigate metabolite concentrations in the dorsolateral prefrontal cortex. They found elevated NAA concentrations (a marker of cortical maturation) in 22q11.2DS compared to controls but no difference in glutamate/glutamine (Glx) concentrations (Shashi *et al.*, 2012). These studies suggest that there are no differences in excitatory neurotransmitter concentrations in people with 22q11.2DS compared with controls, however, despite evidence from multiple sources for abnormal GABAergic function in 22q11.2DS, to my knowledge, no

published studies have investigated GABA concentrations in children with 22q11.2DS.

## 6.3 Rationale, aims and hypotheses

Evidence from cellular, animal and human studies suggest that E-I balance is disrupted in neurodevelopmental disorders and that this may be due to deficits in GABAergic neurotransmission. In Chapters 4 and 5, evidence was found for altered E-I balance in children with 22q11.2DS using MEG. 22q11.2DS is a copy number variant syndrome associated with high-risk across the spectrum of mental disorders and affects genes known to influence GABA metabolism. While there have been previous MRS studies of 22q11.2DS, none have used spectral-editing techniques optimised for the measurement of relative GABA concentrations. This study, therefore, seeks to determine whether there are differences in the concentrations of GABA in the occipital cortices of children with 22q11.2DS compared with controls. This voxel has been chosen because GABA estimation in this region is robust, reproducible and stable over time (Near *et al.*, 2014; Greenhouse *et al.*, 2016; Bai *et al.*, 2017). In addition, it is the same region from which visual gamma oscillations were measured in Chapter 5 and therefore the relationships between gamma power, amplitude, frequency and GABA concentrations can be explored. As in Chapters 4 and 5, attempts have been made to reduce the impact of potential confounding factors by recruiting children who have been ascertained by CNV status rather than by clinical diagnosis together with a control group consisting of siblings of children with neurodevelopmental CNVs. Detailed phenotypic information was collected so that associations between psychiatric, cognitive and demographic variables could also be explored.

The primary aim of this chapter is to compare the concentration of GABA in the occipital cortex (relative to water and creatine) between children with 22q11.2DS and children without neurodevelopmental CNVs. A secondary aim is to explore relationships between GABA concentrations, gamma band responses, cognitive ability and psychopathology. It is hypothesised that, compared to children

without neurodevelopmental CNVs, children with 22q11.2DS have reduced relative GABA concentrations which are associated with visual gamma responses, cognitive impairment and psychopathology.

## 6.4 Methods

### 6.4.1 Participants

Participants were recruited from the Experiences of people with copy number variants (ECHO) cohort at Cardiff University (See Chapters 2 and 3 for a detailed description of recruitment methods and sample characteristics). Children with 22q11.2DS and unaffected siblings of children with neurodevelopmental CNVs were invited to take part. Ethical approval for the study was obtained from South East Wales National Health Service (NHS) Research Ethics Committee. Participants over the age of 16 years with capacity to consent gave written informed consent to participate in the study. Children under the age of 16 years or those over the age of 16 years who lacked the capacity to consent for themselves gave written and/or verbal assent to participate and a parent or carer consented to their participation in the study.

All potential participants were screened over the telephone for contraindications to MRI at 3T. In cases where participants had potential contraindications, advice was sought from CUBRIC's radiographers and laboratory managers and where necessary the participants' doctors were contacted to ensure that it was safe for them to participate. Inclusion criteria were age 10-17 years old with a diagnosis of 22q11.2DS or a genetically-related sibling of a child with a neurodevelopmental CNV. Potential participants were excluded if they had contraindications to MRI at 3T (e.g. presence of a pacemaker or cochlear implant, intracerebral shunt, some types of orthodontic braces, or a history of surgery which may have involved metallic implants not certified as MRI safe (or not known to be safe) at 3T).

Participants fulfilling the criteria for participation in the study and who passed safety screening were invited to visit Cardiff for brain imaging at a time convenient

to them. Data were collected between March 2013 and the closure of CUBRIC's Park Place facility in August 2016. Demographic, psychiatric and cognitive data were collected either during the imaging visit (n=6) or during a home visit (n=20, mean time gap=1.23 months, SD=6.19 months). Psychiatric interviews with the child's primary caregiver were conducted using the Child and Adolescent Psychiatric Assessment [CAPA; (Angold *et al.*, 1995)] to derive DSM-IV-TR diagnoses and symptom counts, and the Autism Diagnostic Interview-Revised [ADI-R; (Lord, Rutter and Le Couteur, 1994)] to derive ASD diagnoses and symptom counts. Cognitive assessments were conducted with participating children using the Wechsler Abbreviated Scale of Intelligence [WASI; (Wechsler, 1999)], the Wisconsin Card Sorting Test [WCST; (Heaton *et al.*, 1993)] and a selection of tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB, Cambridge Cognition Limited UK, 2006; see Chapters 2 and 3 for a description of the subtests used). Parents or carers were also asked to complete a questionnaire about their child and the wider family. This pack included questions about family background (ethnicity, family income and maternal education) and standardised questionnaires including the Social Communication Questionnaire [SCQ; (Rutter, Bailey and Lord, 2003) for ASD symptomatology.

13 Children with 22q11.2 deletion syndrome (probands) and 14 siblings of children with 22q11.2DS (controls) took part in this study. There were five sibling pairs, other children were unrelated.

### 6.4.2 MRI data acquisition

Individual 1mm isotropic, T1-weighted fast spoiled gradient echo (FSPGR) images were acquired on a 3T General Electric MRI system (GE medical systems, Milwaukee, WI) for localisation of the occipital voxel.

GABA was quantified from a 3cm x 3cm x 3cm voxel in the occipital cortex (see figure 6-2) using a Mescher-Garwood Point Resolved Spectroscopy [MEGA-PRESS;

(Mescher *et al.*, 1998)] acquisition (TE/TR=68/2000ms). This sequence enables GABA signals to be separated from the stronger overlying signals of other metabolites by collecting two interleaved datasets, one with a Gaussian editing pulse at 1.9ppm (ON) and the other at 7.5ppm (OFF). Each editing pulse was applied for 16ms. The majority of peaks in the spectrum are unaffected by these pulses so subtraction of the "ON" spectrum from the "OFF" spectrum creates an edited spectrum of those peaks affected by the pulses i.e. GABA signals at 3.0ppm. The GABA signals obtained using this method also contain signal from macromolecules in the voxel so the term 'GABA+' will be used to refer to the concentrations measured.

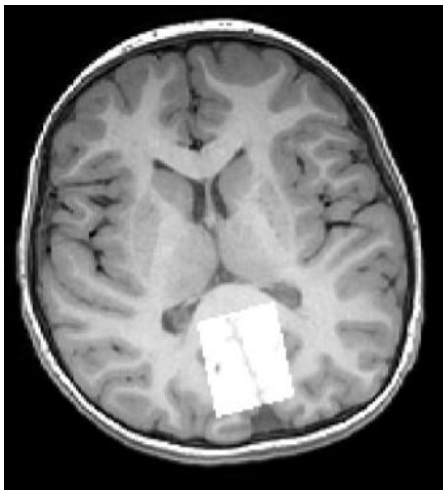

*Figure 6-1 MRS voxel placement*

### 6.4.3 MRS analysis pipeline

GABA+ concentration was quantified using the Gannet toolkit (version 2.0, www.gabamrs.com) in MATLAB (version R2015a, MathWorks). Individual spectra were phase corrected and edited spectra were produced by subtracting "ON" spectra from "OFF" spectra. The edited GABA+ peak at 3.0ppm was then modelled as a single Gaussian and quantification performed by calculating the integral of the area under the peak (Edden *et al.*, 2014).

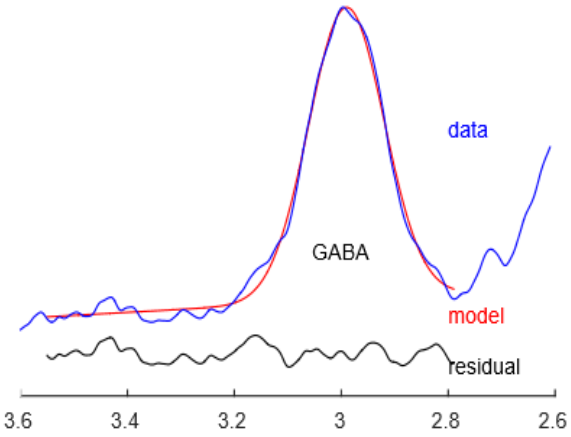


*Figure 6-2 Example of GABA-edited MR spectrum*

GABA+ concentration was estimated relative to creatine and water. The fit error of each estimate was calculated by dividing the standard deviation of the fitting residual by the amplitude of the fitted peak. Each individual spectrum was visually inspected and poorly fitting spectra were excluded from further analysis. As water concentration is affected by tissue composition, GABA+/$H_20$ measurements were corrected for the proportions of grey matter, white matter and cerebrospinal fluid in the voxel (Gasparovic *et al.*, 2006).

### 6.4.4 Statistical analysis

**Between-group analyses**

Statistical analyses were conducted using R Studio (version 1.1.383 for Mac, www.rstudio.com). For continuous data, distributions were first checked using the Shapiro-Wilk's test for normality in order to determine the most appropriate statistical test. Between group-differences in age, cognitive variables, GABA+ concentrations and fit errors were examined using t-tests. Gender and handedness proportions were compared using chi-squared tests. P values were not corrected for multiple testing.

**Relationships between GABA+ concentrations, cognitive ability and psychopathology**

Exploratory analyses of the relationships between GABA+ concentrations, age, cognitive ability and psychopathology were subsequently conducted using linear regression. To reduce the number of comparisons, GABA+/$H_2O$ and GABA+/Cr concentrations were combined by converting the concentrations into z-scores for each participant and averaging these to give a single estimate of GABA concentration. As cognitive ability is strongly associated with group status (proband or control), analyses were conducted in each group separately. Full-scale IQ (FSIQ), CANTAB subtest and WCST scores were used as measures of cognitive ability. Due to low rates of psychopathology in the control group, the relationships between GABA+ concentration and psychopathology were explored in the proband group only. Symptom counts for the most common disorders were used to investigate the relationships with psychopathology. As outlined in Chapter 3, the most common disorders in the sample were anxiety disorders, ADHD and ASD. No correction was performed for multiple testing.

**Relationships between GABA+ concentrations, visual gamma responses and visual evoked responses**

The relationships between GABA+ concentrations, visual gamma responses and visual evoked responses were explored using linear regression with GABA+ z-scores as the dependent variable and sum of gamma power between 35-70Hz, peak gamma amplitude, peak gamma frequency and evoked amplitude as independent variables. These exploratory analyses were not corrected for multiple comparisons.

## 6.5 Results

### 6.5.1 Descriptive data

Following quality control, occipital GABA+ data were available for 13 probands and 13 controls. None of the children who were in included in the final analysis

were taking psychotropic medication. As shown in table 6-1, there was no statistically significant between-group difference in age. Probands had a mean age of 13.85 years (age range=12.13-17.34 years); controls had a mean age of 14.43 years (age range=12.10-17.49 years), p=0.46. The groups were matched for gender and handedness. There were no significant differences in the fit errors for either the water or creatine contrasts.

*Table 6-1 Age, gender, handedness and MRS data quality in probands and controls*

| | **Probands** | **Controls** | **$t/x^2$** | **P** |
|---|---|---|---|---|
| **Age (SD)** | 13.8 (1.7) | 14.4 (1.8) | -0.75 | 0.46 |
| **Gender, female (%)** | 7 (53.8) | 7 (53.8) | <0.01 | >0.99 |
| **Handedness, right (%)** | 12 (92.3) | 12 (92.3) | <0.01 | >0.99 |
| **Fit error $H_2O$ (SD)** | 4.2 (1.4) | 4.2 (1.2) | 0.05 | 0.96 |
| **Fit error Cr (SD)** | 7.9 (1.4) | 7.4 (2.0) | 0.72 | 0.48 |

### 6.5.2 Cognitive and psychiatric data

As reported in Chapter 3 for the whole imaging sample, full scale IQ (FSIQ) was approximately 30 points lower in probands than controls. There were no statistically significant differences between groups in performance on CANTAB tasks or the Wisconsin Card Sorting Test (WCST) at $p<0.05$. In line with data in the whole imaging sample, children included in the GABA+ analyses had high rates of psychopathology. Eight probands (61.5%) had a DSM-IV-TR diagnosis, five were diagnosed with probable ASD (38.5%), three with ADHD (23.1%) and two with anxiety disorders (15.4%). Only one sibling had any DSM-IV-TR diagnoses (social phobia). None of the children included in the final analyses had a history of epilepsy and none were taking psychotropic medication at the time of the study.

*Table 6-2 Cognitive performance in children participating in the MRS study*

| | Probands | | Controls | | Group differences | |
|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **t** | **P** |
| **FSIQ** | 77 | 14.38 | 106 | 7.40 | -6.47 | $4.46 \times 10^{-6}$* |
| **CANTAB** | | | | | | |
| Visual attention (MTS) | 45.09 | 3.39 | 46.58 | 1.78 | -1.30 | 0.21 |
| Processing speed (RTI) | -0.92 | 2.20 | 0.45 | 0.55 | -2.01 | 0.07 |
| Sustained attention (RVP) | -0.45 | 1.15 | 0.28 | 1.21 | -1.47 | 0.15 |
| Spatial planning (SOC) | -1.02 | 0.87 | -0.52 | 1.05 | -1.24 | 0.23 |
| Spatial working memory (SWM) | -0.90 | 0.95 | -0.37 | 0.65 | -1.56 | 0.14 |
| | | | | | | |
| **WCST** | | | | | | |
| Set-shifting ability | 72.20 | 43.76 | 86.17 | 49.50 | -0.70 | 0.49 |

*Abbreviations: FSIQ, full-scale IQ; CANTAB, Cambridge Neuropsychological Test Automated Battery; MTS, Match-to-Sample; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing; SOC, Stockings of Cambridge; SWM, Spatial Working Memory; WCST, Wisconsin Card Sorting Test. P values are uncorrected.*

### 6.5.3 GABA+ concentrations

#### Between-group analyses of GABA+ concentrations

The boxplots in figures 6-3 and 6-4 show GABA+/$H_2O$ and GABA+/Cr concentrations in children with 22q11.2DS and controls.

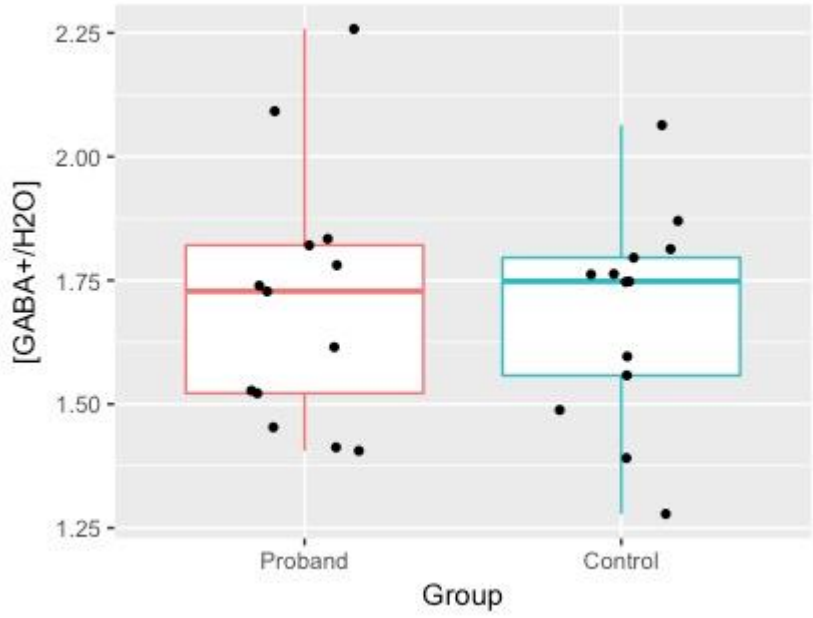


*Figure 6-3 Boxplot showing GABA+/$H_2O$ concentrations in probands and controls*

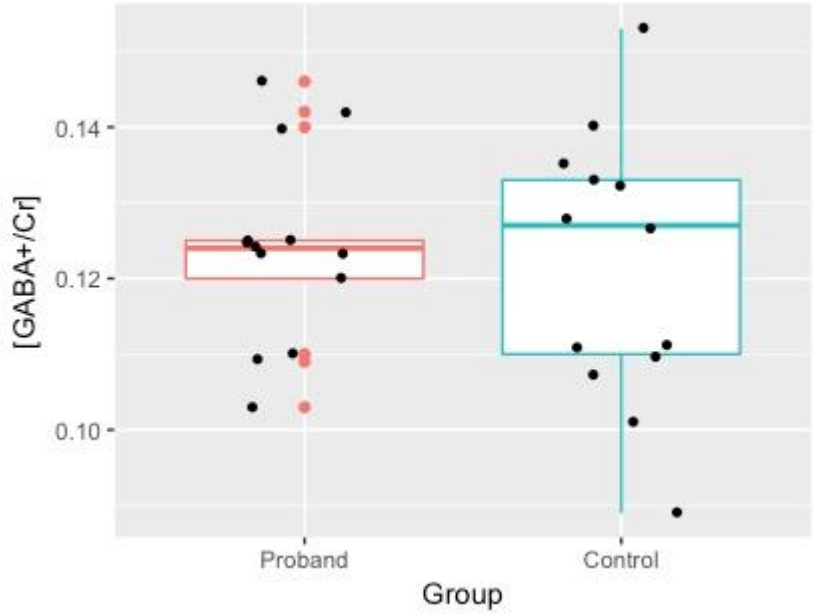


*Figure 6-4 Boxplot showing GABA+/Cr concentrations in probands and controls*

Mean GABA+/$H_2O$ concentration was 1.71 (SD=0.26) for probands and 1.68 (SD=0.01) for controls (t=0.26, p=0.80). Mean GABA+/Cr concentration was 0.12 (SD=0.01) for probands and 0.12 (SD=0.02) for controls (t=0.48, p=0.64). Figure 6-5 shows that across the age range recruited to the study, there were no between-group differences in mean GABA+ concentrations (shown in the figure as a combined z-score of creatine and water ratios).

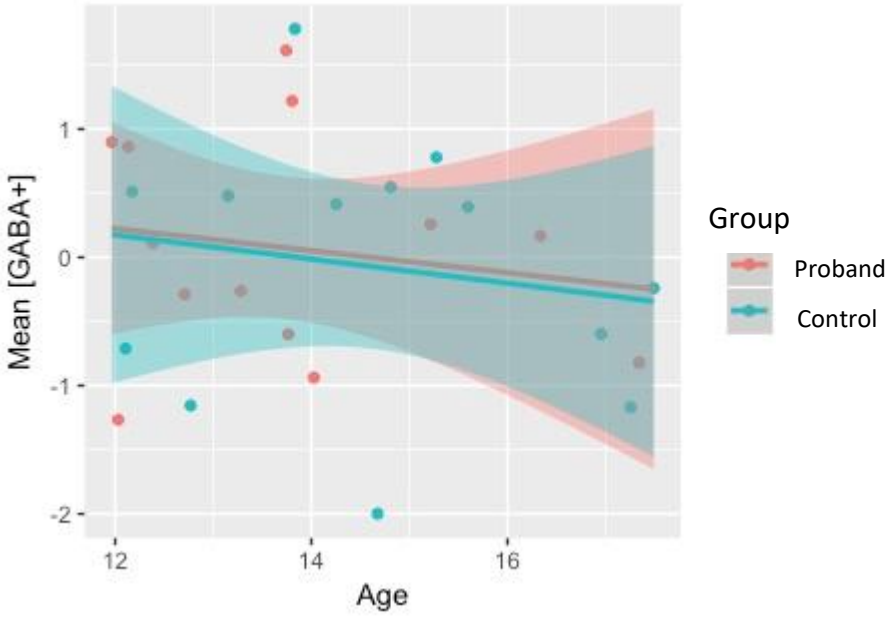


*Figure 6-5 Mean GABA+ z-score by age for probands (red) and controls (blue)*

**Relationships between GABA+ concentrations, cognitive ability and psychopathology**

Table 6-3 shows the results of the exploratory analyses for the associations between GABA+ z-scores and cognitive variables in children with 22q11.2DS. There were no statistically significant associations between GABA+ concentration and any of the cognitive variables tested.

*Table 6-3 Relationships between GABA+ concentration and cognitive function in probands*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **FSIQ** | $2.43x10^{-4}$ | 0.02 | 1.56x10-5 | 0.99 |
| **WCST (Set-shifting ability)** | $-4.37x10^{-3}$ | $6.81x10^{-3}$ | 0.05 | 0.54 |
| **Visual attention (MTS)** | 0.03 | 0.09 | 0.01 | 0.74 |
| **Spatial working memory (SWM)** | 0.33 | 0.30 | 0.12 | 0.30 |
| **Spatial planning (SOC)** | -0.08 | 0.35 | 0.01 | 0.83 |
| **Processing speed (RTI)** | 0.10 | 0.14 | 0.05 | 0.50 |
| **Sustained attention (RVP)** | 0.27 | 0.25 | 0.10 | 0.32 |

*Abbreviations: FSIQ, full-scale IQ; WCST, Wisconsin Card Sorting Test; MTS, Match-to-Sample; SWM, Spatial Working Memory; SOC, Stockings of Cambridge; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing. P values are uncorrected.*

Table 6-4 shows the results of the exploratory analyses between GABA+ z-scores and cognitive variables in controls. As for probands, no significant relationships were observed between GABA+ concentration and cognitive ability.

*Table 6-4 Relationships between GABA+ concentration and cognitive function in siblings*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **FSIQ** | 0.06 | 0.04 | 0.18 | 0.15 |
| **WCST (set-shifting ability)** | $1.83x10^{-3}$ | 6.70x10-3 | $7.47x10^{-3}$ | 0.79 |
| **Visual attention (MTS)** | 0.02 | 0.19 | $1.38x10^{-3}$ | 0.91 |
| **Spatial working memory (SWM)** | -0.13 | 0.51 | $6.17x10^{-3}$ | 0.81 |
| **Spatial planning (SOC)** | -0.13 | 0.31 | 0.02 | 0.68 |
| **Processing speed (RTI)** | 0.69 | 0.56 | 0.13 | 0.24 |
| **Sustained attention (RVP)** | 0.07 | 0.27 | $6.95x10^{-3}$ | 0.80 |

*Abbreviations: FSIQ, full-scale IQ; WCST, Wisconsin Card Sorting Test; MTS, Match-to-Sample; SWM, Spatial Working Memory; SoC, Stockings Of Cambridge; RTI, 5-choice Reaction Time; RVP, Rapid Visual Processing. P values are uncorrected.*

Table 6-5 shows the relationships between GABA+ concentration and psychopathology in the proband group. No significant associations were found between the most common symptoms reported by probands and GABA+ concentration.

*Table 6-5 Relationships between GABA+ concentration and psychopathology in probands*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **ADHD Score** | 0.03 | 0.07 | 0.01 | 0.72 |
| **ADI-R Score** | -0.02 | 0.02 | 0.04 | 0.51 |
| **SCQ Score** | -0.08 | 0.06 | 0.19 | 0.19 |
| **Anxiety Score** | $-8.52 \times 10^{-3}$ | 0.05 | $3.05 \times 10^{-3}$ | 0.86 |

*Abbreviations: ADHD, attention deficit hyperactivity disorder; ADI-R, Autism Diagnostic Interview-Revised; SCQ, Social Communication Questionnaire. P values are uncorrected.*

**Relationships between GABA+ concentrations and visual responses**

Table 6-6 shows the relationships between GABA+ concentrations and visual induced and evoked responses for proband and control groups. No significant associations between these variables were found for either group.

*Table 6-6 Relationship between GABA+ concentration and visual responses in the MEG*

| | Estimate | Standard Error | $R^2$ | P |
|---|---|---|---|---|
| **Probands** | | | | |
| Sum of gamma power | $8.90x10^{-5}$ | $1.04x10^{-4}$ | 0.13 | 0.43 |
| Gamma amplitude | 0.01 | $8.11x10^{-3}$ | 0.28 | 0.23 |
| Gamma frequency | -0.04 | 0.06 | 0.11 | 0.48 |
| Evoked response | $3.83x10^{10}$ | $5.50x10^{11}$ | $9.87x10^{-4}$ | 0.95 |
| **Controls** | | | | |
| Sum of gamma power | $9.66x10^{-5}$ | $1.55x10^{-4}$ | 0.05 | 0.55 |
| Gamma amplitude | $2.84x10^{-3}$ | 0.01 | $6.54x10^{-3}$ | 0.81 |
| Gamma frequency | -0.05 | 0.07 | 0.06 | 0.47 |
| Evoked response | $1.11x10^{12}$ | $6.13x10^{11}$ | 0.29 | 0.11 |

*P values are uncorrected.*

## 6.6 Discussion

In this investigation of occipital GABA+ concentrations, no differences were found in GABA+ concentrations between children with 22q11.2DS and children without neurodevelopmental CNVs. Furthermore, no associations were found between GABA+ concentrations, cognitive performance, psychopathology or visual MEG responses. This was unexpected since murine models of 22q11.2DS point towards abnormalities of GABAergic signaling, and in Chapter 5, alterations were found in visual gamma responses in 22q11.2DS which were expected to be reflected by a reduction in GABA concentration.

The GABA signal measured by MRS reflects the total GABA present in the selected voxel. This includes GABA located both intracellularly and extracellularly. Recently, transcranial magnetic stimulation has been used to better understand

how GABA measured by MRS is related to GABAergic inhibitory function in healthy controls. Data from these studies suggest that GABA concentrations measured by MRS may reflect extra-synaptic inhibitory tone in a brain region rather than GABAergic synaptic transmission (Stagg, Bachtiar and Johansen-Berg, 2011; Dyke *et al.*, 2017). If replicated in larger samples, the results of the current study could indicate that there is no difference in the extra-synaptic inhibitory tone of the occipital cortex between children with 22q11.2DS and controls, however, there may still be differences in phasic inhibition that cannot be measured using MRS.

No relationship was found between GABA+ concentrations and psychopathology. MRS studies in people with neurodevelopmental disorders have produced variable results. In schizophrenia, some studies found GABA reductions in patient groups (Yoon *et al.*, 2010; Marsman *et al.*, 2014; Rowland *et al.*, 2016; Thakkar *et al.*, 2017) while others found no differences between patients and controls (Tayoshi *et al.*, 2010; Brandt *et al.*, 2016). Studies have differed in the brain region investigated, the age of participants recruited, illness stage and medication use which may, at least in part, explain the discrepant findings. However, a recent meta-analysis of MRS studies in schizophrenia found high heterogeneity between studies and small effect sizes (Egerton *et al.*, 2017), and overall no significant differences between patients and controls. MRS studies in children with ASD have found reductions in GABA concentrations in several brain regions (Harada *et al.*, 2011; Kubas *et al.*, 2012; Gaetz *et al.*, 2014; Rojas *et al.*, 2014; Puts *et al.*, 2017). However, findings in adults have conflicted with those in children, reporting that GABA concentrations and $GABA_A$ receptor densities do not differ from controls (Horder *et al.*, 2018). In a recent MRS study of adults with ASD, brain responses to the drug riluzole were found to differ between adults with ASD and controls. In ASD, riluzole increases relative GABA concentration in the prefrontal cortex but decreases it in controls (Ajram *et al.*, 2017). This suggests abnormal responses to the perturbation of neurotransmitter systems in ASD. There has been much less research into the role of GABA in other psychiatric and neurodevelopmental disorders such as ADHD, however, one MRS study of children with ADHD found a reduction in GABA concentration compared with controls (Edden *et al.*, 2012),

while another study of adults and children found no difference between children with ADHD and controls but increased GABA in adults with ADHD (Bollmann *et al.*, 2015). A further study found that stimulant use during childhood but not adulthood alters GABA concentration and responsivity (Solleveld *et al.*, 2017). The relationship between GABA concentration and cognition is similarly unclear. Frontal GABA concentrations have been positively associated with superior cognitive performance in older adults (Porges *et al.*, 2017). However, GABA/glutamate ratios have been negatively associated with working memory performance in young adults (Marsman *et al.*, 2017). In this chapter no evidence was found for associations between GABA concentrations and cognitive variables in either the proband or control group.

As discussed in Chapter 5, the relationships between gamma responses and GABA+ concentrations measured by MRS are not well-elucidated. While Muthukumaraswamy *et al.* (2009) found a correlation between gamma frequency and GABA concentration (finding a positive association), another study failed to replicate this finding (Cousijn *et al.*, 2014). Cousijn *et al.* (2014) found no correlation between gamma activity and either GABA or glutamate concentrations in a large sample of healthy volunteers and suggested that other methods may be needed to explore the relationships between gamma oscillations and GABA/glutamate concentrations in the human brain. These methods could include pharmaco-MEG and PET studies.

## 6.7 Strengths and limitations

To my knowledge, this is the first study to investigate GABA+ concentrations in children with 22q11.2DS. A rigorously phenotyped sample of children of a relatively narrow age range was recruited on the basis of genotype rather than phenotype, minimising some of the biases that occur in clinically ascertained samples. The subsample of children participating in this study was broadly representative of the whole ECHO 22q11.2DS cohort in terms of demographics and levels of impairment. None of the participating children were taking

psychotropic medication. Furthermore, the proband and sibling groups were well-matched for age, gender and handedness, facilitating between-group comparisons. However, a major limitation of this study is the sample size. Due to the closure of CUBRIC's Park Place facility during the data collection period, the time available for recruitment was reduced. Furthermore, a large proportion of the children who wished to take part in the study were unable to do so because of contraindications to MRI at 3T. Some children who were able to tolerate a short structural scan (for MEG registration purposes) were unable to remain in the scanner for the MEGA-PRESS sequence either due to anxiety or difficulty remaining still for this longer acquisition. This resulted in the collection of only a modest dataset for analysis which limited the power of the study to investigate between-group differences and associations with cognition, psychopathology and gamma response variables. The results of the present study should therefore be interpreted cautiously.

The data in this study were collected at 3T as this was the strength of the scanner available at the time the study commenced. However, CUBRIC now benefits from a 7T scanner which has the ability to collect MRS data with higher signal to noise ratio than previously possible. This also enables improved separation of glutamate signals from neighbouring peaks (e.g. glutamine). Furthermore, new sequences are now available that can reduce contamination from macromolecules. High molecular weight macromolecules may account for 40-60% of the observed MRS signal (Behar *et al.*, 1994; Mikkelsen *et al.*, 2016) and so reduction in this contamination will improve the precision of GABA measurements in the future.

## 6.8 Conclusions

In conclusion, in this study of occipital GABA+ concentration in 22q11.2DS no evidence was found for altered GABA+ levels in 22q11.2DS. Furthermore, no associations were found between GABA+ concentrations, cognition, psychopathology or visual MEG responses. The data from this experiment

suggests that if there are alterations in GABAergic activity in 22q11.2DS, these are not reflected by GABA signals measured using MEGA-PRESS at 3T.

# 7 General discussion

In this thesis, magnetoencephalography and magnetic resonance spectroscopy were used to investigate potential neural mechanisms underlying the high risk of psychopathology and cognitive impairment in 22q11.2DS. Specifically, these investigations sought to determine whether 22q11.2DS is associated with alterations in excitatory-inhibitory balance and whether any observed alterations are associated with psychiatric, neurodevelopmental or cognitive problems. Three experiments were used to address these questions: firstly, as discussed in Chapter 4, resting-state MEG was used to investigate whole-brain neural oscillatory patterns; secondly, as discussed in Chapter 5, a simple visual stimulus was used to study induced gamma band responses in the visual cortex; and finally, as discussed in Chapter 6, MRS was used to determine whether GABA concentrations were altered in the occipital cortex.

## 7.1 Summary of findings

Findings from the investigations presented in this thesis contribute to our understanding of the impact of 22q11.2DS on developing neural circuits, particularly its effect on excitatory-inhibitory balance. Chapter 3 showed that the sample of children participating in the brain imaging experiments had psychiatric and cognitive phenotypes which were similar to those of children taking part in research at Cardiff University but who did not take part in the neuroimaging study. The phenotypes were also similar to those reported in previous studies of 22q11.2DS (Schneider *et al.*, 2014), suggesting that the imaging sample is not biased towards a highly functioning phenotype.

In the first MEG experiment, it was found that the low frequency oscillatory patterns that underlie communication between different brain regions at rest, were atypical in children with 22q11.2DS. Globally, children with 22q11.2DS had lower connectivity (as inferred from amplitude-envelope correlations) in the delta, alpha and beta bands than children without neurodevelopmental CNVs. In the alpha band, there was a significant relationship between global connectivity,

anxiety scores and social communication problems, which survived correction for age, gender and handedness. The reductions in amplitude-envelope correlations were most evident in posterior brain regions, with a reduction in the strength of signal connections between the calcarine region and lingual gyrus in both the alpha band (uncorrected for multiple testing) and beta band (corrected for multiple testing). These are key brain regions involved in processing visual information. The calcarine cortex is the primary visual cortex which receives visual information from the lateral geniculate nucleus via the optic radiation. The lingual gyrus also forms part of the visual cortex and is believed to play a role in the recognition of familiar landmarks (Takahashi and Kawamura, 2002; Mendez and Cherrier, 2003) and facial expressions (Kitada *et al.*, 2010), as well as being involved in visual memory (Bogousslavsky *et al.*, 1987; Machielsen *et al.*, 2000) and dreaming (Bischof and Bassetti, 2004). In the delta band there was a statistically significant reduction in the strength of signal connections between the precuneus and inferior parietal cortex in probands compared with controls. The precuneus and inferior parietal cortex form a core part of the 'default-mode network' and are thought to be involved in aspects of consciousness (Cavanna, 2007). The precuneus has a role in episodic memory (Lundstrom *et al.*, 2003; Lundstrom, Ingvar and Petersson, 2005) and visuospatial processing (Cavanna and Trimble, 2006), while the inferior parietal cortex has been associated with the perception of emotions in facial stimuli (Adolphs *et al.*, 1996; Radua *et al.*, 2010), the integration and interpretation of sensory information, visual attention, and the response to novel stimuli (Singh-Curry and Husain, 2009).

The AAL nodes that were most significantly affected by CNV status were the left insula in the delta band, the right precuneus in the alpha band and the right calcarine region in the beta band, which all had a reduction in total z-scores in probands compared with controls. Connectivity in the right precuneus was negatively associated with anxiety scores and social communication problems. The insula is part of the limbic cortex with a role in self-awareness (Critchley *et al.*, 2004), attention (Eckert *et al.*, 2009) and salience (Taylor, Seminowicz and Davis, 2009). As outlined above, the precuneus forms part of the default-mode

network and the calcarine region is the primary visual cortex. These brain regions have all been implicated in neurodevelopmental disorders including schizophrenia, ASD and ADHD (Wylie and Tregellas, 2010; Ahrendts *et al.*, 2011; Nickl-Jockschat *et al.*, 2012; Whitfield-Gabrieli and Ford, 2012; Goodkind *et al.*, 2015; Tohid, Faizan and Faizan, 2015; Mowinckel *et al.*, 2017; Padmanabhan *et al.*, 2017; Brodski-Guerniero *et al.*, 2018; Vetter *et al.*, 2018). Thus the results of this first experiment suggest that resting-state oscillatory patterns, which are dependent on excitatory-inhibitory interactions, are affected by 22q11.2 deletion status and are associated with psychopathology.

In the second MEG experiment, using a simple visual stimulus, it was found that induced responses in the high-frequency gamma range were affected by 22q11.2DS. Visual evoked responses were unaffected by deletion status. Children with the 22q11.2 deletion had lower total induced gamma power between 35 and 70Hz. While there were no statistically significant differences in peak gamma amplitude or peak gamma frequency at $p<0.05$, there was a trend towards a reduction in peak gamma amplitude and an increase in peak gamma frequency in 22q11.2DS. In the proband group, many participants had no discernible gamma peak, limiting the ability to detect between-group differences in peak gamma amplitude and frequency. In subsequent exploratory analyses, low total gamma power was associated with poor performance in tasks of spatial working memory and visual attention as well as social communication problems, indexed by the SCQ. Peak gamma frequency was also associated with social communication problems, those with lower peak frequency had more ASD symptoms as measured by the ADI-R. The results of this study suggests that low level sensory processing of visual stimuli is intact in 22q11.2DS but higher order processes involving integration of sensory input by GABAergic interneurons are altered, with increased excitatory-inhibitory balance and lower signal to noise ratio in these circuits.

Finally, using magnetic resonance spectroscopy, no differences were found in GABA+ concentrations in the occipital cortex between children with 22q11.2DS

and controls and there were no relationships between GABA+ concentrations and psychopathology, cognitive ability, visual evoked or induced gamma band responses. This finding was unexpected since GABA concentration measured using MRS is thought to reflect inhibitory tone, which was hypothesised to be reduced in 22q11.DS. GABA+ concentrations measured by MRS reflect total GABA in the voxel studied. While this can give important insights into the total GABA pool, it does not help to distinguish extracellular and intracellular GABA nor functional GABA activity at the synapse. Furthermore, it does not give insights into dynamic changes in GABA concentrations which may be affected by 22q11.2DS. Alternative methods such as pharmaco-MEG, pharmaco-MRS, transcranial magnetic stimulation (TMS) or PET will be required to further investigate the effects of 22q11.2DS on GABAergic neurotransmission.

## 7.2 Implications of this research

To my knowledge, there have been no previous MEG studies or GABA-edited MRS studies in children with 22q11.2DS. This series of investigations therefore brings novel insights into imaging markers of excitatory-inhibitory balance in children with 22q11.2DS, particularly implicating the visual system.

### 7.2.1 Excitatory-inhibitory balance in 22q11.2 deletion syndrome

In Chapters 4, 5 and 6, excitatory-inhibitory balance in children with 22q11.2DS was investigated. Excitatory-inhibitory balance is a crucial homeostatic mechanism, facilitating coordinated activity within and between different brain regions. Altered excitatory-inhibitory balance, particularly increased excitatory to inhibitory ratio, has been proposed as a potential mechanism underlying neurodevelopmental and psychiatric disorders such as ASD (Gao and Penzes, 2015; Nelson and Valakh, 2015; Foss-Feig *et al.*, 2017; Lee, Lee and Kim, 2017; O'Reilly, Lewis and Elsabbagh, 2017), ADHD (Naaijen *et al.*, 2017), schizophrenia (Lisman, 2012; Gao and Penzes, 2015; Foss-Feig *et al.*, 2017; Grent-'t-Jong *et al.*, 2018) and epilepsy (Fritschy, 2008).

22q11.2DS is highly pleiotropic and there has been intense effort to better understand the mechanisms conferring risk across the spectrum of mental disorders. One plausible mechanism is that haploinsufficiency of genes in the 22q11.2 region alters excitatory-inhibitory balance in favour of excitation. Evidence from post-mortem studies and animal models shows that GABAergic interneurons have abnormal morphology and migration patterns in 22q11.2DS (Kiehl *et al.*, 2009; Meechan *et al.*, 2009, 2012; Mori *et al.*, 2011; Piskorowski *et al.*, 2016). Furthermore, GABAergic excitatory to inhibitory shift is delayed in 22q11.2DS (Amin *et al.*, 2017). The 22q11.2 region contains the gene *PRODH*, which codes for the enzyme proline oxidase, which in turn degrades L-Proline. Mice deficient in *PRODH* have elevated L-Proline which has been shown to inhibit GAD67, which synthesises GABA from glutamate. *PRODH* deficient mice have impaired responses at GABA synapses during sustained stimulation and lower local field potential strength in the gamma frequency range. Elevated plasma L-Proline has also been found in people with 22q11.2DS (Magnée *et al.*, 2011) and a recent EEG study of auditory steady-state responses found reduced gamma power in 22q11.2DS (Larsen, Pellegrino, *et al.*, 2018).

Overall, the results presented in this thesis support the hypothesis of altered excitatory-inhibitory balance in 22q11.2DS. While no between-group differences were found in GABA concentrations, this finding does not exclude the possibility that there are abnormalities of GABA signalling that are not detectable using MRS at 3T. The total power of gamma responses to a simple visual stimulus was lower in 22q11.2DS and there was a trend towards a decrease in peak amplitude. The peak frequency was found to be higher in children with 22q11.2DS, which is consistent with an increased excitatory-inhibitory ratio. Furthermore, longer-range connections in the delta, alpha and beta bands were also impaired in posterior brain regions 22q11.2DS at rest. These connections, typically mediated by lower frequency oscillations, are critically dependent on excitatory-inhibitory interactions. Low and high frequency oscillations have been found to originate from separate cortical layers. High frequency gamma band oscillations emerge from the supragranular layers (2 and 3), while alpha oscillations emerge from the

more superficial layers 5 and 6 (van Kerkoerle *et al.,* 2014). It has been proposed that activity in the different frequency bands interacts via an interlaminar coupling circuit based on excitatory and inhibitory interactions (Mejias *et al*., 2016). Phase amplitude-coupling (PAC) is one of the most extensively studied forms of cross-frequency coupling. The phase of low frequency oscillations (e.g. alpha) has been found to be coupled to the amplitude/power of of high-frequency (e.g. gamma) responses in a number of different brain regions (Voytek *et al.,* 2010, Lega *et al.,* 2014, Cho *et al.,* 2015, Seymour *et al.,* 2017). Deficits in PAC have been proposed to underlie cortical dysconnectivity in ASD (Kessler *et al.,* 2016) and schizophrenia (Kirihara *et al*., 2012), although whether these are driven by aberrant bottom-up or top-down processing or a combination of both remains unclear. Local excitatory-inhibitory imbalance (reflected by atypical gamma band activity) could impede long-distance communication between different brain regions and the development of connections between cortical regions. In addition, atypical oscillatory activity in lower frequency bands could result in the inability of specialised cortical regions to provide feedback to regulate local excitatory-inhibitory balance. Directional measures of connectivity such as Granger causality (Granger, 1969) or dynamic causal modelling (Friston et al., 2003) may help to elucidate the relationships between local and long-range cortical circuitry.

Figure 7.1 shows a proposed model in which 22q11.2DS could result in E-I imbalance and abnormalities in long-range connectivity, and how this in turn may result in psychopathology and impaired cognitive function.

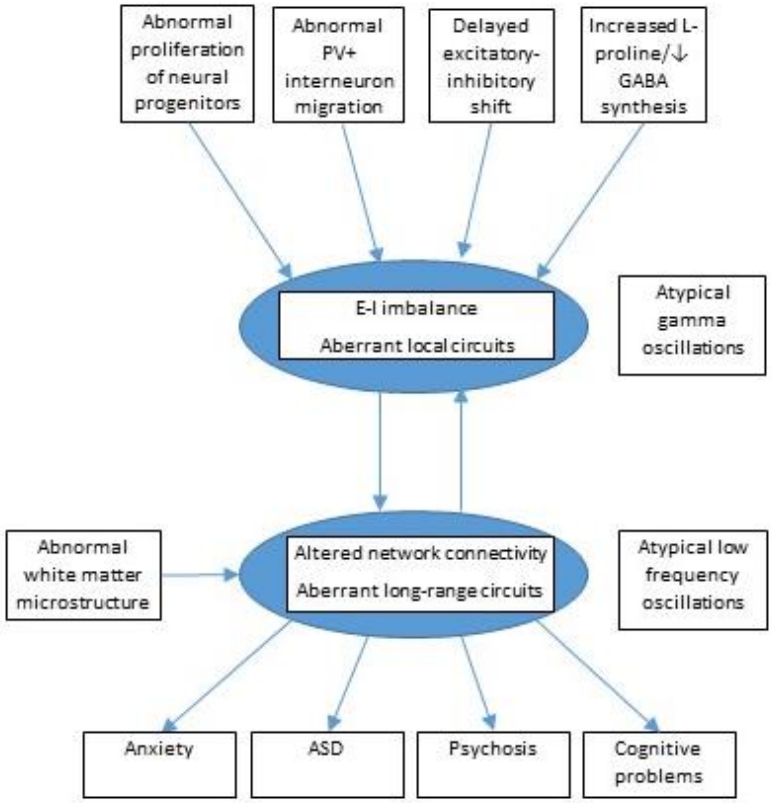


*Figure 7-1 Hypothesised model of the effects of 22q11.2DS on local and long-range cortical circuits. The timing, nature, location and severity of these alterations as well as the effects of other genetic and environmental risk factors will determine the clinical and cognitive phenotype*

### 7.2.2 The visual system in 22q11.2 deletion syndrome

In Chapter 4, a whole-brain AAL atlas-based approach was taken to investigate amplitude-envelope correlation as a marker of connection strength between brain regions and across frequency bands. These analyses showed that reductions in connectivity strength were most prominent in posterior brain regions, particularly the occipital lobe (visual cortex). In Chapters 5 and 6, investigations were focused on the visual system. Visual stimuli have previously been shown to induce strong gamma responses in healthy controls and are highly repeatable within participants (Muthukumaraswamy *et al.*, 2010). The responses are also highly heritable suggesting that they are influenced by genetic factors (van Pelt, Boomsma and Fries, 2012). Abnormal visual gamma oscillations have also been reported in neurodevelopmental disorders and have been associated with clinical

symptoms (Spencer *et al.*, 2008; Stroganova *et al.*, 2015). As described above and in Chapter 5, atypical visually-induced gamma oscillations were found in children with 22q11.2DS which were associated with cognitive and social communication difficulties.

GABA-MRS studies have shown that GABA estimation from an occipital voxel is robust, reproducible and stable over time (Near *et al.*, 2014; Greenhouse *et al.*, 2016; Bai *et al.*, 2017). There have been no published MRS studies of GABA concentrations in 22q11.2DS however, studies of occipital GABA in patient samples e.g. schizophrenia (Yoon *et al.*, 2010) have reported large GABA reductions of up to 10% using an identical sample size to that presented in Chapter 6. The lack of between-group differences in GABA concentrations reported in Chapter 6 suggests that either the effect size is too small to be detected in sample of this size at 3T in 22q11.2DS or that total GABA concentrations in the occipital lobe are not affected by 22q11.2DS. However as discussed previously, measuring GABA concentrations with MRS presents a number of challenges and may not reflect synaptic or dynamic GABA concentrations. In a previous MRS study of 22q11.2DS investigating Glx concentrations, there were no differences between people with 22q11.2DS and controls; group differences were only found between participants with 22q11.2DS and schizophrenia and those without schizophrenia. The findings from this study and that of Yoon *et al.*, suggest that neurotransmitter concentrations may be only be altered in people with established schizophrenia, although whether this is a causal relationship or secondary to the effects of chronic psychosis or antipsychotic use remains unclear. In order to further investigate the relationship between psychosis onset and GABA/Glx concentrations in 22q11.2DS, longitudinal studies will be required which are of sufficient size and have adequate follow-up periods to identify antipsychotic naïve people who develop psychosis and compare them to those who do not.

Taken together, the evidence presented in this thesis suggests abnormal cortical circuitry in the visual cortex in people with 22q11.2DS. This is interesting as visual

processing abnormalities are well-established in schizophrenia and ASD (Vandenbroucke *et al.*, 2008; Baruth *et al.*, 2010; Silverstein *et al.*, 2015), disorders for which 22q11.2DS confers high-risk (Murphy, Jones and Owen, 1999; Niarchou *et al.*, 2014; Schneider *et al.*, 2014; Ousley *et al.*, 2017). While people with 22q11.2DS have known difficulties with visuospatial processing (Bearden *et al.*, 2001; Simon, Bearden, *et al.*, 2005; Magnée *et al.*, 2011; McCabe *et al.*, 2016) and atypical visual networks have been found in fMRI and EEG studies of 22q11.2DS (Andersson *et al.*, 2008; Debbané *et al.*, 2012; Biria *et al.*, 2018), there has been little research into the mechanisms underlying these deficits. The findings presented in this thesis imply that these abnormalities may reflect aberrant top-down modulation of visual responses as a result of excitatory-inhibitory imbalance.

### 7.2.3 Feasibility of brain imaging research in children at high-risk of neurodevelopmental disorders

A final and important implication of the research presented in this thesis concerns the feasibility of conducting research in children with genetic syndromes associated with cognitive impairment and psychopathology. I recruited children and their families over a five year period and encountered several barriers that affected families' ability to participate in brain imaging research. These ranged from practical issues, such as needing to time data collection to coincide with school holidays, to medical and safety considerations such as a history of surgery involving metal implants, the presence of orthodontic braces, claustrophobia, sensory difficulties, attentional problems and anxiety. Despite these issues, there was great willingness among families of children affected by CNVs to engage in research perhaps because of a desire to learn more about the syndrome and to help other families affected by it.

I selected imaging modalities and experimental tasks that were not only relevant to the research questions but which were acceptable to children and their families. Data were collected during two separate sessions (MEG and MRI) and a

lot of time was spent explaining the data collection process to children and their parents/caerers before commencing recording or scanning. In addition, prior to the MRI session, time was dedicated to familiarisation with the MRI environment in a dedicated 'mock' scanner which was invaluable in relieving anxiety and optimising scanning time. A flexible approach was taken during data collection sessions to make the environment as relaxed and comfortable as possible, allowing plenty of time for breaks between recordings or scan sequences. During the MRI session, children were encouraged to watch a movie in the scanner as a distraction in order to improve their experience and minimise head motion. In the MEG session, I selected tasks that were simple to perform and short in duration to maximise the quality of data collected.

As discussed in Chapters 3-6, more children were able to take part in the MEG than the MRI experiments as MRI contraindications were common but did not preclude participation in the MEG session. While it is preferable to co-register MEG data to each individual's anatomical MRI, excluding children with MRI contraindications would have substantially reduced the sample size recruited for the MEG experiments. I therefore developed an alternative strategy in which MEG data from children who were unable to participate in the MRI session or whose MRI data were not of adequate quality were co-registered to another participant's MRI which closely matched the size and shape of the index participant. Inspection of the resulting head models suggested that this strategy worked well and meant that a much larger and more representative sample of children were able to participate than would have been possible if everyone with MRI contraindications was excluded from the MEG study.

As described in Chapters 3-6, different numbers of children completed each of the experiments and the quantity of good quality data varied considerably between these. In the MEG, children found the resting-state MEG recording easy to complete and the proportion of data that survived quality control was high. Children found the visual task more challenging as the paradigm was longer and required active participation in the form of a button press. This resulted in more

artefacts from head motion, eye movement and muscle contraction, with the result that less good quality data were available for analysis. In the MRI, structural data collection took 5 minutes to complete and this was achievable for the majority of participants. GABA MRS data collection was only undertaken until the upgrade of CUBRIC facilities in 2016. The scan sequence was longer at ten minutes but was able to be completed by the majority of children, providing good quality data. The low numbers of participating children for the MRS experiment therefore reflects the high rates of MRI contraindications in this sample and the early termination of data collection due to a change in MRI scanning facilities, rather than issues related to the ability of participants to complete the scans or data quality.

Overall, families affected by CNVs were keen to participate in brain imaging research. Careful selection of tasks and scan sequences as well as taking a flexible approach to data collection can improve the quantity and quality of data collected. Furthermore, alternative approaches for co-registration of MEG data can help to maximise sample sizes in patient groups in which MRI contraindications are prevalent.

## 7.3 Strengths and limitations

### 7.3.1 Participant ascertainment and sample size

22q11.2DS is a rare disorder affecting approximately 1 in 4000 live births (Botto *et al.*, 2003; Oskarsdottir, 2004). In addition to high rates of psychopathology and cognitive impairment, it is associated with a variety of physical health problems including global developmental delay, congenital cardiac abnormalities, cleft palate, renal insufficiency, immune deficiencies and musculoskeletal problems such as scoliosis (McDonald-McGinn *et al.*, 2015). As the majority of cases occur *de novo* and 22q11.2DS is not routinely screened for in the UK, most children are diagnosed by virtue of having one (or more likely several) of the features of 22q11.2DS. Therefore, although I did not select the proband sample according to

any particular phenotype (psychiatric or otherwise), the fact that participants had received a diagnosis in childhood may have biased the sample towards a more severe phenotype. The data presented in Chapter 3 suggest that the sample of children who took part in these investigations did indeed have a high burden of psychopathology. However, only one was prescribed psychotropic medication and few were known to mental health services or had a clinical diagnosis of a psychiatric illness. This suggests that these children were not referred for genetic testing due to psychiatric problems, but for other reasons such as cardiac malformations, cleft palate or developmental delay. The cognitive ability and psychiatric morbidity of children in brain imaging were similar to children who did not take part in brain imaging suggesting that the sample recruited to the investigations presented in this thesis is representative of the whole ECHO cohort.

While 22q11.2DS is not part of current antenatal screening programmes in the UK, the recent introduction of cell-free DNA antenatal screening tests means that non-invasive antenatal testing for 22q11.2DS is now possible (Ravi *et al.*, 2018). Recently, there has also been a call for the introduction of early postnatal screening by heel-prick test (https://hansard.parliament.uk/commons/2018-06-05/debates/18060550000001/DigeorgeSyndrome(ReviewAndNationalHealthServiceDuty)). Introduction of such screening tests would likely increase the reported prevalence of 22q11.2DS and have many important implications for research, increasing the pool of potential participants and facilitating less biased sampling. It would also facilitate the collection of longitudinal data from birth or early infancy which could yield very important insights into brain development in 22q11.2DS.

Recruitment for the investigations presented in this thesis was limited to children taking part in the ECHO study who were aged between 10 and 17 years old in order to balance the need for an adequate sample size with the desire for a narrow age range in order to reduce the confounding effects of brain development on the data collected. The age range selected was also based on the anticipated ability of children to participate in the imaging sessions. During pilot

work, I found that children younger than 10 years old found it much more difficult than older children to remain still during data collection and to attend to the stimuli, therefore a lower age limit of 10 years was selected. From this reduced pool of potential participants, screening was performed to identify contraindications to MEG and MRI. Due to the frequency of surgical and orthodontic implants in the sample, the pool of potential imaging participants was further narrowed to the sample of 39 who were ultimately recruited. While modest, this sample size is comparable to much of the published imaging literature in 22q11.2DS, with the exception of recent large collaborative efforts to which I contributed data (Sun *et al.*, 2018).

I selected the control sample from a cohort of siblings of children with neurodevelopmental CNVs who were also taking part in the ECHO study at Cardiff University. This control sample was chosen for a number of reasons: firstly, siblings of children with neurodevelopmental CNVs have been exposed to a similar home environment as CNV carriers. Although this does not eliminate the potential confounding effects of environmental influences on brain structure, it does, however, reduce between-group differences, for example in socioeconomic status. Secondly, genotypic data was being collected on siblings as part of the wider ECHO study which meant that, in most cases, it was possible to confirm the CNV status of controls prior to their recruitment into the imaging study. Finally, many families travelled long distances to take part in the brain imaging research and typically they would travel to Cardiff with the whole family for several days. This meant that siblings were potentially available to take part in the research without families needing to take additional time off work and school to participate. When possible I tried to recruit sibling pairs, however for many families there was no sibling who met the age criteria. Conversely, some siblings took part in the absence of a participating proband, for example, when the proband did not fit the age criteria or had contraindications to brain imaging. Due to the presence of unmatched sibling pairs, it was not possible to perform pairwise data analysis. In total, I recruited 22 siblings who were related to a child with 22q11.2DS. To increase the sibling sample, I also recruited 4 children who

were taking part in ECHO and who were siblings of children with other neurodevelopmental CNVs. In the future, it would be interesting to compare probands to siblings in a pairwise fashion.

### 7.3.2 Imaging and analysis methods used

EEG and MEG are complementary methods that are commonly used to investigate neural oscillations with advantages and disadvantages to each approach. EEG systems are less costly than MEG systems and, unlike MEG, they are unaffected by magnetic fields in the environment and therefore don't need to be used in a shielded environment. Many EEG systems are portable so experiments can be conducted in participants' homes. However, EEG preparation time is much longer and involves participants wearing a tightly-fitting cap and having gel applied to the scalp. This raises potential issues for children with sensory difficulties. EEG measures signals that arise directly from the electrical potential generated from neuronal currents. These signals have contributions from tangential and radial currents whereas in MEG, only the tangential component contributes to the signal. However, unlike EEG, MEG is not affected by conductance differences and therefore source reconstruction is easier to perform, enabling cortical sources of generated signals to be identified, a factor that was important in the investigations presented in this thesis.

While there are many metrics that could be used to estimate MEG resting-state connectivity, amplitude-envelope coupling is one of the most robust and repeatable and was therefore selected for the investigations presented in Chapter 5. It would be interesting however, to compare alternative approaches and explore cross-frequency coupling. A five-minute eyes-open resting-state paradigm was used in Chapter 4. Longer resting-state recordings have been found to improve the reproducibility of MEG connectivity estimates, however these potential benefits have to be balanced with the ability of participants to remain still for longer periods of time (Liuzzi *et al.*, 2017). Eyes-closed resting-state paradigms are associated with strong alpha power increases but can induce

drowsiness and result in participants falling asleep during recordings (Tagliazucchi and Laufs, 2015). Keeping one's eyes closed for five minutes in an unfamiliar environment would also be daunting for many children. For these reasons, an eyes-open paradigm was used. One of the major concerns in the interpretation of resting-state MEG/EEG data is the potential for spurious correlations or noise to affect the observed results. Several approaches were used to reduce the likelihood of spurious correlations or noise being interpreted as signal, including orthogonalisation of the source-level signals to reduce the effects of signal leakage between adjacent regions and Gaussian mixture modelling to classify connections as signal or noise. These approaches are conservative and may have led to false negatives but importantly they reduced the likelihood of false positives.

To investigate gamma band responses between probands and controls, a simple visual stimulus which has previously been shown to produce strong evoked and induced responses in the occipital cortex was used (Muthukumaraswamy *et al.*, 2010). Despite selecting a task which was not cognitively demanding, many participants found it difficult to remain still for the ~ eight minutes required to complete the MEG recording and a large number of trials were contaminated by artefact, leading to exclusion of participants from the final analyses. Furthermore several children failed to generate peak gamma responses, further reducing the sample size for the between-group analyses. Significant reductions in the sum of gamma power and a non-significant trend towards a reduction in peak gamma amplitude and an increase in peak gamma frequency were found in the proband group. The lack of statistically significant group differences in peak gamma responses may reflect low statistical power in the limited sample who remained in the analyses after quality control. Future work in larger samples will help to clarify whether peak gamma variables are altered in 22q11.2DS.

The lack of observed GABA concentration differences between children with 22q11.2DS and controls may also be due to type II error. This dataset was relatively small (13 participants in each group) and, as GABA concentrations show

high inter-individual variability (Muthukumaraswamy *et al.*, 2010), this study may have lacked statistical power to detect significant differences between groups in the presence of small effect sizes. As with much of the existing literature on GABA MRS in clinical samples, the data for this thesis were collected at 3T which has lower sensitivity to detect GABA signals than higher field strengths (e.g. 7T), thus low signal to noise ratio at 3T may also have limited the ability to detect any group differences, if present. A MEGA-PRESS sequence was used to identify GABA peaks. While this approach was considered to be gold-standard at the time the study commenced, subsequent work has shown that a substantial component of the signal detected using MEGA-PRESS sequences at 3T is due to macromolecules (Mikkelsen *et al.*, 2016). It is possible that differences in macromolecule concentrations between groups could mask differences in GABA+ concentrations. Future research using macromolecule suppression may help to elucidate this.

A potential problem in interpreting between-group differences in neuroimaging studies is the possibility that data quality varies between study groups (e.g. due to higher levels of motion or other artefacts in patients than controls). In the investigations presented in this thesis, rigorous data cleaning and quality control procedures were used, after which there were no significant between-group differences in either the number of good trials or markers of head motion in the MEG experiments, or in MRS data quality (e.g. fit error), suggesting that overall the significant findings are not due to lower data quality in the proband group.

### 7.3.3 Phenotyping

Participants underwent rigorous phenotyping either at the time of imaging data collection or at another time in the participant's home or by telephone. Although every effort was made to complete data collection in a timely fashion, some phenotypic data were nevertheless missing at the time of analysis. This is particularly relevant for ADI-R data which were only available for 26 probands and no controls. SCQ data were available for a larger number of participants (both probands and controls), however comparison between SCQ and ADI-R data

suggested that although these measures are both reported to index social communication problems, they did not correlate well with one another in this sample.

While 22q11.2DS increases the risk of childhood-onset disorders like ASD and ADHD, it is also one of the strongest risk factors for schizophrenia, being associated with a 25-30 fold increase in risk compared to the background population. While data about the presence of psychotic symptoms were collected, these symptoms occurred at too low a rate in this sample to be included in the regression analyses (present in only 16.2% of probands and none met criteria for a DSM-IV psychotic disorder). One would anticipate that in time, as probands enter their late teens and early twenties, these rates will increase. Longitudinal follow-up of these children may help to identify cortical circuit abnormalities associated with psychotic symptoms and potentially the presymptomatic neural correlates of psychosis risk.

## 7.4 Future research directions

### 7.4.1 Larger samples and collaborations

The data presented in this thesis provide interesting insights into neural circuit abnormalities in 22q11.2DS and warrant replication in a larger sample and in a wider age range. There are increasing efforts to combine brain imaging data across different sites internationally to improve the statistical power to interrogate data with a range of questions that could not previously have been addressed in small independent studies For example, through the work of ENIGMA 22q11.2DS (www.enigma.ini.usc.edu/ongoing/enigma-22q-working-group), it has been possible to examine detailed brain structure in 22q11.2DS and to investigate the effects of factors such as deletion size (Sun *et al.*, 2018). Such efforts require standardisation of data collection and analysis strategies. While some positive strides have been made in structural MRI collaboration, the same cannot be said of MEG or indeed MRS collaborations. This is therefore an important area for future development.

### 7.4.2 Longitudinal studies

In order to better understand the biological mechanisms underlying risk of neurodevelopmental and psychiatric disorders, longitudinal studies are needed. While there are several relatively large longitudinal studies of the 22q11.2DS phenotype ( Vorstman *et al.*, 2015; Kates *et al.*, 2015; Chawner *et al.*, 2017, 2019; Tang and Gur, 2018), there are few large longitudinal imaging cohorts (Ramanathan *et al.*, 2017). Collecting MRI and MEG/EEG data from children at a young age (before the onset of symptoms) and following them up into adulthood would yield remarkable insights into brain development in 22q11.2DS and its relationship with psychopathology and cognitive impairment. These studies would have the potential to identify early signatures of risk and may therefore have important clinical implications such as providing diagnostic and prognostic information for clinicians and families. In addition, recognition of brain abnormalities associated with increased risk may help to identify novel therapeutic targets which have long been needed in psychiatry.

### 7.4.3 Other genetic syndromes

As our knowledge of genetic risk factors for psychopathology expands, so do the opportunities for research into the brain mechanisms underlying risk. Syndromes such as 22q11.2DS, Fragile X, Down's syndrome and Rett syndrome have perhaps received most research interest to date. However, a large number of copy number variants associated with psychiatric and cognitive risk have now been identified (Doherty and Owen, 2014) and little is known about their neural substrates. Cross-CNV work has the potential to identify common mechanisms and pathways by which these CNVs may act and could provide important insights into neurobiology that are relevant to idiopathic mental health problems.

### 7.4.4 Translational research

One of the most exciting developments in recent years has been the ability to model genetic syndromes in cellular and animal models. Cells can be taken from patients with 22q11.2DS (e.g. via hair or skin samples) and transformed into induced pluripotent stem cells (iPSCs). Neuronal cells can then be generated from iPSCs and their properties investigated. These techniques have the potential to advance our understanding of the effects of 22q11.2DS on excitatory and inhibitory neurons *in vitro*. Beyond cellular models, animal models of 22q11.2DS can advance our understanding of the effects of the deletion on brain circuits *in vivo* (Sigurdsson *et al.*, 2010). For example, oscillatory dynamics can be examined in model systems and be compared to studies of neural oscillations in humans using MEG/EEG both at rest and during specific task conditions. In addition, structural brain imaging can be used in animals to validate histologically the structural imaging findings from human studies.

### 7.4.5 New technologies

Since data collection for this study commenced, there have been advances in brain imaging both in terms of the hardware available and the development of analysis techniques. CUBRIC now has a 7T MRI scanner and a 3T Connectom scanner in addition to conventional 3T facilities. Higher field and gradient strengths can improve signal to noise ratios and provide more precise measurements than previously thought possible (van der Kolk *et al.*, 2013; Huang *et al.*, 2015; Fan *et al.*, 2016). Novel analysis techniques can minimise the effects of head motion thus improving data quality in samples where head motion is particularly problematic (Federau and Gallichan, 2016). Furthermore, new MEG systems are being developed that do not require a shielded room and that are wearable so potentially less affected by participant head motion (Boto *et al.*, 2016). This would have obvious advantages in the study of children and young people with developmental disorders.

## 7.5 Conclusions

Children with 22q11.2DS have high rates of cognitive impairment and psychopathology. Abnormalities in excitatory-inhibitory balance have been reported across the spectrum of neurodevelopmental and psychiatric disorders but have not previously been explored in detail in children with 22q11.2DS. In this thesis, there was evidence for excitatory-inhibitory imbalance in children with 22q11.2DS as indexed by atypical resting state and visually-induced gamma oscillatory patterns which were associated with some of the cognitive and psychiatric difficulties experienced by affected children. There were no corresponding abnormalities in the concentration of the major inhibitory neurotransmitter GABA as measured by MRS at 3T. The findings from this thesis merit replication in a larger sample size across the age spectrum to compare markers of excitatory-inhibitory balance across different stages of development. In the future, longitudinal studies using advanced imaging techniques will help to elucidate the relationship between excitatory-inhibitory balance and the onset of psychiatric symptoms such as psychosis. Furthermore, cross-CNV work may identify brain mechanisms common to high-risk groups which may identify important therapeutic targets which are not only relevant to people with neurodevelopmental CNVs but also people with idiopathic mental illness.

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