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Functional Connectivity in Youth at High Genetic Risk for Psychosis

A dissertation submitted in partial satisfaction of the

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in Neuroscience

by

Matthew James Schreiner

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ABSTRACT OF THE DISSERTATION

Functional Connectivity in Youth at High Genetic Risk for Psychosis

by

Matthew James Schreiner

Doctor of Philosophy in Neuroscience

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Professor Carrie E. Bearden, Chair

Schizophrenia is a devastating neurodevelopmental disorder that assaults the afflicted with visual and auditory hallucinations, confusion and cognitive problems, emotional withdrawal, a lack of motivation, a misunderstanding of what constitutes appropriate social behavior, and a lower life expectancy. While the majority of schizophrenia cases are of unknown etiology, recent work has highlighted the impact that rare genetic mutations can have on an individual's predisposition to developing a psychotic disorder. 22q11.2 Deletion Syndrome (22q11DS, aka velocardiofacial syndrome or DiGeorge syndrome;), resulting from the loss of a 1.5-3 Megabase portion of chromosome 22, is one such disorder; it imparts a greatly increased risk of psychosis (up to 30%), amongst other psychiatric disorders, for those with the deletion. Concurrently, the use of non-invasive neuroimaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) to investigate the brain-

behavior relationship has rapidly grown to the method of choice in the quest to characterize the neural correlates of normal and abnormal cognition; a large body of literature now exists that reports alterations to functional brain network (both task-oriented and resting-state) across a range of psychiatric disorders. However, despite the clear utility in studying a disorder with a known genetic etiology and the neuroscience community's growing interest in probing the intrinsic connectivity of the human brain and how it relates to behavior and cognition, very little research to date has attempted to characterize the dynamics and extent of resting state networks (RSNs) in 22q11DS. Accordingly, we sought to explore the 22q11DS-related alterations to RSN (if any) via a range of analysis methods, and to uncover the cognitive and behavioral correlates of aberrant connectivity within these RSNs.

An initial region-of-interest-based exploration of the Default Mode Network (DMN), in a cross-sectional analysis of 22q11DS subjects and controls (N=77), showed evidence of weakened long-range connectivity in this network for 22q11DS subjects relative to controls. The strength of long-range connectivity was inversely correlated with scores on the social responsiveness scale, such that individuals with more robust DMN connectivity exhibited improved social behavior. Subsequently, we commenced a model-free investigation of resting state network connectivity in youth with 22q11DS and matched control subjects (N=66), utilizing spatial Independent Components Analysis (ICA) to parse the observed variance in the subjects' data into multiple RSNs at once. Upon identifying networks of interest, rigorous statistical testing yielded group differences of significant within-network hypoconnectivity in 5 RSNs: ACC/Precuneus network, Executive network, DMN, Posterior DMN and Salience network. No cortical RSN tested showed any evidence of within-network hyperconnectivity in 22q11DS. Concurrently, each of the identified RSNs was vectorized and used to train and cross-

validate a sparse diagnostic classifier to differentiate between 22q11DS and controls on the basis of their within-network connectivity alone. Of all RSNs, the within-network connectivity differences between subjects were most robust in the DMN, allowing the associated diagnostic classifier to partition the groups with 100% accuracy during cross-validation. Additionally, the DMN-derived classifier could identify the presence of psychotic symptoms dimensionally and further partition the 22q11DS subjects into low-risk and high-risk cohorts on the basis of their DMN hypoconnectivity alone. The findings of RSN hypoconnectivity and DMN utility as a classifier were replicated in an independent cohort of 22q11DS subjects and controls (N=56), showing that cortical RSN hypoconnectivity, particularly within the DMN, is a stable distinguishing feature of 22q11DS. Finally, a targeted analysis of thalamocortical connectivity, in a cross-sectional cohort (N=79) as well as a subsample of individuals with longitudinal data (N=26), was performed to characterize the subcortical-cortical connectivity differences between 22q11DS and controls. Results were largely consistent with current literature in idiopathic schizophrenia and mouse models of 22q11DS, showing hyperconnectivity between the thalamus and bilateral sensory cortices, and hypoconnectivity between the thalamus, striatum, occipital cortex and cerebellum. The observed hyperconnectivity between thalamus and sensory cortex became significantly exacerbated with time in the patient group, and could be used to predict psychotic symptom severity and prodromal risk status in 22q11DS subjects.

Overall, this collected work provides evidence of aberrant within-RSNs connectivity in 22q11DS and lays the groundwork for future investigations into how changes in developing brain networks can predispose an individual to psychosis.

The dissertation of Matthew James Schreiner is approved.

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2015

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I would like to extend my sincere thanks to all of the people that made this work possible, either by the personalized aid they provided, or as an indirect consequence of their influence on my work and/or me. First and foremost, I would like to express my heartfelt gratitude for the constant support and encouragement supplied by my mentor, Carrie Bearden. I am especially grateful for the countless hours she has spent editing my work, critiquing my analyses, and helping me think through what I have always considered to be the most daunting part of academic research – the writing. Her constant attention to my questions, her willingness to respond to my 3am emails when a grant deadline is looming and her ability to maintain a cheery and supportive attitude, in the face of my distaste for deadlines and throughout my graduate career in general, has been a boon and a true inspiration.

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I am also indebted to my fellow graduate students and lab-mates, especially all of the co-authors mentioned in the Chapter attributions below. In particular, I would like to thank Maria

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I would like to acknowledge that Chapter 1 contains significant content from a published review of 22q11DS, reprinted from Neuropharmacology (vol. 68, Schreiner M, Lazaro M, Jalbrzikowski M, Bearden CE, 'Converging levels of analysis on a genomic hotspot for psychosis: Insights from 22q11.2 Deletion Syndrome,' pp. 157-173, Copyright (2013), doi: 10.1016/j.neuropharm.2012.09.012) with permission from Elsevier. This work was co-authored by Maria T. Lazaro, Maria Jalbrzikowski and Carrie E. Bearden, all of who have consented to the inclusion of this work in this thesis. In this work, I wrote the introduction, most of the body of the review paper, conclusions and future directions. I also edited and compiled the material contributed by my co-authors. Maria Lazaro contributed the section on Mouse models of 22q11DS and Maria Jalbrzikowski contributed the section on Structural Connectivity and Myelination Deficits in 22q11DS. Carrie Bearden oversaw the compilation of this work and provided essential editing throughout the writing process.

Chapter 2 consists primarily of a published manuscript, credited and formatted in accordance with the publishing agreement mandated by the journal; it is a pre-copy-editing, author-produced PDF of an article accepted for publication in Social Cognitive and Affective Neuroscience (SCAN) following peer review. The definitive publisher-authenticated version,

"Schreiner M, Karlsgodt KH, Uddin L, Chow C, Congdon E, Jalbrzikowski M, Bearden CE (2014), 'Default Mode Network Connectivity and Reciprocal Social Behavior in 22q11.2 Deletion Syndrome,' SCAN, vol. 9, no. 9, pp. 1261-1267. doi: 10.1093/scan/nst114. Epub 2013 Aug 2," is available online at: http://scan.oxfordjournals.org/content/9/9/1261.long. This work was co-authored by Kathryn H. Karlsgodt, Lucina Uddin, Carolyn Chow, Eliza Congdon, Maria Jalbrzikowski and Carrie E. Bearden, all of who have consented to the inclusion of this work in this thesis. In this work, I assisted with collection of resting-state fMRI data, preprocessed the data, analyzed the ROI-derived connectivity of the DMN at the group level, wrote the manuscript and generated all tables and figures. Kathryn Karlsgodt and Lucina Uddin contributed valuable advice regarding data preprocessing and interpretation of results. Carolyn Chow assisted with subject enrollment, collecting the majority of the fMRI scans and neurobehavioral assessments from the subjects. Eliza Congdon provided suggestions about the analysis of between-scanner effects. Maria Jalbrzikowski and Carrie Bearden assisted with the editing and writing of the manuscript.

The work in Chapter 3 is adapted from a manuscript in preparation for publication, with the working title, "Resting-Network-Based Classification and Prediction of Psychotic Symptoms in Youth with 22q11.2 Deletions," co-authored by Nurit Hirsh, Leila Kushan, Lucina Uddin, Leah M. Mattiacio, Ioana L. Coman, Wendy R. Kates and Carrie E. Bearden. This work was proposed, performed, analyzed and written up largely by me. Nurit Hirsh provided invaluable assistance during the preprocessing of subject data. Leila Kushan assisted with fMRI scan acquisition. Lucina Uddin provided consultation on the characterization of ICA-derived group differences and implementation of machine learning classifiers on the identified networks. Wendy Kates graciously contributed the SUNY replication dataset (with data preprocessing

provided by Leah Mattiacio and Ioana Coman) for validation of our findings in an independent cohort. Carrie Bearden edited the manuscript and as always, provided indispensible input on the framing of the research question and overall content of the paper.

The work in Chapter 4 is also from a manuscript in preparation, with the working title "Thalamocortical dysconnectivity in 22q11.2 Deletion Syndrome - the subcortical signature of psychosis risk". This work was done in collaboration with Alan Anticevic, who provided essential advice on all levels of the analysis (from the preprocessing pipeline to the interpretation of results yielded at the group level), with oversight provided by Carrie E. Bearden.

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(in press) **Schreiner M**, Jalbrzikowski M, Lazaro M, Bearden CE (2013). Converging Levels of Analysis on a Genomic Hotspot for Psychosis: Insights from 22q11.2 Deletion Syndrome. Neuropharmacology, vol. 68, pp. 157-73 (doi: 10.1016/j.neuropharm.2012.09.012. Epub 2012 Oct 23)
(in preparation) **Schreiner M**, Hirsh N, Kushan L, Uddin L, Mattiacio LM, Coman IL, Kates WR, Bearden CE. "Classification of 22q11.2 Deletion Syndrome by Severity of Resting State Network Dysconnectivity."

PRESENTATIONS & MEETINGS

Schreiner M, Hirsh N, Kushan L, Uddin L, Mattiacio LM, Coman IL, Kates WR, Bearden CE (June 2015). "Classification of 22q11.2 Deletion Syndrome by Severity of Resting State Network Dysconnectivity." Poster to be presented at 2015 OHBM Meeting.
Schreiner M, Karlsgodt KH, Chow C, Jonas R, Montojo C, Jalbrzikowski M, Bearden CE (May 2013). "22q11.2 Deletion Syndrome and the Resting State: An investigation of functional connectivity in the default mode network and its association with social behavior." Poster presented at 2013 SOBP Meeting.
Schreiner M, Karlsgodt KH, Chow C, Jalbrzikowski M, Bearden CE. (Dec 2011). "Resting State Functional Connectivity in 22Q11.2 Deletion Syndrome, A Recurrent Genetic Mutation Associated With Schizophrenia." Poster presented at 2011 ACNP Meeting
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CHAPTER 1:

Converging Levels of Analysis on a Genomic Hotspot for Psychosis: Insights from 22q11.2 Deletion Syndrome

1.1 - Introduction

Although schizophrenia is a highly heritable neurodevelopmental disorder, the precise biochemical pathways by which it wreaks its devastating effects remains elusive. The complexity and heterogeneity of this illness poses enormous challenges to biomedical discovery. While the majority of cases are of unknown etiology (idiopathic), there is increasing evidence that rare genetic mutations may account for a larger proportion of cases than was previously believed (Sebat et al., 2009; Tam et al., 2009; Walsh et al., 2008). While these findings have fundamentally changed our understanding of the genetic architecture of schizophrenia, they do not address the mechanisms by which structural mutations of genes may contribute to the disease. As such, in-depth investigation of a known genetic cause of psychosis offers a unique window into specific biological pathways leading to its development. 22q11.2 Deletion Syndrome (Velocardiofacial/DiGeorge syndrome; 22q11DS) affecting about 1/4000 live births, is one such genetic disorder. This genetic microdeletion syndrome is estimated to account for 1-2% of schizophrenia cases, and currently represents the only known recurrent copy number mutation responsible for introducing new cases of schizophrenia into the population (Karayiorgou and Gogos, 2004). About thirty percent of individuals afflicted by 22q11DS are estimated to meet criteria for a psychotic disorder and up to 25% of these individuals are diagnosed with schizophrenia by adulthood (Murphy et al.,1999; Bassett and Chow, 1999). The phenotypic consequences of this deletion event are complex and varied, ranging from facial dysmorphology, congenital heart defects, hypocalcaemia and cleft palate, to cognitive deficits and neurodevelopmental delays (Drew et al., 2011; McDonald-McGinn et al., 2001). Several of the genes within this region are highly expressed in the brain, and known to affect early neuronal

migration and cortical development (Maynard et al., 2003). As such, this syndrome provides a unique window into gene-brain-behavior relationships.

While the majority of individuals diagnosed with this syndrome have a similar 3 Megabase (Mb) deletion, encompassing about 60 identified genes, an estimated 8-10% of cases have smaller (approximately 1.5 Mb) deletions, a region that includes up to 35 identified genes (Drew et al., 2011; Edelmann et al., 1999) (see Fig. 1.1). Importantly, the smaller and less common deletion seems to contain all of the genes necessary for development of the syndrome (Carlson et al., 1997), and the increased risk of psychosis (Drew et al., 2011; Karayiorgou et al., 1995). Accordingly, this review will focus on the genes implicated in this 1.5 Mb Critical Region, in the context of a unifying theoretical framework from which to understand the biological mechanisms underlying psychotic symptom development in this syndrome. We first review the developmental trajectory of psychopathology in 22q11DS, findings on neurocognitive dysfunction and its ostensible similarities to the cognitive phenotype of schizophrenia, and then discuss the structural and functional neuroanatomic alterations that are characteristic of the disorder. Finally, we highlight recent findings from animal models of the 22q11.2 deletion, which inform our understanding of specific genetic mechanisms relevant to the development of psychosis via their structural and functional consequences, and their overall impact on brain systems involved in motivation, attentional and memory processes.

1.2 - Developmental trajectory of 22q11DS-associated psychopathology

While psychotic symptoms usually evolve during adolescence or early adulthood, non-psychotic psychiatric disorders and behavioral abnormalities are present from early childhood in

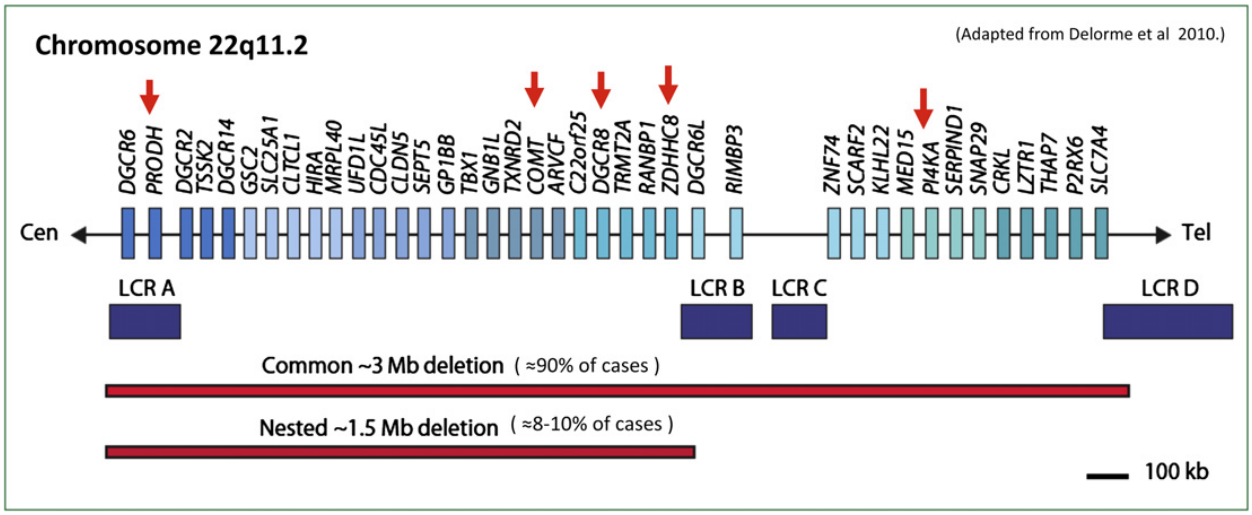


Fig. 1.1 The *del22q11.2* region on chromosome 22, with genes of interest marked by red arrows. Purple blocks represent low-copy repeats (LCRs), which are believed to mediate the common 3 Mb deletion. The common 3 Mb typically deleted region (TDR), present in over 85% of *22q11DS* patients and the 1.5-Mb deletion are shown.

22q11DS, some of which may be premorbid indicators of psychosis susceptibility (Gothelf et al., 2007a). In particular, 14-50% meet autistic spectrum criteria (Antshel et al., 2007; Fine et al., 2005; Niklasson et al., 2001; Vorstman et al., 2006), and attention deficit hyperactivity disorder (ADHD) is diagnosed in 35-55% of children and adolescents with the deletion (Antshel et al., 2005b; Gothelf et al., 2007b; Niklasson et al., 2001). In addition, afflicted individuals exhibit an elevated rate of mood and anxiety disorders (Gothelf et al., 2008; Green et al., 2009). Indeed, in two large cohorts from Israel and Western Europe, Green et al. (2009) found that psychopathology in 22q11DS patients appeared to follow a developmental pattern, with high rates of ADHD in early childhood, and substantially increasing rates of mood and psychotic disorder in adolescence and young adulthood. The spectrum of psychopathology associated with this syndrome, spanning a range of DSM-IV diagnostic categories, suggest a model of genetic pleiotropy, in which the same genetic variant can influence multiple phenotypes. Such findings also suggest that schizophrenia and other neuropsychiatric disorders may share overlapping biological pathways (Sebat et al., 2009).

1.3 - Neurocognition

22q11DS is characterized by a diverse assortment of neurocognitive deficits, ranging from overall reduced IQ, to abnormal results on assays of more specific endophenotypes such as prepulse inhibition (Kiley-Brabeck and Sobin, 2006; Sobin et al., 2005a, 2005b; Vorstman et al., 2009a; Vorstman et al., 2009b) tasks of spatial and attention-switching (Simon et al., 2005a; Sobin et al., 2006), and time perception (Drew et al., 2011). Although 22q11DS patients have lower Full Scale IQ relative to typically developing children, verbal skills tend to be better preserved than non-verbal skills on both IQ and academic achievement measures in children with 22q11DS (Bearden et al., 2001b; Moss et al., 1999; Swillen et al., 1999). 22q11DS patients show a characteristic neurocognitive profile involving marked deficit in visuo-spatial cognition and memory, with corresponding difficulties with arithmetic (Bearden et al., 2001b; Simon et al., 2005b). A key question is whether intermediate cognitive traits characteristic of idiopathic schizophrenia are also characteristic of 22q11DS. While few studies have directly compared these patient groups, two studies to date have directly compared neurocognition in adults with 22q11DS with and without psychosis. The most pronounced differences were seen on tests of abstraction, social cognition, spatial working memory, motor skills and verbal learning, with poorer performance in the 22q11DS schizophrenia subjects, supporting the view that the 22q11DS subtype of schizophrenia shares general characteristics of cognitive expression with idiopathic schizophrenia (Chow et al., 2006; van Amelsvoort et al., 2004). Moreover, Lajiness-O'Neil (2006) found Wisconsin Card Sort Test performance was significantly inversely correlated with the Thought Problems subscale of the Child Behavior Checklist (CBCL) in 22q11DS children, suggesting that executive dysfunction may be an indicator of risk for later-

onset psychopathology. This notion is consistent with the literature on youth with a family history of psychosis, which indicates that executive function deficits may be a vulnerability marker for psychosis (Byrne et al., 2003; Davalos et al., 2004; Whyte et al., 2006).

The study of social cognition is also considered to be a high-priority target in current research in schizophrenia (Green et al., 2008). Social cognition refers to the ability to make accurate judgments about the emotional states of others, infer others' intentions, and understand assumptions about relationships between people. Patients with idiopathic schizophrenia show marked deficits in all of these domains (Bora et al., 2009; Fakra et al., 2008; Kohler et al., 2010) and several studies have shown that social cognition mediates the relationship between neurocognition and real world functioning (Brekke et al., 2005; Sergi et al., 2007). Although social impairment has been consistently identified via parental report in 22q11DS individuals (Kiley-Brabeck and Sobin, 2006; Swillen et al., 1997; Woodin et al., 2001), the literature on 22q11DS and social cognition currently offers only preliminary findings. On an emotion identification task, adolescents with 22q11DS displayed significant impairment in detecting anger, fear, and disgust in comparison to healthy controls, but their ability to recognize happy, neutral, and surprised facial expressions was preserved (Campbell et al., 2010) Furthermore, a comparison of visual scan-path strategies in 22q11DS youth and healthy controls has shown that, in addition to impaired ability to interpret facial cues, 22q11DS individuals fail to alter scanning strategies when switching from a non-facial identification task to a facial one (McCabe et al., 2011). This study provides further evidence that a characteristic cognitive inflexibility may contribute to some degree to the social cognition deficits observed in this population. Another study examining Theory of Mind (ToM) - which refers to the ability to comprehend the intentions of others (Frith and Corcoran, 1996) - found that 22q11DS individuals exhibit ToM

deficits when compared to individuals with another neurogenetic disorder, Williams Syndrome, indicating that the observed deficits were not attributable to non-specific effects of having a genetic syndrome and/or lower IQ (Campbell et al., 2009). More recently, Campbell et al. (2011) found that, in comparison to typically developing siblings, 22q11DS youth (ages 6e16 years) exhibited significant impairments on both emotion identification and cognitive ToM tasks. Additionally, these authors found that in 22q11DS, performance on ToM tasks corresponded to increasing age, providing evidence that development is a crucial factor to consider when conducting future studies. Furthermore, Campbell et al. (2011) also found that social competence in 22q11DS was significantly related to performance on cognitive ToM tasks, suggesting that social cognitive deficits may be a useful target in developing future interventions to remediate the social dysfunction seen in 22q11DS. 22q11DS adults with a diagnosis of schizophrenia also show impairments in ToM, in comparison to non-psychotic 22q11DS individuals (Chow et al., 2006), suggesting a similar pattern of social cognitive deficit to that observed in idiopathic schizophrenia. Finally, considering that greater social impairment has been shown to contribute uniquely to the prediction of psychosis in clinically at-risk adolescents and young adults (Cannon et al., 2008), social cognitive measures may account for observed variability in psychotic symptoms not captured by traditional neurocognitive measures in both 22q11DS and those with idiopathic schizophrenia.

Although few studies have examined longitudinal changes in cognition over time in 22q11DS, results to date are surprisingly consistent. Gothelf et al. (2007a) first reported in a small, longitudinal study that lower verbal IQ (VIQ) at baseline - and decline in VIQ over a 5-year follow-up period - was associated with psychotic symptom severity at follow-up. Kates et al. (2011) also recently reported that verbal IQ decline, in conjunction with temporal lobe gray

matter loss, uniquely predicted the development of positive prodromal symptoms of psychosis in adolescence, in a larger cohort of 72 22q11DS youth. In line with these findings within the 22q11DS population, a study of non-22q11DS but clinically-ascertained high-risk youth identified verbal memory as a significant predictor of psychosis outcome (Seidman et al., 2010). In summary, these findings suggest a characteristic neurocognitive profile of 22q11DS patients overall, which only partially overlaps with that observed in idiopathic schizophrenia. Importantly however, neurocognitive deficits in the same domains as those observed in idiopathic schizophrenia (i.e., working memory/executive function, and social cognition) appear to be relevant to the development of psychotic symptomatology within 22q11DS patients. Additionally, longitudinal findings indicating cognitive decline over time in specific domains suggest that measures of change over time may have greater predictive accuracy than those obtained at a single time point. These findings are consistent with those of epidemiologic studies of schizophrenia risk in the general population, which have observed that a combination of static and dynamic cognitive deficits across childhood and early adolescence is characteristic of individuals who subsequently develop schizophrenia (Reichenberg et al., 2010).

1.3.a - Structural neuroanatomy and evidence for altered neurodevelopment

Several studies over the past decade have attempted to morphologically characterize neuroanatomic alterations in 22q11DS. Recently, a meta-analysis of 22 human structural neuroimaging studies confirmed and consolidated reports of widespread decreases in brain volume in 22q11DS patients compared to healthy controls, from gross measures of total brain volume and cortical regions within the frontal, parietal, occipital and temporal lobes, to

subcortical structures such as the hippocampus and cerebellum (Tan et al., 2009). Interestingly, this meta-analysis also noted that the magnitude of effect sizes reported tended to increase as one moved from the frontal toward occipital regions of the brain, lending further credence to the theory of a "rostro-caudal gradient" of volume reduction in 22q11DS (Gothelf et al., 2008). Although these finding are intriguing, as they suggest a pattern of developmental disruption along the anterioreposterior axis, most of the studies included in the meta-analysis focused on children, and so the extent to which this pattern continues to be characteristic of adult 22q11DS patients is not clear.

The literature describing the structural correlates of 22q11DS reveals some important areas of overlap with neuroanatomic anomalies observed in idiopathic schizophrenia, suggesting that common cerebral alterations may lead to cognitive dysfunction and psychotic symptom development in 22q11DS patients. In particular, prior studies using both traditional volumetric approaches and voxel-based morphometry show that developmental midline anomalies - frequently reported to be presented at elevated rates in patients with schizophrenia (Kwon et al., 1998; Nopoulos et al., 1997) - are also frequent in 22q11DS, including callosal dysmorphology (Antshel et al., 2005a; Machado et al., 2007; Shashi et al., 2004), cerebellar volume reduction (Bish et al., 2006; Eliez et al., 2001b), and increased prevalence of cavum septum pellucidum (Chow et al., 2002; van Amelsvoort et al., 2001).

Few studies to date have examined neuroanatomic differences between psychotic and non-psychotic individuals with 22q11DS; nevertheless, available evidence suggests that subjects with 22q11DS and psychosis demonstrate morphologic abnormalities similar to those commonly observed in idiopathic schizophrenia, including reduced overall brain volume, particularly white

matter, reduced frontal and temporal gray matter volume, and increased ventricular volume (Chow et al., 1999, 2002; van Amelsvoort et al., 2004).

Although younger children with 22q11DS are unlikely to manifest overt psychotic disorder, quantitative indices of psychopathology may be related to differences in brain development in 22q11DS. Using a continuous measure, the CBCL, Bearden et al. (2004) found that reduced temporal gray matter was associated with severity of Thought Problems in non-psychotic youth with 22q11DS. Consistent with this, Campbell et al. (2006) found that severity of schizotypy score was correlated with gray matter density in temporo-occipital regions and the basal ganglia in children with 22q11DS. This study also found that emotional and social problems were associated with gray matter concentration in fronto-striatal regions.

The cingulate gyrus may be critically involved in both executive dysfunction and the expression of positive symptoms in patients with psychosis. Gray matter deficits in the anterior cingulate gyrus of 22q11DS patients have been observed, and reported to be correlated with poorer executive functioning and increased psychotic symptoms (Dufour et al., 2008). These data are consistent with findings from our group of marked cortical thinning in the anterior cingulate and subgenual prefrontal cortex in 22q11DS patients relative to healthy controls (Devinsky et al., 1995; Drevets et al., 1998; Pardo et al., 1990). Notably, a recent longitudinal study of patients with idiopathic first episode psychosis found both baseline differences and progressive changes over 1.5 years, in which schizophrenia patients showed significantly reduced cingulate gray matter vs. healthy controls, with progressive gray matter loss in the cingulate over time, of greatest magnitude in anterior subregions, including the subgenual cingulate (Koo et al., 2008). Baseline anterior cingulate volume differences also predicted time to psychosis onset in a clinical high-risk sample, independent of clinical symptomatology

(Fornito et al., 2008), suggesting that structural alterations of the cingulate gyrus may be particularly relevant to psychotic symptom development. Moreover, findings of alterations in both shape and volume of the fusiform gyrus, a cortical area known to be essential for facial emotion identification, lend additional support to emerging evidence for social cognition deficits in 22q11DS (Glaser et al., 2007).

Reductions in temporal regions, particularly the hippocampus (Debbane et al., 2006; Eliez et al., 2001a) and superior temporal gyrus (Eliez et al., 2001a) have also been reported in 22q11DS. Given that similar findings of alteration in medial temporal structures have been reported in individuals with schizophrenia (Jacobsen et al., 1998; Narr et al., 2001, 2002; Schreiber et al., 1999) and those at-risk for the disorder (Lawrie et al., 2002; van Erp et al., 2004), medial temporal anomalies may represent a vulnerability marker for psychosis in 22q11DS patients (Kates et al., 2006).

1.3.b - Developmental brain changes in 22q11DS

Normal brain maturation takes place last in the frontal regions during adolescence, and is accompanied by both increased synaptic pruning and myelination, leading to a reduction of gray matter volume and a corresponding increase in white matter (Giedd et al., 1999; Sowell et al., 1999; Sowell et al., 2004; Toga et al., 2006). Cortical thinning - observed via MRI scans – likely represents normal pruning of gray matter neuropil, associated with increased cognitive efficiency with increasing age (Johnson and Munakata, 2005). Frontal brain structures appear relatively preserved in children with 22q11DS (Eliez et al., 2000; Kates et al., 2001; Simon et al., 2005a) but substantially reduced in adulthood (van Amelsvoort et al., 2001), suggesting abnormal pruning and maturational processes. Consistent with this notion, we found differential age-

associated cortical thinning in adolescent 22q11DS patients (Bearden et al., 2009) and, in a longitudinal study, Gothelf et al. (2007a) observed an abnormal developmental trajectory in 22q11DS patients overall, involving greater longitudinal increases in white matter, and volumes of the superior temporal gyrus and caudate nucleus, but a differential decrease in amygdala volume, relative to healthy controls (Bearden et al., 2009; Gothelf et al., 2007c). In a larger longitudinal study Schaer et al. (2009) found that preadolescent 22q11DS patients showed increased prefrontal cortical thickness relative to age-matched typically developing controls. However, over a 3-year follow up period, age-related cortical thinning was more pronounced in 22q11DS patients. This trend of delayed thinning in pre-adolescence, leading to an increased rate of thinning during and after the teenage years, is suggestive of a differential developmental trajectory that may derive from aberrant neuronal migration or disruption in the mechanisms of synaptic pruning. This implicates one or several of the deleted genes (see Fig. 1.1) in such processes, and provides a plausible substrate by which the clinical symptoms of 22q11DS and predisposition to psychosis may arise (Fig. 1.2).

Two recent studies suggest a specific association between volumetric reductions in temporal lobe gray matter and prodromal/psychotic symptoms in 22q11DS (Chowet al., 2011; Kates et al., 2011). In particular, Kates et al. (2011) reported that, while progressive volume loss in multiple brain regions was associated with a general increase in symptom severity over a 3 year follow-up period, only decrements in temporal lobe gray matter and verbal IQ were uniquely predictive of increased severity of positive psychotic-like symptoms. Using a receiver operating characteristic (ROC) analysis to determine the accuracy with which subthreshold

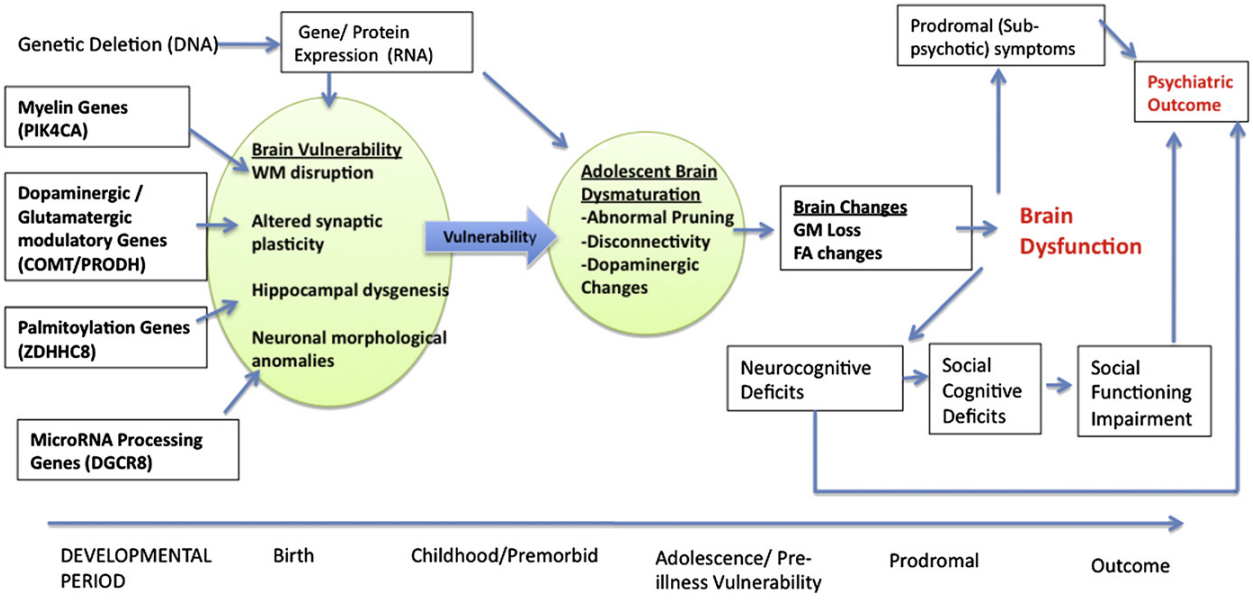


Fig. 1.2. Framework mapping commonly studied measures (boxes) onto the underlying theoretical constructs (shaded circles), with arrows connecting hypothesized genes to relevant neurodevelopmental disturbances. GM = Gray Matter; WM = White Matter; FA = Fractional Anisotropy.

psychotic symptoms could be predicted by neuroanatomic changes, they found that this classification strategy could accurately identify a 22q11DS patient with prodromal psychotic symptoms 86% of the time. This analysis presents a novel approach to looking at predictive accuracy of neuroanatomic markers in the context of this disease model, in which it may be possible to identify larger effects due to reduced heterogeneity (Jalbrzikowski and Bearden, 2011). Further corroborating these findings, Chow et al. (2011) directly compared brain structures of adults with 22q11DS with and without schizophrenia, finding that the expression of schizophrenia in adults with 22q11DS is associated with a selective reduction in gray matter in the superior temporal gyrus (STG; Chow et al., 2011). Taken together, these findings suggest that temporal gray matter loss in adolescence may remain a stable and distinguishing characteristic associated with the expression of psychosis in 22q11DS.These findings are particularly interesting, given their remarkable convergence with the extant literature on youth

with clinical symptoms indicating high risk for developing psychosis (e.g., Pantelis et al., 2003; Takahashi et al., 2009). Findings of selective STG gray matter loss have previously been observed in first-episode schizophrenia (but not in patients with affective psychosis (Kasai et al., 2003)), and volume reduction of these regions, particularly in the left hemisphere, is associated with auditory hallucinations and thought-disorder severity (Shenton et al., 1992). Taken together, these results implicate disruption of temporal regions in disease pathogenesis, and thus may serve as a valuable phenotype for identifying increased psychosis risk in vulnerable individuals.

1.3.c - Structural connectivity and myelination defects in 22q11DS

It has long been proposed that schizophrenia is a disorder of "dysconnectivity," with disruption of white matter integrity affecting developmental processes in the brain (Davis and Haroutunian, 2003). White matter regions of the brain consist of myelin, a sheath that insulates neurons. Because myelination of axons is necessary for efficient transmission of information between brain areas, aberrations in white matter may reflect the absence of - or poorly synchronized - "long distance" connectivity between brain areas Given that the 22q11.2 deletion region includes myelin-related genes (e.g., PIK4CA, RTN4R; Fournier et al., 2001; Jungerius et al., 2008; Vorstman et al., 2009a; Vorstman et al., 2009b;Wang et al., 2002), and white matter has been shown to be disproportionately affected in 22q11DS (Kates et al., 2004, 2001), examining white matter dysconnectivity in 22q11DS may help us better understand how these disturbances contribute to the pathophysiology of schizophrenia.

Diffusion tensor imaging (DTI) is a powerful tool for examining white matter microstructure and coherence in vivo, by measuring the diffusion of water molecules within axons. The degree of fractional anisotropy (FA) in a voxel indicates the directionality and

density of the fiber tracts, and can be viewed as a proxy for white matter or myelin integrity (Basser, 1995) (see Glossary, Table 1.1 and Fig. 1.3). FA is higher in heavily myelinated fiber

Table 1.1 - Glossary of commonly used terms and associated abbreviations referred to throughout this manuscript.

Binding Potential (BP, BP_{ND}) - A commonly reported measure of receptor occupancy from radio-ligand based studies. Assuming the affinity of the radio-ligand for the receptor of interest is significant, a high BP signifies the presence of many unoccupied receptors, while in contrast, a low BP is indicative of less receptor occupancy, ostensibly due to competition at the receptor due to high concentrations of endogenous neurotransmitters.
Catechol-O-methyltransferase (COMT, Comt) - A hemizygously deleted gene within the 1.5 Mb critical region of 22q11DS that codes for a critical enzyme involved in catecholamine breakdown, such as dopamine and norepinephrine. COMT-dependent dopamine degradation is particularly relevant in brain regions with low expression of the presynaptic dopamine transporter (DAT), such as the prefrontal cortex. A common functional polymorphism within this gene, $Val^{158}Met$, results in a roughly four-fold decrease in enzyme activity in the Met variant, and hence, higher extrasynaptic DA levels.
Diffusion Tensor Imaging (DTI) - A magnetic resonance imaging (MRI) technique that maps the passive diffusion of water to quantify white matter microstructure and connectivity across brain regions.
DiGeorge Syndrome Critical Region 8 (DGCR8, Dgcr8) - A gene from the 1.5 Mb critical deleted region of chromosome 22q11.2, DGCR8 encodes a crucial component of a complex involved in miRNA processing, which in turn is responsible for modulating gene expression *in vivo*.
Fractional Anisotropy (FA) - a measure of white matter integrity with values ranging from 0 to 1; a value of zero means that the diffusion is isotropic i.e., that the diffusion occurs in all directions equally and there are no barriers, while barriers to diffusion (e.g., axonal tracts) cause greater diffusion in one direction, resulting in anisotropic diffusion. Higher levels of fractional anisotropy are most often associated with greater the coherence of the white matter tracts.
Magnetic Resonance Imaging (MRI) - A non-invasive neuroimaging modality that enables *in vivo* examinations of brain structure and function in humans and animals.
MicroRNA (miRNA) - a short ribonucleic acid (RNA) molecule found in eukaryotic cells, with a very small number of nucleotides relative to other RNAs. miRNAs are part of the cellular machinery for regulating gene expression/transcription.
Phosphatidylinositol 4-kinase, catalytic, alpha (PIK4CA, PI4KA, pik4ca) - A gene located within the 3 Mb region of 22q11.2, but outside of the 1.5 Mb critical region, that encodes for an enzyme involved in the phosphatidylinositol pathway, and hence, the regulation of intracellular calcium levels, synaptic transmission, exocytosis and vesicle trafficking.
Proline dehydrogenase (PRODH, Prodh) - A hemizygously deleted gene located at cytogenetic band 22q11.21, within the 1.5 Mb critical region, that codes for an enzyme essential for the breakdown of proline, an amino acid that can act as a putative neuromodulator through multiple pathways.
Single Photon Emission Computed Tomography (SPECT) - A versatile nuclear medicine imaging technique that utilizes radioisotopes incorporated into ligands of interest to capture information about the function of the brain. Depending on the choice of radio-ligand, SPECT can map cerebral blood flow, obtain a measure of receptor availability or localize tissues of interest/tumors.
Zinc Finger, DHHC-Type Containing 8 (ZDHHC8) - A palmitoyl-transferase protein produced by a gene from the deleted region, also located at 22q11.21 within the 1.5 Mb critical region.

tracts and increases with progressive myelination during development (Beaulieu, 2002).

Previous research using DTI in 22q11DS suggests that disruption of white matter integrity may be due to reduced FA in widespread brain regions. In a cross sectional study of individuals ranging from 7 to 21 years old (n = 19), Barnea-Goraly (2003) found reductions in the superior longitudinal fasciculus (SLF), which connects the parietal lobe to the frontal lobes, and the inferior longitudinal fasciculus (ILF), which connects the occipital and temporal lobes; however, FA was increased in 22q11DS patients relative to controls in regions spanning the

corpus callosum. Consistent with this, our group also found altered callosal morphology, concomitant with increased FA in midline brain regions in 7-14 year old children with 22q11DS (Simon et al., 2005a). However, in another small sample of 22q11DS youth (n = 11, ages 9-17 years), reduced FA was restricted to many regions of the left hemisphere: the posterior thalamic

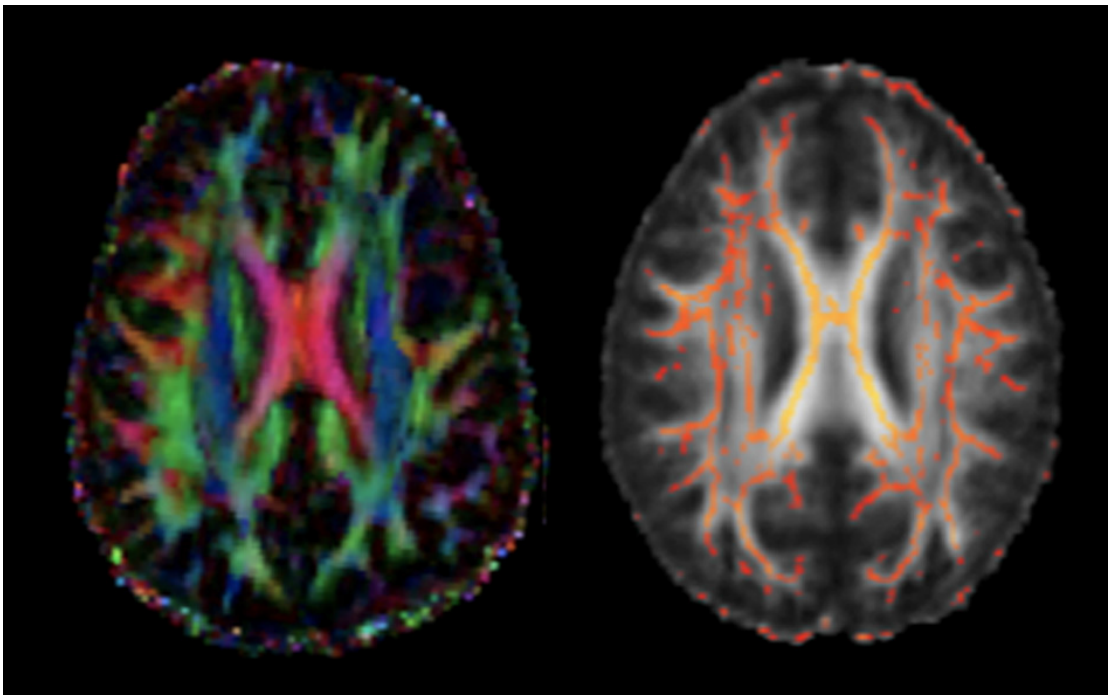


Fig. 1.3. Diffusion tensor imaging (DTI) uses the movement of water to measure white matter tracts in the brain. Left figure displays individual subject data, with color indicating fiber orientation. Right panel is an example of an average fractional anisotropy (FA) image, with tracts common to all participants shown in red and yellow.

radiations, the posterior limb of the internal capsule, the superior region of the corona radiata, and in the arcuate fasciculus, which is often considered to be part of the SLF (Sundram et al., 2010). Others have also found that, in comparison to age-matched healthy controls, adults with 22q11DS had reduced FA in bilateral pre- and post-central brain regions, bilateral parahippocampal regions, and right superior frontal and parietal areas of the brain (da Silva Alves et al., 2011a, 2011b). The variability in DTI findings across studies may be due to the

small sample sizes, differences in age ranges, diverse image processing techniques, and divergent statistical methods. Thus, future studies examining DTI in 22q11DS should examine sub-groups of narrow age ranges, follow longitudinal brain changes over time, and examine the data using multiple processing and statistical methods.

Studies have also identified relationships between FA and cognitive measures or clinical symptomatology in 22q11DS. Barnea-Goraly et al. (2005) found that better arithmetic performance in children and adolescents with 22q11DS was associated with higher FA in parietal areas, brain regions critical for visuospatial cognition and arithmetic function. In 22q11DS youth, increased schizotypy scores, which reflect an increased susceptibility for psychosis, were associated with decreased FA in the internal capsule and corpus callosum (Sundram et al., 2010). More recently, increased psychotic symptom severity as assessed by the Positive and Negative Symptom Scale (PANSS, Kay et al., 1987), was shown to be correlated with reduced FA in multiple frontal, cingulate, and temporal regions in adults with 22q11DS (da Silva Alves et al., 2011a, 2011b). These findings provide preliminary evidence that disruption of white matter integrity may underlie for cognitive deficits and psychotic symptoms in 22q11DS.

Higher FA is typically associated with better cognitive processing in healthy individuals (Grieve et al., 2007). However, the same studies finding widespread FA reductions have also found increased FA in localized regions when comparing individuals with 22q11DS to age matched controls. For example, Barnea-Goraly (2003) found increased FA in 22q11DS youth in tracts connecting the splenium to the occipital lobe. Additionally increased FA relative to controls has been observed in the anterior cingulate, in both children (Simon et al., 2005c) and adults with 22q11DS (da Silva Alves et al., 2011a, 2011b). These findings suggest that aberrant white matter may also be reflected through increased FA and could be due to a variety of factors,

such as reduced dendritic branching, and/or smaller axonal diameter. Neuropathological studies and/or studies in animal models are needed in order to determine the underlying basis of the observed in vivo white matter alterations.

1.3.d - Human neurotransmitter studies

As disruptions in dopamine (DA) neurotransmission have long been reported to be associated with the development of psychiatric disorders (Del Campo et al., 2011; Howes et al., 2012) and genes within the 22q11.2 locus are involved in modulating DA levels (Karayiorgou and Gogos, 2004), there is an intense interest in understanding the contribution of DA dysregulation to the 22q11DS psychiatric phenotype. Yet, few studies to date have directly investigated this link, most of which have assessed the catechol-O-methyltransferase (COMT) gene (see Glossary, Table 1.1) which is hemizygously deleted in patients with 22q11DS. This gene encodes for an enzyme that is responsible for dopamine metabolism, particularly in the frontal cortex (Yavich et al., 2007), has been found to strongly influence the brain and behavior.

A challenge study by Boot et al. (2008) was the first investigation of putative DA-ergic dysfunction in 22q11DS. This study demonstrated that individuals with 22q11DS had higher peripheral DA at baseline and lower concentrations of the primary DA metabolite homovanillic acid (HVA) than controls. Following the DA challenge/ depletion regimen, 22q11DS subjects showed lower urine and plasma HVA levels and a reduced prolactin response, suggesting that affected individuals have higher tonic dopaminergic activity, ostensibly as a consequence of decreased dopamine metabolism due to COMT haploinsufficiency. Moreover, the ratio of DA concentration to HVA concentration was found to be significantly higher in 22q11DS subjects at

both baseline and following the depletion regimen, and as this ratio is inversely related to the rate of DA turnover, it is indicative of impaired DA metabolism in 22q11DS.

To follow up on this intriguing evidence from peripheral and neuroendocrine indices of DA function, Boot et al. next examined central DA disruption in neuroleptic-naïve 22q11DS patients using single photon emission computed tomography (SPECT) (see Glossary, Table 1.1), to compare striatal D2/3 Dopamine Receptor ($D_{2/3}R$) availability, relative to healthy controls. Contrary to their predictions, the authors found no evidence of statistically significant difference in Binding Potential (BP_{ND}) (see Glossary, Table 1.1) between the two groups when utilizing the common SPECT radiotracer [^{123}I]IBZM (Boot et al., 2010). While this would suggest that striatal DA function is not altered in 22q11DS, it is important to keep in mind that striatal $D_{2/3}R$ availability depends not only on endogenous DA levels, but also on receptor affinity, and neuroreceptor density. Accordingly, tonically high DA concentrations and the resultant overstimulation of the receptors may have resulted in the up-regulation of striatal $D_{2/3}R$ expression as a compensatory mechanism, which could conceivably mask differences in BP_{ND} between 22q11DS patients and healthy controls. In addition, prolactin release is typically repressed by DA (Schlegel et al., 1996); this relationship was observed in healthy controls, but was absent in individuals with 22q11DS, suggesting that dopaminergic dysregulation may also exist at another level in 22q11DS, perhaps pre-frontally or within the midbrain (Boot et al., 2010).

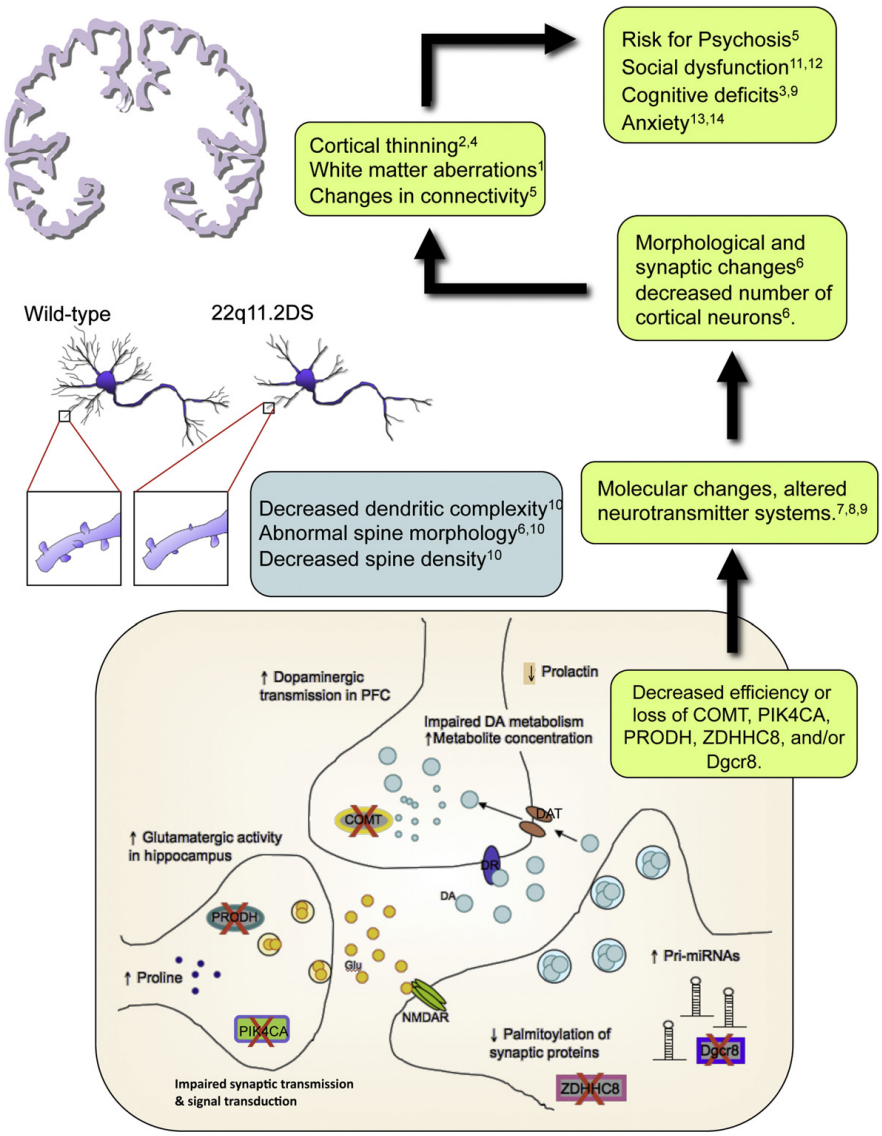
Interestingly, later work by the same group investigating the impact of the COMT Val^{158}Met polymorphism on striatal BP_{ND} in 22q11DS patients only, found evidence of a difference between 22q11DS patients as a function of genotype, with Met hemizygotes exhibiting significantly lower BP_{ND} than Val carriers, and presumably, higher levels of synaptic

DA (Boot et al., 2011). As the major mechanism of synaptic DA clearance within the striatum is ostensibly via the Dopamine Transporter (DAT), rather than degradation by COMT, haploinsufficiency of this particular gene may not play a key role in striatal DA function, except in cases where polymorphisms such as this deleteriously affect the activity of the single remaining allele. Functional variants such as the COMT Val^{158}Met polymorphism within the 22q11.2 locus could further assault the neural architecture necessary for normal structural and functional development, conceivably contributing to variability in dopaminergic neurotransmission, and in turn, the cognitive and neuropsychiatric phenotype. Notably, case reports on the development of early onset Parkinson's Disease (which is characterized by reduced DA in the Substantia Nigra and Striatum) in this population have recently emerged, further highlighting the complex but integral involvement of the DA-ergic system in the phenotype of 22q11DS (Booij et al., 2010; Zaleski et al., 2009) Recent work utilizing Proton Magnetic Resonance Spectroscopy to track metabolite concentrations in the brain has investigated the putative association between glutamatergic dysfunction, the 22q11DS phenotype and risk of psychosis. This study offers new evidence of significant excesses of glutamate, the major excitatory neurotransmitter in the brain, within the hippocampus of 22q11DS patients with schizophrenia, compared to those of healthy controls (da Silva Alves et al., 2011a, 2011b). Moreover, hippocampal glutamate was significantly increased in 22q11DS patients with a diagnosis of schizophrenia compared to 22q11DS patients without schizophrenia, suggesting that glutamatergic dysfunction may play an integral role in the development of psychosis in this population. Given the key involvement of the hippocampus in learning and memory functions, increased hippocampal glutamate may also be related to the increased cognitive impairment observed in 22q11DS patients with schizophrenia. Overall, the picture that emerges from these

radioligand and neurotransmitter-oriented studies is, one of subtly impaired function in the DA-ergic and glutamatergic systems, but the currently available literature is inconclusive and far from complete; further research is clearly warranted to better understand the full extent and mechanisms of this dysfunction.

1.4 - A theoretical model of psychotic symptom development in 22q11DS

Although the mechanisms underlying the development of psychotic symptoms in 22q11DS are not well understood at present, our working hypothesis is that a central component of the neuropathology underlying emergence of these symptoms during adolescence is a process of neuronal volume reduction, resulting in reduced cortical connectivity (Feinberg, 1982; McGlashan and Hoffman, 2000; Selemon and Goldman-Rakic, 1999; Weinberger, 1987). Fig. 1.4 presents a schematic diagram of several known genetic factors that contribute to disturbances in brain function and may lead to behavioral alterations. The basic view reflected in this model is of a life-long biological vulnerability (i.e., reduced synaptic plasticity and connectivity) that results from haploinsufficiency for particular genes that are critical for primary brain development. This, when combined with subsequent alteration of predetermined biological events (i.e., abnormal pruning and increased dopaminergic innervation during adolescence),



References: (1) Barnea-Goraly et al. 2003 (2) Schaer et al. 2009 (3) Bearden et al. 2001 (4) Bearden et al 2009 (5) Gothelf et al. 2008 (6) Fenelon et al. 2011 (7) Boot et al. 2010 (8) Boot et al. 2011 (9) Paterlini et al. 2005 (10) Mukai et al. 2008 (11) Chow et al. 2006 (12) Baker & Skuse 2005 (13) Jolin et al 2009 (14) Gogos et al. 1998

Fig. 1.4 - Schematic depiction of molecular, cellular, anatomical, and behavioral alterations that result from deficiencies in five genes within the 22q11.2 locus: COMT, PRODH, ZDHHC8, DGCR8 and PIK4A. Evidence from mouse models shows that deficient COMT activity results in slower dopamine (DA) metabolism and altered dopaminergic neurotransmission in the prefrontal cortex (PFC) (Gogos et al., 1998; Yavich et al., 2007), which also correlates with decreased prolactin levels in plasma (Boot et al., 2010); reduced Prodh activity leads to proline accumulation and increased glutamatergic neurotransmission in the hippocampus, which can also cause dopaminergic overflow in PFC (Paterlini et al., 2005); deficiency in ZDHHC8 activity decreases palmitoylation of synaptic protein (Mukai et al., 2008), and altered Dgcr8 activity leads to pri-miRNA overexpression (Stark et al., 2008; Fenelon et al., 2011), both affecting the integrity of dendrites and spines. These changes in molecular processes and cell morphology potentially impact brain stability at many levels, resulting in changes in neuronal connectivity and aberrant cortical development (Schaer et al., 2009; Bearden et al., 2007, 2009; Barnea-Goraly, 2003; Gothelf et al., 2007d). This ultimately leads to an array of behavioral phenotypes that increase susceptibility to psychosis (e.g., Gothelf et al., 2007a, 2007b, 2007c; Chow et al., 2006; Baker and Skuse, 2005; Bearden et al., 2005; Paterlini et al., 2005).

leads to a range of measurable changes in brain structure (e.g., reductions in gray matter), endophenotypes (e.g., executive function deficits), clinical symptoms and functional disturbances. A "two hit" theoretical view is proposed, in which the first 'hit' results in reduced neuropil and disrupted white matter integrity during early development (Cannon et al., 2003; Weinberger, 1987), and the second constitutes abnormally aggressive synaptic pruning, possibly associated with dopaminergic changes in adolescence (Feinberg, 1982).

Abnormal synaptic pruning is believed to be relevant to the development of psychotic symptoms (Hoffman and McGlashan, 2001). This is supported by findings of reductions of cortical synaptic density (Selemon and Goldman-Rakic, 1999) and decreases in gray matter in schizophrenia patients (Cannon et al., 1998, 2002, 2003). Computer modeling has demonstrated how abnormally thinned neuronal networks can generate symptoms characteristic of psychosis (Hoffman and McGlashan, 2001; McGlashan and Hoffman, 2000). Although the causes of abnormal pruning are unknown, gene expression studies suggest that the 22q11.2 microdeletion could lead to atypical neural maturation and excessive synaptic pruning (Maynard et al., 2003; McGlashan and Hoffman, 2000). Moreover, heightened dopaminergic neurotransmission during adolescence - likely involving COMT haploinsufficiency, and possibly in combination with other genetic or epigenetic factors - may contribute to this dysmaturational process (Boot et al., 2008).

As indicated by the model, brain dysmaturation, indexed by gray matter loss and white matter disruption, results in brain dysfunction, as assessed by neurocognitive measures. Excessive frontal gray matter loss during adolescence would, in turn, be expected to result in cognitive declines over this time period, especially in executive control, working memory, and attentional functions (Brewer et al., 2006). Deficits in these cognitive domains are also observed in children and adolescents with 22q11DS (Bearden et al., 2001a; Debbane et al., 2006;

Lewandowski et al., 2007; Simon et al., 2005a). Moreover, the severity of abnormalities in these cognitive domains predict conversion to psychosis in youth with clinical symptoms indicating a behaviorally defined psychosis risk syndrome (Pukrop et al., 2006). Previous research also suggests that cognitive deficits contribute to the profoundly impaired social functioning that characterizes psychosis, including during the prodromal phase (Addington et al., 2007; Cornblatt et al., 1992). Impaired social functioning is a robust predictor of subsequent psychosis (Cannon et al., 2008) as well as long term disability and may be mediated by social cognitive deficits such as emotion perception, and theory of mind. Thus, an evolving pattern of brain dysfunction is hypothesized to underlie a range of emergent positive and negative prodromal or psychotic symptoms and functional difficulties in 22q11DS patients (Table 1.2).

1.5 - Mouse models of 22q11DS

Animal models provide an invaluable tool for understanding the pathophysiology of complex neuropsychiatric disorders. Technological advances in the field have provided us with the ability to recapitulate specific aspects of human disease in rodents, mainly by means of targeted genetic modifications, such as introduction of specific mutations or deletions of putatively causal genes. In the case of 22q11DS, a complex disorder with variable phenotypes caused by heterozygous loss of multiple genes, this tool has provided away to dissect several of the specific traits at the single gene level (Karayiorgou and Gogos, 2004). Targeted deletions of single genes that span the syntenic mouse locus have allowed us to determine which specific deficits might potentially arise from deficiencies in their corresponding proteins. There have also been successful attempts to assess the impact of hemizygous deletion of multiple genes within the 1.5Mb critical region in mice, and we attempt to give an overview of the results of these

studies, providing a distinction between any human findings relevant to 22q11DS and to idiopathic schizophrenia wherever appropriate.

Here, we will concentrate on mouse model investigations of several of the individual genes within this critical region for the mouse orthologs of COMT, PRODH, DGCR8, and ZDHHC8, beginning with a discussion of the findings involving a full-microdeletion model, the

Table 1.2 - Summary of selected studies of the prevalence and functional consequences of psychotic symptoms in 22q11DS.

Study	Measure	N	Mean age [range]	Prevalence of psychotic symptoms	Psychotic symptoms associated with.
Feinstein et al., 2002	Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present & Lifetime (KSADS-PL), Diagnostic Interview for Children and Adolescents (DICA-P)	28	12.3 ± 3.9 [6-19]	4 (14.3%) with some evidence of psychotic symptoms (delusions or hallucinations), no individuals met criteria for a full psychotic disorder	
Baker and Skuse, 2005	Child and Adolescent Psychiatric Assessment (CAPA), Junior Schizotypy Schedule	25	16.4 ± 2 [13-25]	21 (84%) reported schizotypal symptoms; 12/25 (48%) reported psychosis-like phenomena	Premorbid adjustment (e.g., lower scores on sociability, peer relations, and interests)
Gothelf et al., 2005a, 2005b	Brief Psychiatric Rating Scale (BPRS)	24	13.3 ± 3.7	Time 1: no participants met criteria for a psychotic disorder Time 2: 7/24 (30%) met criteria for a psychotic disorder vs. 1(4%) of IQ-matched controls	Significant decline in verbal IQ from Time 1 to Time 2, COMT Met genotype, decrease in prefrontal cortex (PFC) volume
Vorstman et al., 2006	KSADS-PL	60	13.4 ± 2.7 [9-20]	16 (27%) reported the presence of hallucinations and/or delusions, 7 (11.7%) met full criteria for a psychotic disorder	Significantly lower IQ score

Campbell et al., 2006	Schizotypy Scale [a]	39	11 ± 3[b]	Significantly higher Schizotypy scores in 22q vs. Sibling controls ($p < 0.01$)	Gray matter volume in temporo-occipital regions and corpus striatum
Chow et al., 2006	SCID-IV	56	27.8 ± 8.8[b]	27/56 (48%) Met criteria for Schizophrenia or Schizoaffective disorder	Poorer performance on neurocognitive tests of motor skills, verbal learning, verbal recognition and social cognition
Debbane et al., 2006	Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) DICA-P, KSADS-PL	43	10.6 ± 11.2 [6-19]	12 (28%) reported the presence of positive symptoms	Decreased verbal IQ, increased social withdrawal, reduced adaptive socialization skills, higher anxiety/depression
Gothelf et al., 2007a, 2007b, 2007c	Brief Psychiatric Rating Scale (BPRS)	28	17 ± 4 [12-24]	Time 1: No participants met criteria for a psychotic disorder Time 2: 7/19 (37%) met criteria for a psychotic disorder	Baseline sub-threshold psychotic symptoms, anxiety/depression, lower verbal IQ and COMT Met genotype associated with psychotic symptom severity at follow-up
Dufour et al., 2008	DICA-P/KSADS-PL SCID-I	42	17 ± 4 [12-24]	24 (57%) had lifetime history of hallucinations or delusions	Reduced gray matter in right cingulate gyrus
Schaer et al., 2009	Not listed	59	15.9 ± 8.9 [6-40]	6/59 (10%) Met criteria for schizophrenia, 24/59 (41%) reported the presence of hallucinations and/or delusions	Cortical thinning in right fusiform/lingual region, and left superior frontal gyrus
Stoddard et al., 2010	Structured Interview of Prodromal Symptoms (SIPS), KSADS-PL	20	15.1 ± 4.3 [12-22]	9/20 (45%) with prodromal or psychotic symptoms (>3 on SIPS)	Lower socioeconomic status
Gothelf et al., 2011	BPRS	19	13.1 ± 3.9 [9-28]	Time 1: no participants had a psychotic disorder	Decrease in gray matter

				Time 2: 6/19 (32%) of participants met full criteria for a psychotic disorder	volume in left dorsolateral prefrontal cortex (dIPFC); whole brain GM reductions and whole brain WM reductions as significant predictors of psychotic symptoms (94.7% accuracy)
Antshel et al., 2010	KSADS-PL, Scale of Prodromal Symptoms (SOPS)	70	11.9 ± 2.2[b]	No participants had a psychotic disorder diagnosis at Time 1 or Time 2. Average SOPS Positive Symptoms at Time 2: 1.3 ± 2.9	Combined information from non-perseverative errors on the Wisconsin Card Soring Task and Atypicality Score on the Behavioral Assessment Scale for Children (BASC) from Time 1 resulted in 79% sensitivity and 95% specificity for positive symptoms at Time 2
Kates et al., 2011	SOPS	72	11.8 ± 2.1[b]	No subjects had a psychotic disorder diagnosis at Time 1 or Time 2. Average SOPS Positive Symptoms at Time 2: 1.2 ± 2.7	Decreases in Verbal IQ and temporal lobe gray matter volume
Chow et al., 2011	Positive and Negative Syndrome Scale (PANSS); SCID-IV	63	30.7 ± 8	29/63 (46%) met criteria for Schizophrenia or Schizoaffective disorder	Reduced gray matter in left superior temporal gyrus

[a] Follow-up study of subjects initially described in Feinstein et al, [b] Dimensional measure of schizotypal traits based on DSM-IV.

DF(16)A+/- mouse model. We will focus on the relevance of each of these models for understanding neurotransmitter system abnormalities, pathophysiological correlates of psychosis, and the biological pathways that increase risk of psychosis.

1.5.a - Full deletion models

Studies in DF(16)A+/- mice, the murine homolog of the 1.5 Mb human deletion, have found compelling evidence for changes in neuronal cell properties that could potentially underlie disease pathology in affected individuals, and have provided us with insights on how clinical manifestations arise. On a broader scale, DF(16)A+/- mice recapitulate several behavioral phenotypes observed in human microdeletion carriers, including deficits in prepulse inhibition

(an index of sensory processing abnormalities), learning, as well as hyperactivity and anxiety (Stark et al., 2008). This model has also demonstrated reduced prefrontal-hippocampal synchrony, analogous to the impaired functional connectivity observed in 22q11DS patients, which goes in hand with deficits in working memory (Sigurdsson et al., 2010).

Furthermore, anatomical and physiological characterization of the DF(16)A knockout identified morphological abnormalities in the dendritic arbors of hippocampal pyramidal cells, including a reduction in the density and size of mushroom spines and an overall "simplification of dendritic complexity/branching", both in vitro and in vivo (Mukai et al., 2008). It is known that changes in cell morphology, dendritic complexity, and spine density have significant effects on the overall properties of neurons and circuits (Calabrese et al., 2006; Henze et al., 1996). The 22q11.2 deletion could thus lead to alterations in excitatory neurotransmission in the hippocampus, which can cause seizures and underlie the range of cognitive defects observed in a subset of schizophrenic and 22q11DS patients (Henze et al., 1996; Raux et al., 2007). In addition, these changes could conceivably prevent proper integration and relay of salient sensory information, reminiscent of the sensorimotor gating deficits observed in individuals with or at risk of psychosis (Braff et al., 1992). A reduction in neuronal complexity could also explain reductions in brain volume of 22q11DS patients (Gothelf et al., 2005b). Moreover, the DF(16)+/- model has demonstrated reduced prefrontal-hippocampal neural synchrony, suggesting impaired functional connectivity between these two regions, which may underlie the working memory deficits observed in 22q11DS (Sigurdsson et al., 2010).

Interestingly, the morphological abnormalities observed in DF(16)A+/- primary cultured neurons were reversed with transfection of ZDHHC8, a gene that is also found in the 22q11.2 critical region (Mukai et al., 2008). Thus, loss of ZDHHC8 may play a significant role in altering

neuronal morphologies that, on a larger scale, affect behavior (see below). Overall, this evidence suggests a potential substrate by which aberrant neural networks in human patients with 22q11DS develop, mainly as a result of altered neural connections that jeopardize the computational efficiency and information processing capacity of the brain.

1.5.b - COMT and dopaminergic dysregulation

One of the more well-researched genes within the 22q11.2 locus is COMT (see Glossary, Tables 1.1 and A1.3). Both human and mouse studies have found that COMT has sexually dimorphic effects on brain function (Lewine et al., 1990; Tunbridge and Harrison, 2010). COMT deficiency is believed to primarily affect behavior via decreased dopamine metabolism, most notably in the frontal cortex, a brain region critical for higher-order cognitive functions, including executive control and emotion regulation (Fig. 1.4) (Gogos et al., 1998).

Studies in COMT-null mice have shown that alterations in COMT-mediated modulation of dopaminergic neurotransmission in this region also lead to increased anxiety and aggressive behaviors (Gogos et al., 1998). Moreover, dopamine metabolite overflow in the PFC has been proposed to exacerbate the onset of psychotic symptoms and schizophrenia in 22q11DS patients, perhaps by making the region less responsive to other forms of neuromodulation and less able to process and filter newly-incoming sensory inputs (Blasi et al., 2010; Yavich et al., 2007).

As previously discussed, the $Val^{158}Met$ polymorphism encodes for a less active COMT enzyme, which results in decreased clearance of dopamine metabolites. This low activity COMT genotype has also been associated with violent behavior in schizophrenia, suggesting that an alteration in the dopaminergic system influences behavior and is a potential therapeutic target (Lachman et al., 1998). Early work in COMT mouse knockouts recapitulated this aggressive

behavior, which also manifested in a sexually dimorphic manner, being more pronounced in males (Gogos et al., 1998). Alternatively, the same study found that COMT also modulates anxiety, most notably in females. This sexual dichotomy in behavior could be due to the influence of sex hormones. Estrogen, for example, decreases COMT activity and may exert protective effects against the development of psychosis in females (Hafner et al., 1993; Harrison and Tunbridge, 2008).

Consistent with human studies Huotari et al. (2004) found that, in the COMT knockout mouse, amphetamine administration increased DA metabolites (but not dopamine) in a way that was inversely proportional to COMT dosage and, again, that this effect was more robust in the cortex, as compared to striatum and hypothalamus. Sexually dimorphic effects were also observed in these mice, as males were more affected than females in terms of increased locomotion after D-amphetamine administration. This is also comparable with sex-specific differences observed in 22q11DS and schizophrenia, as studies have found sex-related differences in behavior and brain anatomy in function of the COMT polymorphism. For instance, Coman et al. (2010) found a gender-specific difference in emotional processing in 22q11DS patients that was influenced by an interaction between sex and COMT genotype, as female carriers of the Val allele and male carriers of the Met allele both showed increased activation of inferior frontal regions when processing pleasant stimuli, whereas Met females and Val males showed increased activation of limbic regions in response to unpleasant stimuli. Thus, this differential regulation of region-specific activity could in turn predispose certain individuals (e.g., male carriers of the Met allele) to develop psychiatric symptoms as a result of decreased frontal regulation of limbic responses (Gothelf et al., 2005a, 2005b).

The COMT allele can also influence brain anatomy in a gender-specific manner, as Kates et al. (2006) found a tendency for hemizygous Met allele females and hemizygous Val allele males to have increased dorsal prefrontal volumes relative to Val hemizygous females and Met hemizygous males, which exhibit larger orbital frontal volumes. Thus, while there is some evidence that COMT genotype can modulate behavior, and brain structure and function in a gender-specific manner, further work is needed to elucidate the precise causal mechanisms underlying these relationships. It is also notable that factors other than sexual dimorphism could affect COMT activity, such as age and developmental stage (for further discussion on this topic see Tunbridge and Harrison, 2010).

The above mentioned studies provide evidence that baseline dopamine metabolism is reduced as a function of COMT gene activity and dosage. Since 22q11DS patients only carry one copy of the gene, this might predict that variation in the remaining allele could predispose them to exacerbated effects of decreased dopamine metabolism and advance the onset of psychosis. Accordingly, studies on 22q11.2 microdeletion carriers that contain the low activity COMT allele have shown that these subjects exhibit a higher predisposition for developing psychotic symptoms, along with a decrease in prefrontal cortical volume and Verbal IQ and worsening clinical symptomatology, as measured by the Brief Psychiatric Rating Scale (BPRS) (Gothelf et al., 2005a).

A possible mechanism by which this might occur comes from the work of Yavich et al. (2007), who demonstrated that dopamine is removed two-fold slower in the prefrontal cortex of COMT-deficient mice (as compared to wild-type controls), suggesting a dopamine overflow in this region (Yavich et al., 2007). Such "overflow" could cause the region to become less responsive to alternating neuromodulation and lead to difficulties processing and filtering newly-

incoming sensory information. This could consequently lead to psychotic symptoms and thought disorder.

1.5.c - PRODH and the glutamate-dopamine theory of psychosis

The PRODH gene, also found within the 22q11.2 critical region, encodes for the proline dehydrogenase enzyme, which is involved in the degradation of proline, an agonist of glutamatergic receptors and potentiator of excitatory neurotransmission (Wang and Brandriss, 1987; Henzi et al., 1992; Cohen and Nadler, 1997). Research on murine knockouts of Prodh has shown that these mice have deficits in sensorimotor gating, as compared to wild type animals (Gogos et al., 1999). In addition, Prodh null mutant mice also presented decreased biosynthesis of glutamate, GABA, and aspartate, and these effects were more pronounced in the frontal cortex (Table A1.3, Fig. 1.4) (Gogos et al., 1999). These studies suggested that increased proline levels, resulting from decreased proline metabolism, can adversely impact tonic neurotransmitter concentrations and may have a bearing on epilepsy, mental retardation, and psychosis, perhaps by adversely modifying neural connections and excitatory neuronal activity (Raux et al., 2007).

Along these lines, a considerable proportion of both patients with 22q11DS and idiopathic schizophrenia are hyperprolinemic (Goodman et al., 2000; Jacquet et al., 2005, 2002). It is thus possible that altered proline metabolism, resulting from aberrant PRODH activity, can change brain physiology and function, with detrimental effects on behavior. For example, patients with 22q11DS show deficits in cognitive functions that rely on frontal cortical function, such as working memory and emotion regulation (Kiley-Brabeck and Sobin, 2006). Further evidence supporting the involvement of PRODH in idiopathic psychosis comes from human linkage disequilibrium studies in family-based samples, which have identified a schizophrenia

susceptibility locus in the PRODH/Dgcr6 region (Liu et al., 2002b). Further, the observation association was stronger among those with an early onset of psychosis, suggesting that PRODH may be particularly relevant to early-onset forms of the illness. This, taken together with the fact that proline metabolism disruption is more pronounced in regions within or projecting to frontal cortex, suggests a mechanism for psychosis predisposition and/or precipitation. This has led some investigators to speculate that promoting a low-proline diet for at-risk individuals, in order to reduce overall proline levels and protect against the putative neurotransmitter dysfunction intrinsic to the syndrome, may be a potential approach for reducing psychosis risk (Jaksic et al., 1990).

Another interesting perspective on mechanisms involved in the onset of psychosis derives from animal models, which indicate that excess striatal dopaminergic activity may be driven by dysfunctional glutamatergic transmission in the hippocampus (Lisman et al., 2008). This relationship has led to the glutamate-dopamine theory of psychosis, which is supported by evidence that the relationship between hippocampal glutamate and striatal dopamine systems is disrupted in individuals at clinical high risk for psychosis, and that the degree of disruption is related to increased risk of conversion to overt psychosis (Stone et al., 2010). In individuals with 22q11DS, Raux et al. (2007) showed that cognitive performance was inversely correlated with plasma proline levels; further, hyperprolinemic 22q11DS patients carrying the COMT Met (low-activity) allele had a 2.8-fold increased risk for psychosis. Interestingly, it is believed that decreased inhibition in the hippocampus, perhaps due to NMDA receptor hypo-function, may result in increased glutamatergic inputs onto the striatum and an increase in limbic dopaminergic neurotransmission, which can then precipitate psychotic symptoms (Stone et al., 2010).

1.5.d - Evidence for epistatic interactions: COMT and PRODH

As mentioned above, hyperprolinemic 22q11DS patients are at increased risk for psychosis if they carry the Met allele of the COMT gene (Raux et al., 2007). Evidence from gene expression profiling of brain tissue taken from PRODH knockout (KO) mice indicates that increased proline levels induce COMT overexpression in the frontal cortex, perhaps as a feedback mechanism to increase dopaminergic transmission (Paterlini et al., 2005). The fact that 22q11.2 microdeletion carriers lack one copy of both PRODH and COMT suggests that they are unable to compensate for loss of PRODH (and the subsequent increase in proline levels) by means of COMT up-regulation. In fact, it has been shown that 22q11DS patients with the Met allele are more likely to have elevated serum proline levels and perform poorly on smooth pursuit eye movement (SPEM) tasks (Vorstman et al., 2009a; Vorstman et al., 2009b).

PRODH KO mice are also less sensitive to NMDA blockade, perhaps due to higher tonic glutamate concentration or because of congenital desensitization (Paterlini et al., 2005). In addition, they exhibit learning deficits, and are more sensitive to amphetamines, which is also the case for patients with schizophrenia (Breier et al., 1997). These features should be taken into consideration when therapeutically targeting specific neurotransmitter systems. Thus, it is possible that the PRODH-COMT interaction modulates the penetrance of psychiatric features in 22q11DS patients.

1.5.e - ZDHHC8-role in neural cell morphology and synaptic transmission

The zinc finger domain-containing protein (ZDHHC8) gene putatively encodes for a palmitoyltransferase enzyme, which is highly expressed in the brain and is responsible for palmotylation of proteins (see Glossary, Table 1.1). It has been shown to play an important role

in regulating nervous system development, dendritic morphology, spine density, synaptic proteins, and glutamatergic neurotransmission (el-Husseini Ael and Bredt, 2002). Previous associations have been reported between with ZDHHC8-truncating variants and schizophrenia in the general population (Liu et al., 2002a).

Notably, both Zdhhc8+/- and Zdhhc8-/- mice reproduce aberrant neural cell morphology and synaptic abnormalities observed in DF(16)A+/- mice, as well as many of the behavioral phenotypes observed in 22q11DS patients (Mukai et al., 2008). In particular, these mice show a decreased density of dendritic spines and glutamatergic synapses in primary hippocampal neurons, as well as impaired dendritic growth (Fig. 1.4). These deficits were reversed by re-introduction of enzymatically active ZDHHC8 protein, a putative palmitoyltransferase encoded by a gene in the 22q11.2 locus, and were also observed in primary cultures from Zdhhc8-deficient mice. Also, a detailed assessment of the molecular effect of ZDHHC8 deficiency found that it causes a reduction of PSD95 staining, an important synaptic protein that modulates spine and dendrite morphology. These structural and functional changes represent possible predisposing factors to the psychiatric and cognitive symptoms associated with the 22q11.2 microdeletion, and further suggest that impaired neuronal protein palmitoylation may contribute to these deficits.

1.5.f - Other models of interest

Recent findings have generated intense interest on the role of molecules that regulate gene expression changes in the nervous system and their role on disease. Such is the role of microRNAs (miRNAs), non-coding RNA segments that are about 22 bp in length, that bind to

untranslated mRNA transcripts and play a role in inhibiting or promoting their expression (Ambros, 2004) (see Glossary, Table 1.1). Work done by Stark et al. (2008) on the full-length knockout of the mouse "critical region," (Df(16)A+/- mice), revealed that these animals have upregulated miRNA expression in the brain. Further sequence characterization of these molecules confirmed that, in fact, many of these miRNAs were in fact pri-miRNAs, a premature and - to some extent - less active form of miRNAs (Stark et al., 2008). Not surprisingly, Dgcr8, a gene that is also knocked out in the critical region, encodes for a miRNA-processing molecule (see Glossary, Table 1.1, Fig. 1.4).

Characterization of the Dgcr8 +/- mouse shows that haploinsufficiency of this sole gene leads to altered miRNA expression, just as in the full-length knockout. In addition, this model recapitulates some of the behavioral deficits observed in Df(16)A+/-, such as decreased dendritic complexity, which might influence functional connectivity, and could play a role in the emergence of restrictive/repetitive behavior and reported deficits in PPI. Another study showed that the knockout has a decreased number of cortical neurons along with white matter abnormalities, and that the animals exhibit a deficit in synaptic potentiation and short-term plasticity (Fenelon et al., 2011), which could explain the deficits in learning and memory observed in 22q11DS individuals.

These findings have opened a new window into the understanding of gene dynamics and their role in modulating psychiatric disorders. Recent work by Moreau et al. has showed that there is indeed a dysregulation of miRNAs in post-mortem brain tissue of individuals with a diagnosis of schizophrenia, as well as those with bipolar disorder (Moreau et al., 2011). It would be interesting to assess miRNA expression changes in 22q11DS patients, which may be related to increased risk for psychosis. Any interruption or change in the orchestration of gene

expression, such as that mediated by miRNAs, could potentially lead to altered cellular function, aberrant network properties, and changes in systemic functions, which could also contribute to the phenotypic variability of psychiatric disease. Future research should be driven toward understanding these changes, in conjunction with their effect on dynamics of neurotransmission, brain function, and behavior.

1.6 - Future directions and conclusions

A consistent picture is emerging regarding the neurobehavioral phenotype of the 22q11.2 Deletion Syndrome. Nevertheless, much work remains to be done to fill the gaps in knowledge regarding the functional correlates and the precise mechanisms by which each of the deleted genes contributes to the overall 22q11DS phenotype. Future research should endeavor to bridge these existing gaps with multidisciplinary and cutting edge approaches to the disease, from the molecular level up to the functional. The development of conditional knockout mouse models, in which the investigators can control the precise timing and/or cell type for which a gene of interest is silenced, offers great potential as a technique for elucidating the molecular underpinnings of 22q11DS-associated psychosis. Additionally, the integrity of neuronal network and microcircuit activity can be further assessed via 2-photon microscopy; alterations in brain synchronization have been revealed using these methods in mouse models of psychiatric disorders (Penagarikano et al., 2011). Future radioligand-based studies utilizing techniques like PET and SPECT, should endeavor to explore dysfunction in the Glutamatergic and GABA-ergic systems, in addition to DA-ergic neurotransmission, in both animal models and human patients with 22q11DS. Functional MRI data, obtained both during and in the absence of an overt cognitive task (i.e., Resting State fMRI) will prove essential in unraveling the functional

consequences of the 22q11DS-specific structural and neurofunctional abnormalities, and will create the foundation for future work on brain-behavior relationships in 22q11DS. In addition, there is a clear need for large-scale, prospective longitudinal studies, paralleling those of behaviorally defined clinical high risk studies (Cannon et al., 2008; Seidman et al., 2010), in order to determine neurobiological and clinical risk factors for psychosis in the context of this syndrome. This is the first step in developing targeted interventions that can be applied early in the course of illness, leading to exponentially improved outcomes (McGorry et al., 2002). Eventually, through rigorous investigation and informed experiments, research on 22q11DS can serve as a model for how a well-characterized genetic anomaly can lead to a cascade of abnormal neurodevelopmental processes, which disrupt brain structure and function, and manifest as early disturbances of emotion, cognition, and behavior. Collectively, the translational evidence reviewed here offers a powerful example of how this line of inquiry can provide clues into the biological mechanisms underlying development of psychotic illness in the broader population.

In light of the current dearth of literature on resting state fMRI in 22q11DS, I have chosen to focus my dissertation project on the alterations to these individuals' intrinsic connectivity networks, with an emphasis on characterizing the network dysconnectivity that is most associated with risk for developing schizophrenia.

CHAPTER 2:

Default Mode Network connectivity and reciprocal social behavior in 22q11DS

2.1 - Abstract

22q11.2 deletion syndrome (22q11DS) is a genetic mutation associated with disorders of cortical connectivity and social dysfunction. However, little is known about the functional connectivity (FC) of the resting brain in 22q11DS and its relationship with social behavior. A seed-based analysis of resting-state functional magnetic resonance imaging data was used to investigate FC associated with the posterior cingulate cortex (PCC), in 26 youth with 22q11DS and 51 demographically matched controls. Subsequently, the relationship between PCC connectivity and Social Responsiveness Scale (SRS) scores was examined in 22q11DS participants. Relative to 22q11DS participants, controls showed significantly stronger FC between the PCC and other default mode network (DMN) nodes, including the precuneus, precentral gyrus and left frontal pole. 22q11DS patients did not show age-associated FC changes observed in typically developing controls. Increased connectivity between PCC, medial prefrontal regions and the anterior cingulate cortex, was associated with lower SRS scores (i.e. improved social competence) in 22q11DS. DMN integrity may play a key role in social information processing. We observed disrupted DMN connectivity in 22q11DS, paralleling reports from idiopathic autism and schizophrenia. Increased strength of long-range DMN connectivity was associated with improved social functioning in 22q11DS. These findings support a 'developmental-disconnection' hypothesis of symptom development in this disorder.

2.2 – Introduction

Neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia are increasingly conceptualized as disorders of cortical connectivity, and current evidence suggests that both of these conditions involve inappropriate circuit formation due to aberrant

neurodevelopment (Insel, 2010; Meechan et al., 2012). 22q11.2 deletion syndrome (velocardiofacial/DiGeorge syndrome; 22q11DS) is a genetic disorder that represents one of the most significant genetic risk factors known for the development of these 'connectopathies' (Karayiorgou et al., 2010). This microdeletion afflicts about 1 in 4000 live births, and is estimated to account for 1–2% of schizophrenia cases, representing the only known recurrent copy number mutation responsible for introducing new cases of schizophrenia into the population (Karayiorgou et al., 2010). Furthermore, the prevalence of ASD in children with 22q11DS ranges from 24% to 50%, indicating that disorders associated with social behavioral dysfunction are a highly penetrant aspect of the 22q11DS phenotype. The deletion encompasses between 1.5 and 3Mb, encoding 30–60 known genes. Phenotypic consequences of the deletion are variable, ranging from cardiac defects and immunodeficiency to language delays and cognitive impairment (Drew et al., 2011). At present, the genetic and neurobiological mechanisms accounting for elevated psychiatric risk in 22q11DS have yet to be fully elucidated. Despite existing evidence for social and neurocognitive dysfunction in 22q11DS (Jalbrzikowski et al., 2012) and the plausibility of aberrant intrinsic brain connectivity as an integral factor in these aspects of the phenotype, direct assays of the impact of the deletion on brain function in humans have only recently been initiated (Gothelf et al., 2007; Debbane´ et al., 2012).

Since the first reports of synchronized functional correlations in low frequency blood oxygen level dependent (BOLD) signal within the motor system at rest (Biswal et al., 1997), interest in the potential of resting-state functional magnetic resonance imaging (rs-fMRI) to characterize the brain's intrinsic functional architecture has grown exponentially. Functional connectivity (FC) mapping approaches have led to the discovery of several putative resting state

networks (RSNs), which have been shown to be robust and reproducible across participants and time (Damoiseaux et al., 2006; De Luca et al., 2006; Kalcher et al., 2012). The best characterized of these is the default mode network (DMN), a collection of spatially distinct regions spanning the medial prefrontal, lateral parietal and posterior cingulate cortices (PCCs), that is more active in the absence of an overt cognitive task and is implicated in social cognition, mind wandering and self-referential thought (Raichle et al., 2001; Greicius et al., 2003; Rosazza et al., 2011). Aberrant connectivity within the DMN has been implicated in a number of neuropsychiatric disorders, and accordingly, the dynamics of this RSN and the degree of network dysfunction may serve as a valuable biomarker for nascent psychiatric disorders in at-risk individuals (Broyd et al., 2009; Soddu et al., 2011; Whitfield-Gabrieli and Ford, 2012). As such, mapping the functional architecture of the brain in 22q11DS may help to elucidate gene–brain–behavior relationships. We hypothesized that individuals with 22q11DS would show reduced intra-network FC between the major hub regions of the DMN, the PCC and ventromedial prefrontal cortex (Uddin et al., 2009), in accordance with existing evidence for DMN dysfunction in both idiopathic schizophrenia and ASD (Broyd et al., 2009; Assaf et al., 2010; Rudie et al., 2012). Secondly, given known developmental shifts in patterns of functional brain connectivity (Uddin et al., 2010), we investigated age effects on DMN connectivity, with the hypothesis that 22q11DS patients would fail to show the typical developmental pattern of 'local to distributed' organization with increasing age (Fair et al., 2009). Finally, as social impairment is a fundamental aspect of both idiopathic schizophrenia and ASD, we investigated whether abnormalities in DMN connectivity were associated with impairment in reciprocal social behavior in 22q11DS. Given the DMN's critical role in social information processing, we

hypothesized that increased connectivity within DMN regions would be associated with better social competence.

2.3 - Methods

2.3.a - Participants

The total (initial) sample consisting of 87 participants aged 6–28 years (31 patients with a molecularly confirmed diagnosis of a 22q11.2 microdeletion and 56 age- and sex-matched typically developing controls) was recruited from an ongoing longitudinal study at the University of California, Los Angeles. Exclusion criteria for all study participants were: additional neurological or medical condition that might affect imaging measures, insufficient fluency in English, endorsement of substance or alcohol abuse and/or dependence within the past 6 months and any condition that is a contraindication for MRI (pregnancy, claustrophobia, etc.). Healthy controls additionally did not meet criteria for any major mental disorder, based on information gathered during administration of the Structured Clinical Interview for DSM-IV Axis I Disorders [SCID; (First et al., 1996)], with an additional developmental disorders module, as applied by Addington et al. (2012) (for participants over the age of 16 years) and/or the Computerized Diagnostic Interview Schedule for Children [C-DISC; (Jensen et al., 1995)] for participants aged <16 years. Diagnoses of autism spectrum disorder were determined using the Autism Diagnostic Observation Schedule (Lord et al., 2000) administered to the child and the Autism Diagnostic Interview-Revised (Lord et al., 1994), administered to the subject's parent/primary caretaker. All clinical interviews were conducted by highly trained MA- or PhD-level psychologists; inter-rater reliability and case consensus procedures have been described in detail elsewhere (Meyer et al., 2005; Ho et al., 2012). All participants and/or their parents underwent a verbal and written informed consent process after complete description of the study. The UCLA Institutional

Review Board approved all study protocols. Table 2.1 provides demographic information for all participants included in our group-level fMRI analyses, following exclusion of subjects with excess motion (see below) during their scan.

2.3.b - Neurobehavioral measures

Estimates of general intellectual functioning were obtained for all participants from the two-subtest (vocabulary and matrix reasoning) version of the Wechsler Abbreviated Scale of Intelligence (Lord et al., 1994). Parents of study participants completed the Social Responsiveness Scale (SRS; Meyer et al., 2005), a quantitative measure of reciprocal social behavior that has been extensively validated in both clinically ascertained and population-based samples. The measure represents the three criterion domains for autism and correlates strongly with a gold standard diagnosis of ASD based on the Autism Diagnostic Interview (Constantino et al., 2003).

2.3.c - fMRI data acquisition

Structural and functional scans were acquired at either the Ahmanson–Lovelace Brain Mapping Center (BMC) or the Staglin Center for Cognitive Neuroscience (CCN) in Los Angeles, CA, USA. Both sites had an identical three Tesla Siemens Tim Trio system, utilizing a 12 channel head coil. The primary structural scan used for registration purposes consisted of a matched-bandwidth high-resolution T1 image (voxel size 1.5x1.5x4.0 mm, echo time (TE)=34 ms, repetition time (TR)=5000 ms, echo spacing=0.89 ms, 34 axial slices, slice thickness 4.0 mm, slice spacing 0 mm, flip angle 90º, field of view (FOV)=210, matrix size=128x128). Subsequently, a 5 min resting state functional scan was acquired, during which a black screen

Table 2.1 - Subject Demographics [a]

	22q11DS (N=26)	Controls (N=51)	p-value
Age [mean ± s.d. (range)]	15.9 ± 4.9 (8–26)	14.4 ± 6.4 (6–28)	0.273
Gender, N male (%)	17 (65)	28 (55)	0.384
Full Scale IQ (mean ± s.d.)	78.6 ± 15.8	114.1 ± 20.8	<0.0001
SRS t–score[b] (mean ± s.d.)	70.8 ± 16.7	47.5 ± 10.4	<0.0001
Autism spectrum disorder diagnosis, n (%)	10 (38)	0 (0)	<0.0001
Psychotic disorder diagnosis, n (%)	2 (8)	0 (0)	0.0454
ADHD diagnosis, n (%)	14 (54)	0 (0)	<0.0001
ODD diagnosis, n (%)	1 (4)	0 (0)	0.1628
Anxiety disorder diagnosis, n (%)	7 (27)	0 (0)	<0.0001
Mood disorder diagnosis, n (%)	5 (19)	0 (0)	0.001
Current antipsychotic treatment, n (%)	3 (12)	0 (0)	0.013
Current antidepressant treatment, n (%)	8 (31)	2 (4)	0.0007
Current psychostimulant treatment, n (%)	2 (8)	0 (0)	0.0454
Scanner Site[c]	16 (62) BMC 10 (38) CCN	31 (61) BMC 20 (39) CCN	0.9497

[a]Five 22q11DS patients and five controls were excluded (from initial sample) due to excessive motion; [b]SRS data were not available for 16 controls; [c]no significant difference between distribution of medicated subjects between the two scanner sites. ADHD, attention deficit hyperactivity disorder ODD, oppositional defiant disorder

was presented and participants were instructed to keep their eyes open, remain relaxed and attempt to avoid falling asleep. The resting state scan consisted of 152 BOLD 3D images (voxel size 3.0x3.0x4.0 mm, TE=30 ms, TR=2000 ms, echo spacing=0.79 ms, 34 axial slices, slice thickness 4.0 mm, slice spacing 0 mm, flip angle 90º, FOV=192, matrix size=64x64).

2.3.d - fMRI data pre-processing

All data were pre-processed and analyzed with tools from the FMRIB Software Library (FSL; http://fsl.fmrib.ox.ac.uk/fsl/). Scans were obtained with an interleaved slice acquisition sequence. Each subject's full functional scan was motion-corrected by registering each image to the middle volume as a reference, using FMRIB's linear image registration tool. Any subject with >2mm of translational motion or 28º of rotational motion was excluded from further analysis (controls=5; 22q11DS=5), resulting in 51 controls and 26 22q11DS patients with useable data. For the remaining subjects, there were no significant differences in rotational or translational motion between the two groups ($p>0.05$). The 4D functional images were skull stripped using FSL's Brain Extraction Tool and spatially smoothed with a 5mm full width half maximum isotropic Gaussian kernel and bandpass temporal filtering ($0.005 \text{ Hz} < f < 0.1 \text{ Hz}$). An initial first level analysis was run in FMRI Expert Analysis Tool (FEAT), modeling the global signal and the six motion parameters, and the data were registered to the matched-bandwidth high-resolution T1 and then to Montreal Neurological Institute (MNI-152) space. Next, the residuals from this analysis were normalized to prepare for extraction of the time series, resulting in a mean of 0 and standard deviation of 1. A 6mm region of interest (ROI) was placed in the posterior cingulate (PCC: MNI coordinates: 0, -52, 30), following previously published work (De Luca et al., 2006; Uddin et al., 2009). These ROIs were registered to each subject's functional dataset (utilizing the transformation matrix derived from the earlier subject-to-standard space registration) and the mean time-series within the registered ROI was extracted from the original residuals file. For the primary connectivity analysis, the average time course of the PCC-seed was normalized and was entered into FEAT as an explanatory variable for the scaled residuals generated following pre-processing. Finally, the statistical contrast output from

FEAT for each subject was normalized via Fisher's z-transformation and used as an input to the group-level analyses (described below). Additionally, we used statistical maps generated from the PCC ROI and extracted an index of the correlation of the PCC with the ventromedial prefrontal cortex (vmPFC; 6mm diameter sphere centered at MNI coordinates: 4, 56, -12) to obtain a metric of PCC-to-vmPFC connectivity at rest and quantify the degree of correlation within the DMN between these two hub regions.

2.3.e - Group-level analyses

In order to rule out potential scanner-related differences, we checked for differences between scanners in each group, then for interactions between scanner and any covariates of interest in each group, for our two group-level analyses. Once it was determined that there were no differences between scanners, nor were there significant interactions between scanner and our model parameters, in any ROI (all $p > 0.05$), all subsequent group-level analyses were conducted with scanner location included in the model as a covariate. In order to investigate group-level effects on DMN connectivity, outputs from the participant-level analysis for each subject were entered into an ordinary least squares analysis. First, a between-groups comparison was conducted to investigate differences in whole-brain PCC-derived functional connectivity between 22q11DS patients vs controls (controlling for age, gender and scanner location); secondly, an interaction term (Age_x_Diagnosis) was added to the group comparison model, in order to explore differential effects of age on DMN connectivity between the two groups. Two-tailed t-tests were used to compare the strength of the z-transformed Pearson's correlation between the PCC seed and vmPFC seed between groups. Finally, a regression analysis within the 22q11DS group was conducted to investigate the association between SRS t-scores and PCC-

derived FC (controlling for age, gender and scanner location). In all analyses, covariates (age, gender and SRS t-score) were demeaned. Cluster correction for multiple comparisons was carried out using Gaussian random field theory (min $z > 2.3$; cluster significance: $P < 0.05$, corrected).

2.4 – Results

2.4.a - Group differences in PCC connectivity

In both healthy controls and 22q11DS participants, regions in which spontaneous BOLD fluctuations were significantly correlated with the PCC include the precuneus, the vmPFC and portions of inferior/lateral parietal cortex; areas classically considered part of the DMN (Figure 2.1a). However, group comparisons revealed significant differences in both the spatial extent and magnitude of DMN connectivity. Controls showed significantly stronger FC between the PCC and other DMN regions, including the precuneus, the left and right precentral gyrus, the left frontal pole and left lateral occipito-parietal regions (Figure 2.1b). In contrast, 22q11DS participants displayed a more diffuse pattern of FC with the PCC (Figure 2.1b), involving significantly stronger connectivity with the right inferior frontal gyrus (IFG). Information on all cluster locations, sizes and significance can be found in Table 2.2.

2.4.b - Functional connectivity between DMN hubs

The strength of correlation between seed regions in the PCC and vmPFC was significantly greater for controls than for 22q11DS participants ($p=0.0358$), providing further evidence of diminished within-network connectivity in 22q11DS.

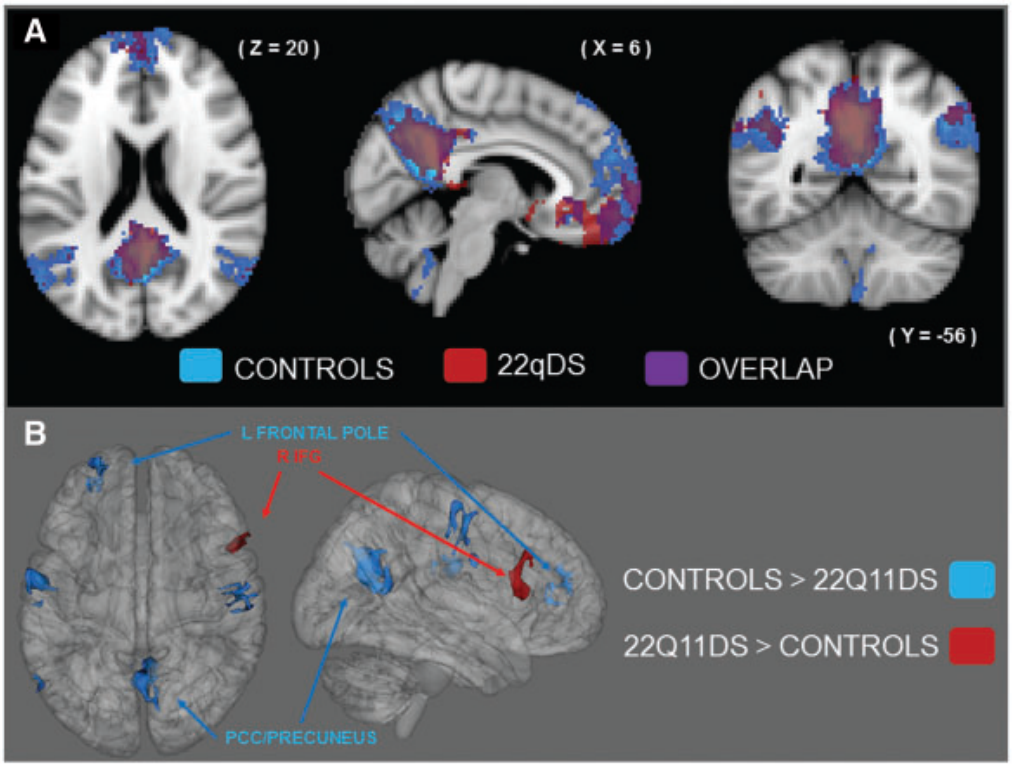


Fig. 2.1 - PCC functional connectivity in 22q11DS patients and healthy controls. Top panel (2.1a) depicts within-group functional connectivity. Red colors indicate regions of significant functional connectivity to the PCC in patients with 22q11DS, while blue colors indicate regions of significant functional connectivity to the PCC in healthy controls and purple indicates areas of overlap across groups. As seen here, both healthy controls and 22q11DS patients exhibit functional connectivity to areas classically considered part of the DMN, though the strength of the DMN network in 22q11DS patients, especially along long-distance connections, appears less robust. The bottom panel (2.1b) depicts significant group differences in PCC connectivity between 22q11DS patients vs controls. As depicted here, controls showed significantly stronger functional connectivity between the PCC and other DMN regions, including the precuneus, the left and right precentral gyrus, the left frontal pole and left lateral occipito-parietal regions. In contrast, 22q11DS patients displayed a different pattern of functional connectivity within the DMN, involving significantly stronger correlations between the PCC and right inferior frontal gyrus.

2.4.c - Age effects

22q11DS participants showed a distinct pattern of age-associated changes in FC with the PCC, relative to controls (Figure 2.2a). Group contrasts show that compared to 22q11DS participants, controls exhibited significantly increased connectivity between the PCC seed and right middle temporo-occipital cortex with increasing age, relative to 22q11DS participants (Table 2.2; Figure 2.2b). In contrast, 22q11DS participants show increased connectivity between

the PCC seed, the right temporal pole/parahippocampal gyrus and inferior portions of the right lateral frontal cortex, with increasing age (Table 2.2; Figure 2.2b).

Table 2.2 - Significant Cluster Locations from Group Analyses

Contrast	Cluster Index	Cluster Size (voxels)	X	Y	Z	p-value	Anatomical Region
CONT > 22q11DS (Figure 2.1b)	1	432	16	-48	16	2.56E-06	Precuneus
	2	349	44	-12	48	3.22E-05	Right precentral gyrus
	3	284	-58	-6	28	2.69E-04	Left precentral gyrus
	4	227	-22	48	6	0.00198	Left frontal pole
	5	167	-54	-64	20	0.0195	Left lateral occipitoparietal regions
22q11DS > CONT (Figure 2.1b)	1	174	54	18	10	0.0147	Right inferior frontal gyrus
CONT Age > 22q11DS age (Figure 2.2b)	1	189	66	-48	-4	0.00837	Right middle temperoccipital cortex
22q11DS Age > CONT age (Figure 2.2b)	1	284	24	6	-24	2.76E-04	Right temporal pole/parahippocampal gyrus
	2	163	34	32	-12	0.00233	Right lateral frontal cortex
22q11DS participants only, SRS (Figure 2.3)	1	405	10	40	-4	1.19E-07	Anterior cingulate/paracingulate gyrus
	2	277	26	62	-2	2.03E-05	Right lateral frontal cortex
	3	250	12	66	24	6.38E-05	Right medial frontal cortex
	4	179	-12	72	1	0.00162	Left medial frontal pole
CONT > 22q11DS (med exclusion)	1	498	0	-60	32	5.96E-08	Precuneus
	2	462	-22	48	12	2.38E-07	Frontal pole

2.4.d - Association with social behavior in 22q11DS

Cortical areas with significant connectivity to the PCC that were also significantly associated with lower SRS scores (i.e. better social competence) were predominantly constrained

to the right frontal cortex, including lateral and medial regions as well as portions of the anterior cingulate cortex (ACC) and paracingulate gyrus and left medial frontal pole (Table 2.2; Figure 2.3).

2.4.e - Medication effects

In order to account for any potentially confounding influence of psychotropic medications on our findings, the analyses described above were re-run excluding any subjects currently taking antipsychotic, psychostimulant or antidepressant medications (11 patients, 2 controls). The observed group differences remained significant, with controls displaying significantly greater FC than 22q11DS participants between the PCC seed and the frontal pole. Correlations between the PCC and vmPFC seed regions remained significantly stronger for controls relative to 22q11DS participants ($p=0.0132$). Regarding age effects, controls continued to show increased PCC connectivity to the ACC, right lateral parietal and superior frontal cortex with increasing age. However, the Age_x_Diagnosis interaction effects changed slightly upon removal of medicated subjects. Specifically, differential age-associated increases in FC with the PCC in controls no longer reached the threshold for statistical significance. Relative to controls, patients showed differential age-associated increases in connectivity between the PCC and right inferior temporal cortex. Finally, the observed association between increased PCC to frontal connectivity and lower SRS scores remained significant, after excluding 22q11DS participants on medications.

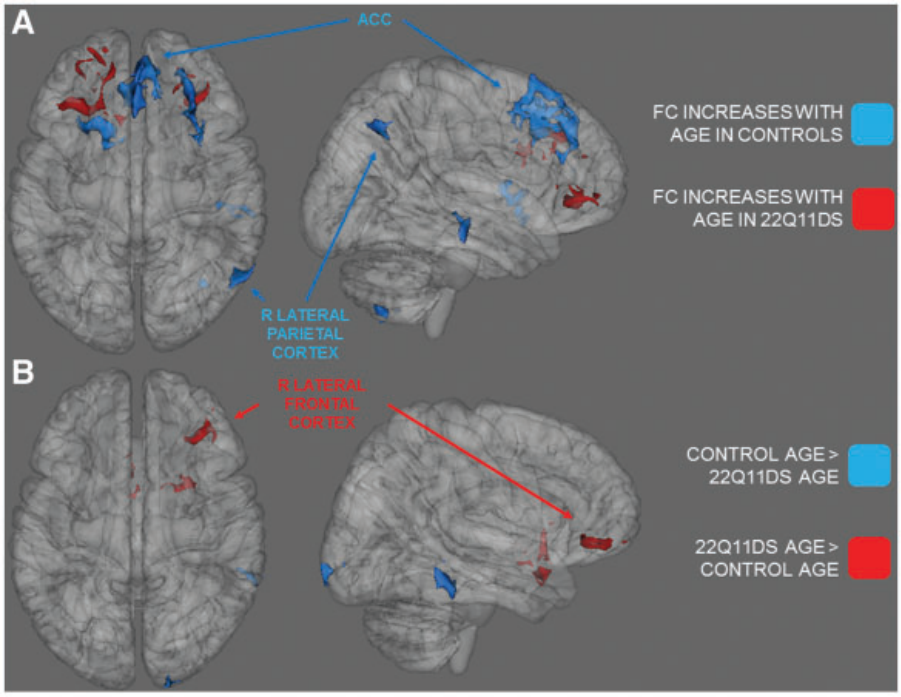


Fig. 2.2 - Developmental effects on PCC connectivity. Figure 2.2a depicts brain regions in which greater functional connectivity with the PCC is associated with increasing age in 22q11DS patients [red_right (R) vmPFC, left (L) frontal cortex] and controls [blue_paracingulate gyrus, anterior cingulate gyrus (ACC), R superior frontal gryus, L putamen, R lateral temporal cortex, R lateral parietal lobe, R cerebellum]. Figure 2.2b depicts regions in which there is a significant Age_x_Diagnosis interaction [i.e. differential PCC connectivity with increasing age for 22q11DS patients (22q11DS) vs controls (Cont)]. Red colors indicate regions in which there is differentially increased connectivity in 22q11DS patients (R vmPFC, Subcallosal cortex, R orbitofrontal cortex), whereas blue colors indicate differentially increased PCC functional connectivity with increasing age in controls (Cont_occipital pole, R lateral temporal cortex).

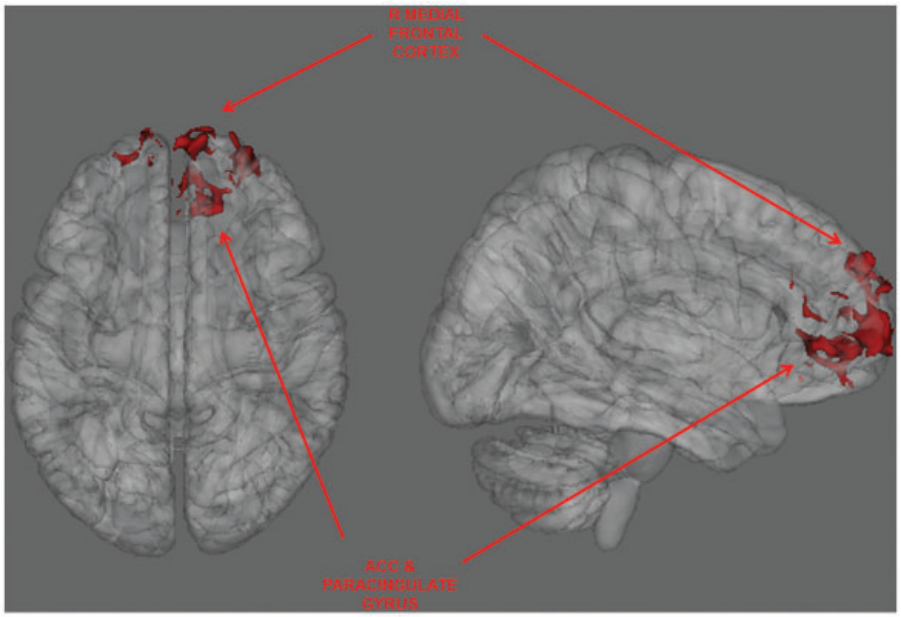


Fig. 2.3 - Association between PCC connectivity and SRS. Regions in which PCC connectivity is significantly associated with a lower SRS score in 22q11DS patients. As shown, connectivity between the PCC and diffuse frontal regions, including the vmPFC, is associated with a lower SRS score (and hence, improved social functioning) in 22q11DS.

2.5 - Discussion

22q11DS is a recurrent genetic mutation associated with defects in cortical circuit formation and high rates of neuropsychiatric disorders characterized by marked social impairment. Evidence from animal models of 22q11DS suggests that disruptions in long-range neural synchrony may be a fundamental component of the disorder (Sigurdsson et al. 2010); nevertheless, very little is known about the functional architecture of the resting brain in human subjects with 22q11DS. Using a seed-based approach, we observed a pattern of reduced long-range connectivity between the PCC and other DMN nodes in 22q11DS participants, consistent with a 'developmental-disconnection' model of the disorder. Furthermore, the strength of PCC-frontal connectivity was associated with increased social competence, thus linking integrity of DMN connectivity with social behavior in 22q11DS. As default network regions are implicated in social information processing and internal representations of self (Uddin et al., 2007), our findings offer new evidence that within-network robustness is an integral factor in modulating the severity of the behavioral phenotype in 22q11DS.

Our findings accord with recent neuroimaging and electrophysiological studies in adults with idiopathic autism spectrum disorders, which have revealed a pattern of altered intrinsic connectivity of long-range DMN circuitry (Murias et al., 2007; Kennedy and Courchesne, 2008). The majority of studies of adolescents and adults with ASD have found reduced functional connectivity of the DMN (Kennedy and Courchesne, 2008; Assaf et al., 2010; Weng et al., 2010); however, a recent study of children with ASD found a pattern of hyper-connectivity of the PCC with medial and anterolateral temporal cortex as well as local hypo-connectivity within posteromedial cortex (Lynch et al., 2013) suggesting that the pattern of DMN alterations in idiopathic ASD may vary as a function of developmental stage (Di Martino et al., 2009b). A

meta-analysis of the functional neuroimaging literature also indicated that the PCC is consistently hypo-activated in social tasks in idiopathic ASD relative to typically developing individuals (Di Martino et al., 2009a), suggesting a neural basis for self-referential processing deficits in both task-positive and task-negative (i.e. resting state) contexts. Moreover, our observed association between long-range DMN connectivity and social behavior in 22q11DS is consistent with a previous study in healthy adults (Di Martino et al., 2009b), reporting that lower levels of autistic traits, as assessed by the SRS, were related to increased FC between the pregenual ACC and anterior mid-insula, brain regions important for social processing previously shown to be hypo-active in ASD patients (Di Martino et al., 2009a). Collectively, these findings support the notion that DMN integrity may be a candidate marker of social competence, in both clinical and non-clinical populations.

Our results are also consistent with those of animal models of 22q11DS, which have reported dramatically reduced neural synchrony between anatomically distant brain regions, suggesting a cellular basis for our findings of disrupted long-range FC (Sigurdsson et al., 2010). In the mouse model, reduced hippocampal-prefrontal synchrony was associated with working memory deficits; a core feature of psychosis. The contribution of disrupted connectivity to other aspects of the phenotype (i.e. social deficits) has not yet been explored in the 22q11DS mouse model, but would be an important extension of this study.

As our sample included a large proportion of young children, we did not see a high rate of psychotic disorder in our sample, and thus we did not analyze DMN activity as a direct predictor of psychotic symptoms; this is an active area of investigation for our future longitudinal studies. Multiple studies have now been conducted indicating aberrant network connectivity,

both in the DMN and globally, in patients with idiopathic schizophrenia as well as their first degree relatives, indicating that altered FC during rest may be associated with genetic risk for the illness (Whitfield-Gabrieli and Ford, 2012; Williamson and Allman 2012; Alexander-Bloch et al., 2013). Remarkably consistent with our findings, (Woodward et al., 2011.) recently reported increased connectivity between the PCC and the left IFG - a brain region typically considered part of the salience network - in patients with schizophrenia relative to controls, suggesting that the functional topography of the DMN may be similarly altered in patients with idiopathic schizophrenia and 22q11DS.

To our knowledge, only one prior study of resting state fMRI in 22q11DS has been conducted (Debbane et al., 2012). Using independent components analysis, an exploratory data-driven approach to analysis of resting-state fMRI data that is an alternative to model-based (ROI) approaches, Debbane et al. (2012) identified group differences in several networks, including the DMN. Interestingly, their findings are largely congruent with those reported here, with 22q11DS participants showing greater connectivity to frontal regions not typically considered part of the DMN, and controls exhibiting stronger connectivity between frontal and posterior regions such as the precuneus and PCC. This study also reported associations between altered connectivity and psychotic symptom severity (Debbane et al., 2012). In contrast, our analysis focuses on a dimensional indicator of social behavior, which is likely to cut across multiple diagnostic categories involving social impairment (i.e. psychosis and ASD).

The strength of FC within DMN nodes may rely on underlying structural connectivity between brain regions. Diffusion tensor imaging studies have identified reduced white matter integrity in long-range fiber tracts in 22q11DS (see Schreiner et al., 2012 for a review), suggesting altered white matter microstructure in tracts connecting these brain regions. Notably,

a recent study of healthy adolescents found that FC between the mPFC and PCC depends upon the maturation of the underlying cingulum white matter tract, suggesting that structural connectivity defects may contribute to the observed reductions in functional connectivity in 22q11DS participants (Gordon et al., 2011). Multimodal imaging studies are now underway, in order to address this question.

The wide age range and relatively large number of typically developing controls in our study allowed us to investigate developmental effects on resting state connectivity. The differential increase in connectivity between the PCC and right lateral temporal cortex evident in controls is consistent with previous literature indicating increases in long-range connectivity within the DMN with maturation (Supekar et al., 2010). In contrast, 22q11DS participants showed evidence of more diffuse frontal connectivity with the PCC, to regions outside of the classic DMN, with increasing age. These findings suggest an altered developmental trajectory of resting state network development in 22q11DS, which may be relevant to the emergence of psychopathology in adolescence. However, given that medication status had an impact on the age_diagnosis interaction results, further investigation is needed. Prospective longitudinal studies are required in order to confirm the intriguing possibilities raised by our cross-sectional findings.

These findings must be interpreted in the light of several caveats. First, we chose to use global signal regression (GSR) in order to account for sources of any physiological, non-neuronal noise in the data. Previous studies have raised the concern that adjusting for global signal may artificially induce, or inflate the strength of, negative functional correlations in resting state networks (Cole et al., 2010); however, there is no currently accepted consensus in the field on whether or not to apply GSR to rs-fMRI data. Given these concerns, and to avoid such interpretive difficulties regarding anticorrelations, we focused our analyses only on positive

correlations. Second, the possibility of differential motion between experimental groups has become a growing concern for interpretation of group differences in resting-state data (Power et al., 2012). Though there are limitations to any method of addressing this, we used a conservative threshold for motion exclusion and ensured that there were no differences in motion between diagnostic groups. Thus, motion artifacts are not likely to account for the observed group differences in connectivity patterns. Finally, the two diagnostic groups differed significantly in terms of Full Scale IQ. Intellectual disability is a known and well-characterized aspect of the 22q11DS phenotype, and prior rs-fMRI studies in 22q11DS and other cognitive impaired populations (e.g. idiopathic schizophrenia) have typically not accounted for IQ differences (Yu et al., 2011; Debbane et al., 2012). In this context, IQ is considered a group defining variable and thus could not be included in our group comparison models (Ho et al., 2012). Thus, we cannot rule out the possibility that variability in IQ (or anxiety or mood disorder diagnoses for that matter) may have had an effect on the results of group comparisons. The relationship between IQ and DMN connectivity is a complex developmental issue, which warrants further consideration in future studies including both 22q11DS patients and other neurodevelopmental disorders. However, it is important to note that SRS scores were not associated with IQ 'within' our 22q11DS sample, and thus could not account for the within-group relationships observed.

In summary, this study reveals dysfunction in long-range connectivity within DMN regions in 22q11DS, a recurrent genetic mutation associated with abnormal neuronal migration and high rates of schizophrenia and ASD. Consistent with previous findings in the general population (Di Martino et al., 2009b), we found that increased long-range connectivity in 22q11DS was associated with the SRS, a continuous measure of autistic traits, suggesting that:

(i) DMN circuitry is a clinically relevant locus of dysfunction in this syndrome, which may have predictive validity for subsequent development of psychopathology and (ii) that alterations in FC identified in the context of this syndrome may fall on a continuum with the broader population. Future work will concentrate on characterizing how resting state functional connectivity may differ across multiple networks in 22q11DS.

CHAPTER 3:

Resting Network –Based Classification and Prediction of Psychotic Symptoms in Youth with 22q11.2 Deletions

3.1 - Abstract

While disturbances in the resting state functional architecture of 22q11DS have been described at a group level, the replicability of such findings and their ability to predict symptom severity on an individual basis are unknown. Here we sought to investigate connectivity of large-scale resting networks and to determine whether connectivity patterns within specific networks can distinguish youth with 22q11DS from typically developing youth.

Group spatial independent components analyses (ICA) were used to extract resting state networks (RSNs) from two independent cohorts, the first ascertained at UCLA (N=33 22q11DS and N=33 demographically-matched controls, ages 8-20) and the second at SUNY Upstate (N=28 22q11DS and N=28 demographically-matched controls, ages 18-26). A dual regression approach was used to generate subject-specific versions of the group components and each RSN was tested for group differences. Subsequently, the identified RSNs from the UCLA cohort were used to train a diagnostic classifier that could: 1) differentiate between 22q11DS and controls on the basis of within-network connectivity in an independent cohort; and 2) predict positive symptom severity and prodromal risk status on an individual basis.

In the UCLA cohort, we observed stronger within-network connectivity in controls relative to 22q11DS patients in five RSNs (ACC/Precuneus, Combined Executive, Default Mode, Posterior Default Mode and Salience Networks; $p<0.05$, corrected). In contrast, no network showed within-network hyperconnectivity in 22q11DS relative to controls. These observed patterns of within-network hypoconnectivity in 22q11DS relative to controls were replicated in the independent SUNY cohort of older individuals. A diagnostic classifier based on default mode network (DMN) connectivity performed best, with an average classification accuracy of 100% ($p<0.05$), regardless of how the groups were partitioned during leave-one-out

cross validation performed during training. This DMN-based classifier was then tested on the SUNY cohort, correctly identifying 27 of 28 patients (96% sensitivity) and 13 of 28 controls (46% specificity), achieving a 71.4% classification accuracy ($p<0.003$) overall. Furthermore, in the UCLA cohort, DMN within-network connectivity predicted SIPS positive symptom severity (a dimensional measure of psychosis; adjusted $R^2 = 0.69$, $p=0.0002$, Bonferroni-corrected), and could distinguish high-risk vs. low-risk 22q11DS patients with a classification accuracy of 79% ($p=0.01$).

These results indicate that widespread within-network hypoconnectivity across several RSNs implicated in higher-order cognition is a defining characteristic of 22q11DS during adolescence. Furthermore, this observed hypoconnectivity appears to persist into early adulthood and presents in a largely consistent set of RSNs. DMN hypoconnectivity was a particularly distinguishing factor, allowing us to correctly identify 22q11.2 deletion carriers in an independent cohort, and to predict psychotic symptom severity. These findings suggest that loss of coherence within the DMN may be a valuable biomarker for individual prediction of psychosis risk in patients with this highly penetrant mutation; future investigation of its utility in the broader population is warranted.

3.2 - Introduction

22q11.2 Deletion Syndrome (22q11DS), also known as DiGeorge Syndrome or Velocardiofacial syndrome is a genetic disorder resulting from a variable length deletion, (comprising some 30-60 genes) on chromosome 22 at a locus designated q11.2. While the deletion confers a wide range of phenotypic consequences, from renal anomalies to congenital heart disease, it is most notable for the cognitive dysfunction and heightened risk of developmental neuropsychiatric disease (particularly psychotic spectrum disorder) it imparts

(Schneider et al., 2014). Accordingly, 22q11DS offers a unique opportunity to examine early neural biomarkers relevant to the development of psychosis in a genetically vulnerable population (Schreiner et al., 2013).

Resting state functional MRI (rs-fMRI) holds promise as such a biomarker; since the first reports of correlations in spontaneous brain activity across spatially distinct areas of the cortex, the analysis of rs-fMRI data has rapidly grown to become an established field in its own right (Biswal et al., 1995, Van Den Heuvel & Hulshoff, 2010). Leveraging this technology, a large number of studies have now documented disrupted resting functional architecture in individuals suffering from idiopathic psychotic illness, and repeatedly shown associations between intrinsic functional neural architecture and behavioral outcomes (Broyd et al., 2009; Littow et al., 2015; Anticevic et al., 2014). Similarly, recent work in 22q11DS has characterized alterations in resting state networks (RSN) and the behavioral correlates of these aberrations via a range of analysis methods, from classic region of interest (ROI) approaches to Independent Component Analysis (ICA) and Graph Theory connectivity analysis. Our group's initial examination of RSN connectivity of 22q11DS used an *a priori*, seed-based approach to uncover evidence of weakened long-range connectivity between the posterior cingulate (PCC) and ventromedial prefrontal cortex, the two major hubs of the default mode network (DMN), and an association between the strength of this long-range connectivity and social cognition (Schreiner et al. 2014). The first published reports of RSN connectivity alterations in 22q11DS utilized dimension-restricted ICA to identify hypo- and hyper-connectivity within several RSNs (including the DMN) and showed a relationship between prodromal symptom severity and DMN strength (Debbane et al. 2012). More recently, this same group applied a support vector machine (SVM) classifier to graph theory connectivity matrices to distinguish between 22q11DS subjects and

controls, showing that the strength of functional connections largely within the frontal lobe are the strongest predictor of group membership (Scariati, 2014). However, these findings were not validated in an independent cohort, and previous research has shown that regularized logistic models that encourage sparsity provide a more robust and accurate prediction of diagnostic status than can be achieved within an SVM-framework (Ryali et al., 2010). Despite the promising groundwork laid by these initial forays, the small sample sizes, wide variation in processing streams and patchwork combination of techniques across studies, highlights the need for robust, reliable biomarkers that can be independently validated. Here we investigated the complete range of resting state networks in youth with 22q11.2 deletions and typically developing controls using an unbiased, data driven approach (Beckmann & Smith, 2004). Since the subtle changes that initially predispose an individual to psychosis-risk (or other psychiatric diagnosis) could conceivably be distributed across the cortex in a sparse fashion rather than localized in one discrete region (as analyses leveraging Gaussian random field theory and cluster correction methods intrinsically assume), an alterative statistical approach is needed to capture this potential effect (Rizk-Jackson et al., 2011, Lemm et al., 2011). To this end, we also sought to determine whether measures of within-network connectivity could be used to: 1) distinguish youth with 22q11DS from typically developing youth; and 2) predict positive symptom severity and prodromal risk status on an individual basis, via sparse classifiers derived from a machine-learning framework.

3.3 - Methods

3.3.a - Participants

The initial sample, consisting of 66 participants aged 8–20 years (33 patients with a molecularly confirmed diagnosis of a 22q11.2 microdeletion and 33 age- and sex-matched typically developing controls), was recruited from an ongoing longitudinal study at the University of California, Los Angeles. Exclusion criteria for all study participants were: additional neurological or medical condition that might affect neuroimaging measures, insufficient fluency in English, substance or alcohol abuse and/or dependence within the past 6 months, and/or any condition that is a contraindication for MRI (pregnancy, claustrophobia, etc.). Healthy controls additionally did not meet criteria for any major mental disorder, based on information gathered during administration of the Structured Clinical Interview for DSM-IV Axis I Disorders [SCID; (First et al., 1996)], with an additional developmental disorders module, as applied by Addington et al. (2012) (for participants over the age of 16 years) and/or the Computerized Diagnostic Interview Schedule for Children [C-DISC; (Jensen et al., 1995)] for participants aged ≤16 years. Healthy controls additionally could not meet criteria for a prodromal state, as assessed by the Structured Interview for Prodromal Symptoms (SIPS; Miller et al., 2003) all clinical interviews were conducted by highly trained MA- or PhD-level psychologists; inter-rater reliability and case consensus procedures have been described in detail elsewhere (Jalbrzikowski et al. 2012, Ho et al. 2012). All participants and/or their parents underwent a verbal and written informed consent process after complete description of the study. The UCLA Institutional Review Board approved all study protocols and all participants provided informed consent.

The unique replication dataset consisted of 56 participants aged 18-26 (28 patients with a molecularly confirmed diagnosis of a 22q11.2 microdeletion and 28 age- and sex-matched typically developing controls) ascertained at SUNY Upstate Medical University from an ongoing longitudinal study. Exclusion criteria matched that described above. The SUNY Institutional Review Board approved all study protocols and all participants provided informed consent. Table 3.1 provides demographic information for all participants included in our analyses, following exclusion of subjects with excess motion (see below) during their scan.

Table 3.1 - Subject demographics, for UCLA and SUNY cohorts

(UCLA COHORT)			
	22q11DS	**CONTROL**	**p-value**
N =	33	33	--
AGE RANGE (MEAN ± SD)	8 - 20 (14.03 ± 3.75)	8 - 20 (13.61 ± 3.44)	0.6337
% MALE	42	52	0.4671
MEAN MOTION in mm	0.6	0.53	0.672
SIPS(+) SCORE, MEAN ± SD	5.2 ± 5.6	1.2 ± 1.5	0.0007
IQ STD SCORE, MEAN ± SD	79.8 ± 14.6	106.9 ± 19.2	<0.0000001
MEDICATION STATUS	7 AntiDep, 2 AntiPsy	1 AntiDepressant	<0.0000001
(SUNY COHORT)			
N =	28	28	--
AGE RANGE (MEAN ± SD)	18 - 26 (21.29 ± 2.46)	18 - 26 (20.79 ± 1.52)	0.365
% MALE	60.7	53.6	0.597
MEAN MOTION in mm	0.31	0.28	0.511

3.3.b - Neurobehavioral Measures

All subjects from the UCLA sample over 10 years old (N=56; 28 22q11DS, 28 CONT) were interviewed with the SIPS (Miller et al., 2003) by a trained clinical interviewer, to assess the presence and severity of psychotic symptoms. Further, 22q11DS individuals were categorically defined as being at High Risk of conversion to psychosis by the presence of any positive symptom item scored in the prodromal or psychotic range, i.e. a rating of 3 or higher on any item in the positive symptom subscale of the SIPS (22q-HR, N=10). Low Risk was defined as having no positive symptoms in the prodromal /psychotic range (22q-LR, N=18).

3.3.c - fMRI data acquisition

(UCLA) Structural and functional scans for the UCLA sample were acquired at either the Ahmanson–Lovelace Brain Mapping Center (BMC) or the Staglin Center for Cognitive Neuroscience (CCN) in Los Angeles, CA, USA. Both sites had an identical 3T Siemens Tim Trio system, utilizing a 12-channel head coil. The primary structural scan used for registration purposes consisted of a matched-bandwidth high resolution T1 image [voxel size 1.5x1.5x4.0 mm, echo time (TE) = 34 ms, repetition time (TR) = 5000 ms, echo spacing = 0.89 ms, 34 axial slices, slice thickness 4.0 mm, slice spacing 0 mm, flip angle 90°, field of view (FOV) = 210, matrix size = 128x128]. Subsequently, a 5 min resting state functional scan was acquired, during which a black screen was presented and participants were instructed to keep their eyes open, remain relaxed and attempt to avoid falling asleep. The resting state scan consisted of 152 BOLD 3D images (voxel size 3.0x3.0x4.0 mm, TE = 30 ms, TR = 2000 ms, echo spacing = 0.79 ms, 34

axial slices, slice thickness 4.0 mm, slice spacing 0 mm, flip angle 90°, FOV = 192, matrix size = 64x64).

(*SUNY Upstate*) Structural and functional scans for the SUNY sample were acquired at SUNY Upstate Medical University, Syracuse, NY, USA. The scan site utilized a 3 Tesla Siemens Tim Trio system, with an 8-channel head coil. The primary structural scan used for registration purposes consisted of a matched-bandwidth high resolution T1 image [voxel size 1.0x1.0x1.0mm, echo time (TE) = 3.31 ms, repetition time (TR) = 2530 ms, echo spacing = 7.6 ms, 176 axial slices, slice thickness 1.0 mm, slice spacing 7 mm, flip angle 7°, field of view (FOV) = 256, matrix size = 256x256]. Subsequently, a 5 min resting state functional scan was acquired, during which a black screen was presented and participants were instructed to keep their eyes open, remain relaxed and attempt to avoid falling asleep. The resting state scan consisted of 152 BOLD 3D images (voxel size 4.0x4.0x4.0 mm, TE = 30 ms, TR = 2000 ms, echo spacing = 0.79 ms, 34 axial slices, slice thickness 4.0 mm, slice spacing 0 mm, flip angle 90°, FOV = 192, matrix size = 64x64).

3.3.d - fMRI data pre-processing

All data (from both the UCLA and SUNY cohorts) were pre-processed and analyzed blind to diagnostic status with tools from the FMRIB Software Library (FSL; fsl.fmrib.ox.ac.uk/fsl/). Each subject's full functional scan was motion-corrected by registering each image to the middle volume as a reference, using FMRIB's linear image registration tool. Any subject with >2mm of translational motion or >2° of rotational motion was excluded from further analysis and there were no significant differences in rotational or translational motion between the patient and control groups ($p>0.05$). The 4D functional images were skull-stripped

using FSL's Brain Extraction Tool, spatially smoothed with a 5mm full width half maximum (FWHM) isotropic Gaussian kernel, bandpass temporal filtered (0.005 Hz < f < 0.1 Hz) and registered to structural data and MNI standard space.

3.3.e - MELODIC De-noising

Following initial preprocessing, the individual fMRI scans were entered into FSL's MELODIC toolbox, for single-session Independent Component Analysis (ssICA). ssICA decomposed each individual's 4D scan into a series of different spatial and temporal components, some of which represented underlying resting state networks and some of which represented artifacts related to motion, scanner drift and/or extraneous physiological noise. Each of the components for a given subject was manually inspected and classified as noise or signal, based off of its temporal profile, frequency power spectrum and spatial characteristics (Kelly et al. 2010, Griffanti et al. 2014). Following classification by trained raters blind to diagnostic status, all noise components were regressed out of the data and the signal components were recombined via fsl_regfilt (fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC#fsl_regfilt_command-line_program). This detailed denoising process was performed in lieu of the recently proposed "motion-scrubbing" procedure (by which specific volumes/TRs associated with spiking motion are removed from the data), in light of the minimum recommended scan length for connectivity analyses (~5min) following any potential removal of TRs and the constraints defined by our own raw data (~5min) (Powers et al. 2012).

3.3.f - Group ICA, network identification and dual regression

The de-noised fMRI scans were concatenated across all subjects in the UCLA cohort and entered into a group ICA (gICA) session implementing Automatic Dimensionality Estimation (ADE) to determine the number of components in the sample, by applying the Laplace approximation to the Bayesian evidence of the model order (Beckmann et al., 2004). The resultant components were upsampled from the default $4mm^3$ to $2mm^3$ resolution, to aid in template matching and subsequent statistical testing. Network templates from the Brain Nexus database (www.brainnexus.com/resources/resting-state-fmri-templates - derived from an age-appropriate sample of N=62 children ages 9-15) were used to identify plausible RSNs from among these gICA components via spatial correlation, and a Dual Regression approach was used to generate subject-specific versions of the relevant gICA components (Beckmann et al., 2009). First, for each subject, the gICA components of interest were regressed (as spatial regressors in a multiple regression) into each subject's 4D space-time dataset. This resulted in a set of subject-specific timeseries, one per gICA component. Next, those timeseries were regressed (as temporal regressors, again in a multiple regression) into the same 4D dataset, resulting in a set of subject-specific spatial maps, one per gICA component. Non-parametric permutation tests (10000 permutations) were performed using FSL's Randomise tool (Winkler et al., 2014) to build a distribution to test against, and each of the RSNs was tested for group differences in both directions (22q11DS>CONT and CONT>22q11DS) via two-sample unpaired t-tests. Significant clusters were identified via threshold-free cluster enhancement and corrected for all multiple comparisons. Results presented herein are thresholded at $p<0.05$, corrected for 24 comparisons ($p<0.0021$).

3.3.g - Network-derived classifiers

For each of the 66 participants from the UCLA cohort, the subject-specific spatial map of a given RSN was restricted to a thresholded (Z>4.3) mask of the group-average RSN from the original gICA, vectorized, and entered into a regularized logistic regression classifier, to analyze the ability of that specific RSN to accurately distinguish between patients and controls. The classification algorithm used was lassoglm, a tool bundled with the Statistics Toolbox for MATLAB (www.mathworks.com/help/stats/lassoglm.html). Lassoglm implemented Lasso/L1-regularization [Ng 2004, Uddin et al., 2013] with Leave-One-Out Cross Validation (LOOCV) to generate a sparse model of in-network voxels that robustly predicted group membership. We report number of subjects misclassified, average cross-validation classification accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each RSN-based classifier. Inference was accomplished by computing a bootstrap distribution of null classifier models, to aid in calculation of p-values that assess significance of a given RSNs classification accuracy.

3.3.h - Classifier Assessment of symptom severity

To examine any potential relationships between within-network connectivity and psychotic symptom severity, we applied a variation of the sparse regression classifier algorithm described above, to the subset of UCLA-22q11DS subjects with SIPS score data (N=28). Forward stepwise regression was performed upon the subset of voxels previously identified by the L1-regularized logistic regression classifier as strongly predictive of 22QvsCON group membership. This linear stepwise regression modeled the association between total positive SIPS score and within-network voxels, to generate a sparse model that predicts prodromal symptom

severity for a patient from the extent of their within-network connectivity alterations. Subsequently, we modified this algorithm to accept a binomial distribution, to see if the identified model could accurately partition these same patients into "HighRisk" (22q-HR) or "Low Risk" (22q-LR) categories solely on the basis of their SIPS scores and within-network connectivity. The regression algorithms used were stepwiseglm (www.mathworks.com/help/stats/stepwiseglm.html) and fitglm (www.mathworks.com/help/stats/fitglm.html), tools bundled as part of the Statistics Toolbox for MATLAB. We report adjusted R^2 and p-value as measures of the performance of the linear-regression classifier in predicting prodromal symptom severity, and sensitivity, specificity and overall classification accuracy as performance metrics for the logistic-regression classifier's ability to correctly partition patients into 22q-HR and 22q-LR categories. As before, the reported p-value for the logistic-regression classifier was calculated from a bootstrap distribution of null models, and is Bonferroni corrected.

3.3.i - Validation of findings with SUNY dataset

Single-subject de-noising, gICA, RSN identification and dual regression were performed on the SUNY cohort in an identical manner to the procedures described above for the UCLA data. Subsequently, the UCLA-trained diagnostic classifier associated with a given network of interest was applied to the subjects from the SUNY cohort, to test the generalizability of any observed connectivity differences to this independent cohort of young adult 22q11DS patients and controls.

3.4 - Results

3.4.a - Group differences in resting state network connectivity

gICA on the UCLA sample of N=66 subjects yielded 32 ICs when utilizing ADE to extract the optimal number of components to describe the data. From these 32 components, the template-matching procedure identified 12 putative RSNs of interest: Default Mode Network (DMN), Posterior DMN, Anterior Cingulate (ACC)/Precuneus Network, a merged Auditory/Middle Temporal Network, Visual Network, Motor Network, Supplementary Motor Network, Left and Right lateralized Executive Networks, Combined Executive Network, Parietal Association Network and Salience Network (Fig 3.1). No [22q11DS>CONT] differences survived the multiple comparisons correction and stringent FWE-rate criteria applied by Randomise, but significant [CONT>22q11DS] differences were found in 5 of the 12 networks: ACC/Precuneus Network, DMN, Combined Executive Network, Posterior DMN and Salience Network. Within the ACC/Precuneus network, significantly greater functional connectivity was localized to the paracingulate gyrus, medial prefrontal cortex (mPFC), superior frontal gyrus and vmPFC (Fig 3.2). Within the Combined Executive network, significantly greater functional connectivity was localized to the right orbitofrontal Cortex (OFC) and frontal pole, bilaterally (Fig 3.2). Within the DMN, significantly greater functional connectivity was localized to the precuneus bilaterally (Fig 3.2). Within the Posterior DMN, significantly greater functional connectivity was localized to the precuneus and posterior cingulate cortex (PCC), bilaterally (Fig 3.2). Within the Salience Network, significantly greater functional connectivity was localized to the left OFC, ACC, PCC, paracingulate gyrus, precuneus, vmPFC, and pre- and post-central gyri (Fig 3.2). Location, size and significance of clusters surviving multiple comparison correction

are reported in Table 3.2. A highly consistent pattern of within-network hypoconnectivity for 22q11DS subjects was found in the RSNs extracted from the SUNY dataset.

3.4.b - Logistic Regression Classifier Performance

Of the 12 RSNs defined above, 10 were able to correctly discern between 22q11DS patients and controls at a greater than chance (50%) level, on the basis of within-network connectivity alone (Fig 3.3). The average classification accuracy was 87% across all RSN derived classifiers (including those trained on connectivity from within the Right Executive network and Motor Network, the two classifiers with error rates of 50%). The DMN-based classifier proved the most accurate and robust, correctly identifying 22q11DS vs Control status 100% of the time (p=0.021), regardless of how subjects were partitioned during cross-validation. For each RSN-derived classifier, we report total subjects misclassified, average cross-validation accuracy,

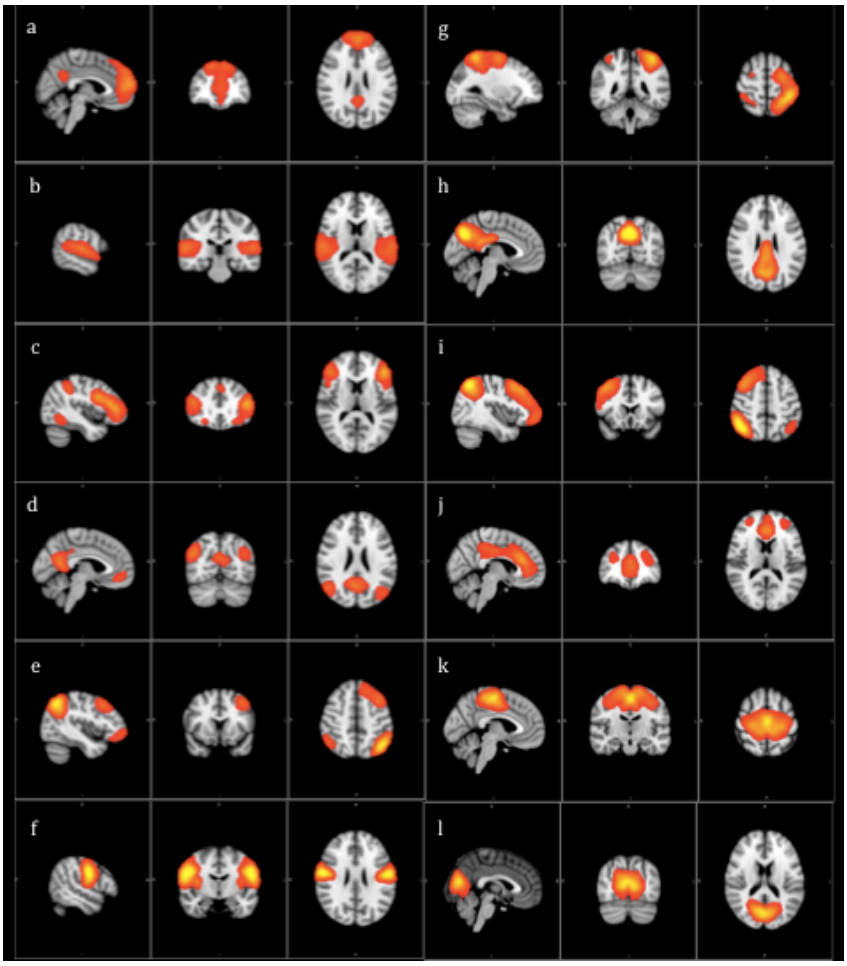


Fig 3.1 - Resting State Networks identified via gICA in the UCLA sample, implemented with ADE a) ACC / Precuneus b) Auditory / Temporal c) Combined Executive d) DMN e) Left Executive f) Motor g) Parietal Association h) Posterior DMN i) Right Executive j) Salience k) Supplementary Motor l) Visual

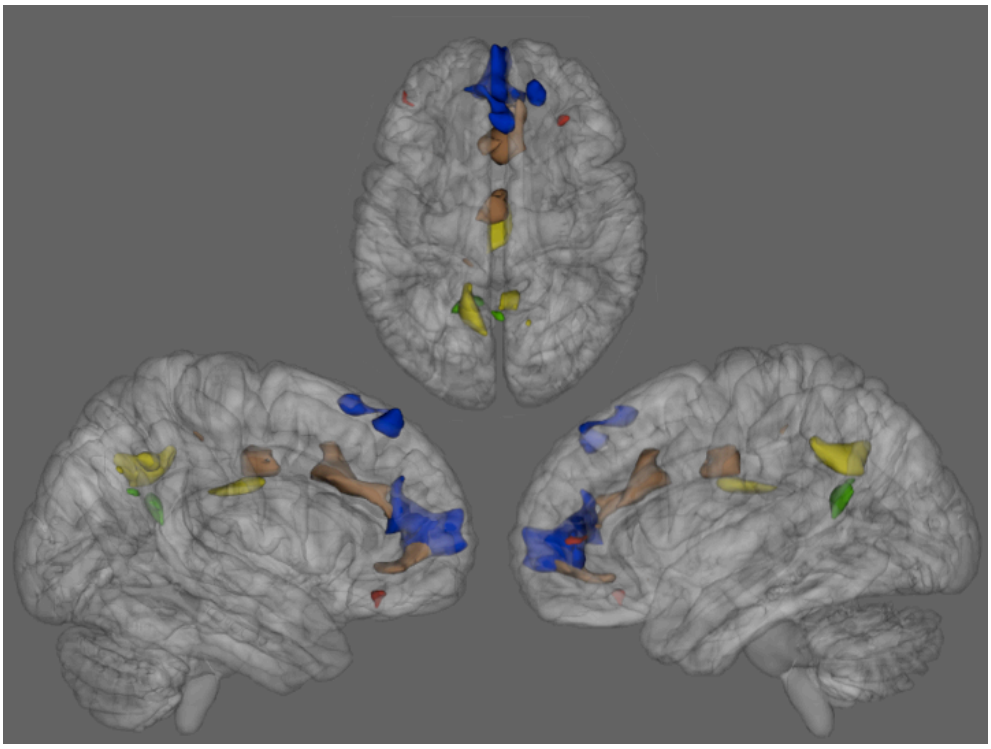


Fig 3.2 - Cluster locations of significant 22q11DS hypoconnectivity for various networks. ACC/Precuneus = Blue. Combined Exec = Red. DMN = Green. Posterior DMN = Yellow. Salience = Brown.

sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in Table 3.3.

3.4.c - Classifier assessment of symptom severity

Upon selection of the DMN as the network with the optimal classifier performance when separating 22q11DS from Control subjects, we investigated the association between network dysconnectivity and prodromal psychotic symptom severity within the 22q11DS subjects. Sparse linear regression was able to generate a compact model from the DMN-based classifier, which predicted positive prodromal symptom severity in UCLA-22q11DS patients (N=28; Adjusted R^2 = 0.6943, p=0.0002, Bonferroni corrected), and could correctly identify 22q-HR vs 22q-LR

prodromal risk status of these 22q11DS patients with 79% accuracy overall (p=0.0107, 60% sensitivity, 89% specificity).

3.4.d - Classifier validation with SUNY dataset

Following identification of the DMN as the most robust and reliable of the resting state networks for training a diagnostic classifier, the UCLA-trained DMN-based classifier was applied to the N=56 SUNY cohort, correctly identifying 27 of 28 patients with 22q11DS (96% sensitivity) and 13 of 28 controls (46% specificity), achieving an overall 71.4% classification accuracy ($p<0.0029$) despite the noted age difference between the subjects in the UCLA and SUNY cohorts.

Table 3.2 – Information on significant clusters surviving multiple comparison correction, in UCLA sample

NETWORK	Cluster Index	Voxels	p-value	X	Y	Z	MNI LOCATION
ACC/Precuneus	4	933	<0.001	-2	48	-4	Paracingulate gyrus
	3	213	0.001	4	26	52	Superior frontal gyrus
	2	147	0.001	18	42	44	Right frontal pole
	1	1	0.002	2	48	-12	mPFC
Combined Executive	2	50	0.001	32	32	-12	Right OFC
	1	28	0.002	-46	44	2	Left frontal pole
DMN	2	185	<0.001	-16	-56	18	Left precuneus
	1	1	0.002	18	-54	24	Right precuneus
Posterior DMN	4	316	<0.001	-14	-56	28	Left precuneus
	3	226	<0.001	2	-16	30	PCC
	2	119	<0.001	6	-52	38	Right precuneus
	1	18	0.002	14	-62	34	Right precuneus
Salience	5	573	<0.001	4	16	28	ACC
	4	402	<0.001	0	50	-4	Paracingulate gyrus
	3	276	0.001	-2	-12	30	ACC, PCC
	2	19	0.002	-12	-36	46	Precentral, postcentral gyrus
	1	3	0.002	-32	18	-12	Left OFC

Table 3.3 – Cross-validated classifier performance metrics for each RSN

NETWORK CLASSIFIER	SUBJECTS INCORRECTLY CLASSIFIED	% ACCURACY	SENSITIVITY	SPECIFICITY	PPV	NPV
Visual	11	83.33	0.9394	0.7273	0.775	0.9231
Right Exec	33	50	1	0	0.5	--
Post DMN	5	92.42	0.9697	0.8788	0.8889	0.9667
Left Exec	1	98.48	1	0.9697	0.9706	1
DMN	0	100	1	1	1	1
Supp Motor	11	83.33	0.8788	0.7879	0.8056	0.8667
Comb. Exec.	3	95.45	0.9697	0.9394	0.9412	0.9688
Motor	33	50	1	0	0.5	--
ACC/Precunes	2	96.97	1	0.9394	0.9429	1
Parietal Assoc.	1	98.48	1	0.9697	0.9706	1
Auditory/Temporal	3	95.45	0.9697	0.9394	0.9412	0.9688
Salience	2	96.97	1	0.9394	0.9429	1

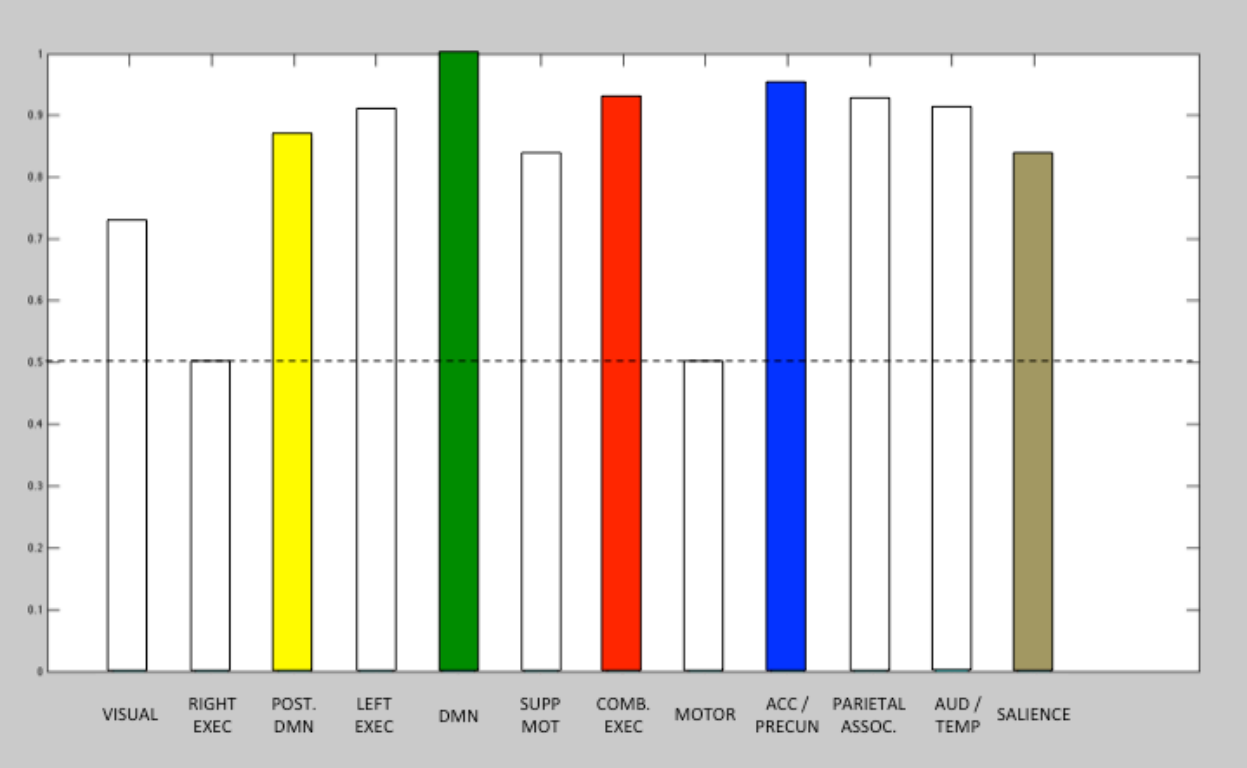


Fig 3.3 - Average cross-validated classifier accuracy for each RSN. Color scheme is consistent with Fig 3.2 for reference.

3.5 - Conclusions

3.5.a - Discussion

This study is the first to investigate multiple resting state networks in two independent samples of patients with 22q11.2 deletions and typically developing controls. Several novel findings emerged, in particular: (1) Hypoconnectivity within networks largely responsible for higher order cognition is a hallmark of 22q11DS during adolescence, (2) the degree of dysconnectivity within the DMN is more severe than that of any other RSN in youth with 22q11DS and (3) the specific patterns of DMN dysconnectivity that distinguish 22q11DS from controls can be used to predict prodromal symptom severity in the 22q11DS group and differentiate between "HighRisk" and "LowRisk" prodromes.

Our finding of within-network hypoconnectivity in 22q11DS across several RSNs is largely consistent with established literature in 22q11DS and idiopathic SZ (Broyd et al., 2009, Scariati et al., 2014). Furthermore, our results from the SUNY replication dataset exhibit striking parallels to our primary findings from the UCLA cohort, with evidence of hypoconnectivity within a very similar set of resting state networks and a complete absence of any observed hyperconnectivity within the RSNs. The consistency of our findings across these two unique datasets supports the notion of resting state network hypoconnectivity as a defining characteristic of 22q11DS. A previous study by our group, utilizing a seed-based approach to characterize the DMN, has shown that the functional connectivity within this network is significantly reduced in subjects with 22q11DS, relative to age- and gender-matched controls (Schreiner et al., 2014). The first report of RSN dysfunction in 22q11DS subjects utilizing an ICA approach (Debbane et al., 2012) with pre-defined dimensionality, and showed within-network connectivity differences in both directions, with 22q11DS showing regions of relative hypoconnectivty in a "high level

visual processing network" and the DMN, but also clusters of hyperconnectivity in a "visuo-spatial processing network", a "sensorimotor network" and the DMN. In contrast, any initially apparent [22q11DS>CONT] differences from our analyses failed to survive correction for multiple comparisons and stringent FWE-rate criteria. Despite these differences, the cluster locations of significant hypoconnectivity in 22q11DS patients reported by Debbane et al. are similar to those we report in the present work. Accordingly, while the initial reports of network dysconnectivity in 22q11DS are not entirely convergent with the findings presented here, the numerous differences in our processing streams and analysis choices (from our larger sample size, 1:1 ratio of 22q11DS to control subjects, more stringent cutoff criteria for maximum subject motion, the addition of an extensive subject-level de-noising protocol, use of ADE to estimate the optimal number of components that describe the data rather than manually constraining the dimensionality the data *a priori*, identification of potential RSNs with age-appropriate network templates, increased resolution, different software, etc.) could account for the discrepancies between our two studies.

With regard to the diagnostic classifiers generated here, our finding that the within-network connectivity of the DMN yields the best performance in separating 22q11DS from Controls is notable in that it highlights the critical impact of DMN dysfunction in this population. Applications of network-based diagnostic classifiers have become an area of intense research and interest in the neuroimaging community as of late, and recent work has shown that regularized logistic regression approaches that encourage sparse models tend to outperform a SVM-framework for classification problems involving fMRI data (Ryali et al., 2010; Rosa et al., 2015). As such, our ability to not only achieve an average cross-validation classification accuracy of 100% when applying training the DMN-derived classifier on the UCLA cohort, but

also to successfully apply it to the completely independent SUNY validation cohort with 71% accuracy is notable. In contrast, previous work on classifying 22q11DS vs controls was only able to achieve a maximal cross-validation classification accuracy of 88% within an SVM-framework applied to graph theory connectivity matrices, and neglected to test the performance of this classifier on a replication dataset (Scariati et al., 2014). Past research has consistently demonstrated that alterations to DMN extent and strength are associated with a range of cognitive deficits and psychotic symptoms, both in 22q11DS and idiopathic SZ (Broyd et al., 2009, Debbane et al., 2012, Schreiner et al., 2013), and our present results serve to underline the crucial role of this network in normal and aberrant cognition, and the structural regions that anchor it. Furthermore, the logistic regression models trained on the five RSNs that showed robust group differences even after multiple comparison correction (DMN, Posterior DMN, ACC/Precuneus, Combined Executive and Salience Networks) each had classification accuracies above 92%, and represented functional networks responsible for higher order cognition rather than those for basic sensory or motor processing, paralleling reports of the cognitive and behavioral deficits characteristic of the syndrome. The robust performance of the DMN-based diagnostic classifier on the replication dataset, in spite of the noted age difference between the UCLA training cohort and SUNY testing cohort, suggests that the persistence of these network alterations through youth into early adulthood is a defining characteristic of 22q11DS.

Our observation of an association between positive SIPS score and within-DMN dysconnectivity, as assessed by a sparse linear regression classifier, replicates the early reports of a relationship between prodromal symptom severity and aberrant connectivity in the DMN (Debbane et al., 2012). Furthermore, the ability of our DMN-based classifier to not only distinguish between 22q11DS patients and controls, but also to accurately partition the 22q11DS

group into “High Risk” and “Low Risk” categories echoes the significant impact that DMN dynamics play in the emergence of psychotic symptoms. In contrast, previous work had established that individuals with a High Risk status could be identified from amongst 22q11DS subjects, but the authors of that research failed to find any overlap between the “discriminative connections” identified by their 22q11DS-vs-CONT classifier and the salient features selected by their HighRisk-vs-LowRisk classifier (Scariati et al., 2014).

3.5.b - Limitations and Future Directions

Despite the overall agreement of results across both the UCLA and SUNY cohorts, we witnessed splitting and/or merging of several common RSNs in the SUNY sample. However, this apparent splitting/merging of the RSNs in the older SUNY sample (ages 18-26) is to be expected, considering the maturation of resting state networks with age and our decision to apply the same BrainNexus templates (derived from youths aged 9-15) used to identify networks from the UCLA sample for consistency.

Despite our use of regularization to avoid over-fitting the classifier models to the input data, there are a number of caveats that should accompany the interpretation of these results. Firstly, when Lasso/L1 regularization algorithms encounter two predictors that are strongly correlated, they will tend to select one and eliminate the other, rather than jointly shrinking the two coefficients and keeping both, as in L2-regularization/Ridge-regression (Ng, 2004). This process results in sparser models, but will not consistently select the same predictors each time it is run on the same input data. For this reason, it is unsuited to reliably identifying specific features (i.e., in-network voxels) that differ between 22q11DS patients and controls. Future analyses of our RSN-derived diagnostic classifiers will attempt to implement elastic-net

algorithms that compromise between L1- and L2-regularization (Zou & Hastie, 2005, Ryali et al., 2010) to identify the specific cortical regions that differentiate 22q11DS from matched controls, and overcome this current limitation.

The findings presented in this work highlight the within-network connectivity deficits that appear to be a hallmark of 22q11DS throughout the critical period of adolescence, when individuals are most at risk of developing psychotic disorder. Of all diagnostic classifiers trained on RSN connectivity, the classifier derived from the DMN was the most robust, highlighting the severe degree of DMN dysfunction in youth with 22q11DS. The DMN-based classifier could generalize to a unique dataset of older individuals, suggesting that DMN hypoconnectivity is a stable distinguishing feature of 22q11DS over time. Finally, the robust and predictable variation in DMN coherence across diagnostic groups allows the rapid and reliable identification of individuals with a high risk of developing a psychotic disorder. These findings will hopefully add to the growing literature on salient, dependable biomarkers that aid in the identification of a nascent psychiatric disorder.

CHAPTER 4:

Thalamocortical connectivity in 22q11DS - the subcortical signature of psychosis

4.1 - Abstract

Despite the growing interest in investigating the dynamics of cortical RSNs in ~~of~~ 22q11DS, no work to date has attempted to directly investigate potential alterations to RSNs associated with the thalamus, a known structural and functional hub. We performed a region of interest analysis of thalamocortical (TC) connectivity in subjects with 22q11DS and demographically matched controls, to assay whether there was any evidence of hyperconnectivity or hypoconnectivity, cross-sectionally (N=79, 40 22q11DS, 39 CONT) and/or longitudinally (subsample, N=26, N=13 22q11DS, N=13 CONT). Cross-sectionally, we observed significant clusters of [22q11DS>CONT] (i.e., 22q11DS TC-hyperconnectivity) to bilateral sensory cortices and significant clusters of [CONT>22q11DS] (i.e., 22q11DS TC-hypoconnectivity) to the striatum, occipital cortex and cerebellum, consistent with recent reports in idiopathic schizophrenia. In order to investigate the ability of the TC-hyperconnectivity patterns to predict risk-status in the patient group with an alternative statistical approach to that discussed above, we also trained a diagnostic classifier (as before, via lasso-regularized logistic regression and leave-one-out cross-validation) on statistical contrasts representing changes in TC-connectivity between baseline and follow-up assessments for each subject. In doing so, we were able to show that these observed longitudinal changes in TC connectivity predict group membership and clinical risk status at follow-up.

4.2 - Introduction

Despite the growing interest in characterizing the cortical connectivity of 22q11DS, no work to date has attempted to directly investigate potential alterations to RSNs anchored more deeply within the brain. The thalamus is a one of the more prominent subcortical structures and

plays a significant role as a structural and functional hub in the relay of sensory information to the cortex for further processing. A cross-sectional analysis of a large sample of schizophrenic subjects recently showed evidence of differential patterns of thalamocortical (TC) connectivity between schizophrenic patients and matched controls; namely, TC-hyperconnectivity to bilateral sensory-motor cortices that was positively associated with psychotic symptom severity and TC-hypoconnectivity to striatal, prefrontal and cerebellar regions (Anticevic et al., 2014). These specific patterns of TC-connectivity alterations were used to train a linear-SVM to distinguish between schizophrenic subjects and healthy controls. Their tuned classifier could correctly identify subject's diagnostic status with an average cross-validation accuracy of 74% ($p<0.001$), and was applied to an independent validation cohort with a 72% success rate ($p<0.001$).

Simultaneously, reports emerged of aberrant thalamic input to the auditory cortex in a mouse model of 22q11DS, due to an up-regulation of $D_{2/3}$Rs in the thalamus mediated by Dgcr8, a previously highlighted gene from the deleted region (Chun et al., 2014). The authors showed that there is a specific deficit in synaptic transmission from TC projections that results in behavioral consequences for the afflicted mouse, and that the cellular and behavioral effects of this deficit could be alleviated by application of the antipsychotics haloperidol and clozapine. Accordingly, we sought to determine the RSN correlates of these findings, both cross-sectionally and longitudinally, in 22q11DS. As in the work detailed in chapter 3, we attempted to assess the performance of a sparse classifier trained on TC-connectivity patterns concurrent with our use of more classical statistical approaches (i.e., via gaussian random field theory and cluster correction) for characterizing significant group differences.

4.3 - Methods

(See previous chapters for info on Participants (i.e., sections 2.3.a and 3.3.a), Neurobehavioral Assessment (2.3.b & 3.3.b) and fMRI Data Acquisition protocols (2.3.c & 3.3.c))

4.3.a - fMRI data preprocessing

We elected to once again utilize a ROI-approach to characterize the thalamocortical connectivity of 22q11DS subjects relative to age and gender matched controls, due to the ease of targeting our analysis and interpreting results. Preprocessing of data was performed as previously described in chapters 2 and 3, with the addition of a variation on the "motion-scrubbing" procedure, in accordance with suggested treatment of subject motion from the current literature (Powers et al., 2014). Specifically, following motion correction, skull-stripping spatial smoothing and bandpass temporal filtering, an initial first level analysis was run in FEAT to model out the effect of nuisance covariates. These covariates included a minimum of 25 nuisance regressors as explanatory variables (EVs) for each subject, modeling the mean global signal and 24 motion parameters (including the 6 standard motion parameters, their first derivatives, the squares of the 6 standard motion parameters and the squares of the derivatives). In addition to these 25 minimum confound EVs, confound files were generated by the fsl_motion_outliers tool for each volume in which a significant spike in framewise displacement occurred. In consideration of the limited number of volumes available for connectivity analysis, these confound files were used as additional nuisance regressors in lieu of explicit timepoint-removal. Subsequently, the residuals from this analysis were normalized, resulting in an image with a mean of 0 and standard deviation of 1. An ROI localized to the bilateral thalamus was registered from MNI standard space to each subject's data and the mean time-series from within

this ROI was extracted from the original residuals file. For the primary connectivity analysis, the average time course from the thalamus was normalized an entered into FEAT as an explanatory variable for the scaled residuals generated during pre-processing, to generate a voxel-resolution map of the connectivity between the bilateral thalamus and a given region. This statistical contrast output from FEAT for each subject was normalized and scaled via Fisher's z-transformation and used as an input to the group-level analyses.

4.3.b - Cross-sectional analyses

Preprocessed subject data for N=79 subjects (N=40 22q11DS, N=39 CONT) was entered into a higher-level analysis structured and mediated by FSL's Local Analysis of Mixed Effects (FLAME; fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT/UserGuide#Group_Statistics). FLAME implemented Mixed Effects (aka "Random Effects") variance modeling, and after determining there was no effect of scanner, included age and gender (both de-meaned) as covariates. Initially, [22q11DS > CONT] and [CONT > 22q11DS] contrasts were created, tested, thresholded and examined to assess differences in whole-brain TC-connectivity between 22q11DS subjects and matched controls. Statistical testing was restricted to the gray matter by masking, and cluster correction for multiple comparisons was carried out using Gaussian random field theory (min Z > 2.3; cluster significance: $P < 0.05$, corrected). The demographic information for all subjects included in the cross-sectional analyses is presented in Table 4.1.

4.3.c - Longitudinal analyses

Preprocessed subject data for the subset of patients with useable 1-year follow-up scan data (N=13 22q11DS, N=13 Controls) was entered into a multi-level analysis for further inquiry.

The first level entailed creating a statistical image, for each subject alone, that represented the within-subject changes in TC-connectivity over time. This was achieved by entering each subject's baseline scan ("T1") and 1-year follow-up scan ("T2") into a Fixed Effects analysis mediated by FLAME and generating [T1 > T2] and [T2 > T1] contrast for each subject individually. The [T2>T1] contrast for each subject was retained for further inquiry, and used as the input to the second level, in which a Mixed Effects FLAME analysis computed the between-group effects of changes in TC connectivity between T2 and T1. As before, covariates included were age (average between T1 and T2, demeaned) and gender (constant between T1 and T2, demeaned). The demographic information for all subjects included in the longitudinal analyses is presented in Table 4.2.

4.3.d – Association with psychotic symptoms and prodromal risk status

The TC-connectivity [T2>T1] maps of the 22q11DS subjects from the longitudinal analysis were analyzed in a patients-only (N=13) analysis probing the relationship of prodromal psychotic symptoms to regions of deteriorating TC-connectivity. This analysis was executed via FLAME, using age (averaged between T1 and T2), gender and positive SIPS score (sum of item scores in the positive symptom subscale of SIPS). All covariates were demeaned, and statistical analyses were restricted to regions of identified hyperconnectivity and/or hypoconnectivity from the longitudinal analysis in 22q11DS and CONTROLS (N=26, *section 4.3.c*). The 22q11DS subjects were further partitioned into "High Risk" (22q-HR, N=5) and "Low Risk" (22q-LR, N=8) cohorts on the basis of their prodromal symptom severity (as before, see *section 3.3.b - Neurobehavioral Measures*) at their 1-year follow-up scan and assessment, to see if their

experienced changes in TC-connectivity were salient predictors of psychosis risk (categorically, in addition to dimensionally).

4.3.e - Longitudinal diagnostic classifiers analysis

The [T2>T1] contrasts of TC-connectivity for each subject included in the longitudinal analysis (N=26) were vectorized and entered into a L1-Regularized logistic regression classifier, to assess how well the changes in TC-connectivity over time could to discriminate between 22q11DS and controls. Classifiers were also applied to the 22q11DS subjects alone (N=13), to interrogate the ability of the longitudinal TC-connectivity alterations to distinguish 22q-HR (N=5) from 22q-LR (N=8) patients. We report average cross-validation classification accuracy of the Minimum Deviance model and "1SE" model (i.e., the simplest model within 1 standard error of the minimum deviance model), and their respective complexities as a percentage of the input feature space.

Table 4.1 - Subject demographics for cross-sectional analyses

	22q11DS	**CONTROL**	**p-value**
N =	40	39	--
AGE RANGE (MEAN ± SD)	10 - 26 (16.8 ± 4.6)	10 - 26 (16.1 ± 4.6)	0.472
% MALE	40%	51%	0.3203
MEAN MOTION in mm	0.57	0.6	0.8321
SIPS(+) SCORE, MEAN ± SD	5.7 ± 6.3	1 ± 1.4	<0.00001
IQ STD SCORE, MEAN ± SD	78.3 ± 14.7	108.7 ± 19.9	<0.00001

Table 4.2 - Subject demographics for longitudinal analyses

	22q11DS	**CONTROL**	**p-value**
N =	13	13	--
AGE RANGE (MEAN ± SD)	11 - 22 (17.2 ±3.1)	11 - 20 (15.6 ± 2.7)	0.171
% MALE	46%	52%	0.7088
MEAN MOTION in mm	0.52	0.41	0.334
SIPS(+) SCORE, MEAN ± SD	5.1 ±7.8	0.5 ± 0.05	0.0489
IQ STD SCORE, MEAN ± SD	73.6 ± 12.8	113.6 ± 20.4	<0.00001

4.4 - Results

4.4.a - Cross-Sectional Analyses

Significant [22q11DS>CONT] connectivity (i.e., TC-hyperconnectivity) was witnessed in the bilateral sensorimotor cortices (postcentral gyrus, precentral gyrus, superior parietal lobule) and bilateral auditory cortices (heschl's gyrus, planum temporale, middle temporal gyrus, superior temporal gyrus, parietal operculum cortex) (Figure 4.1). Significant [CONT>22q11DS] connectivity (i.e., TC-hypoconnectivity) was witnessed in the occipital cortex (occipital pole intracalcarine cortex, occipital fusiform gyrus), striatum (left caudate), the PCC and left cerebellum (Figure 4.1).

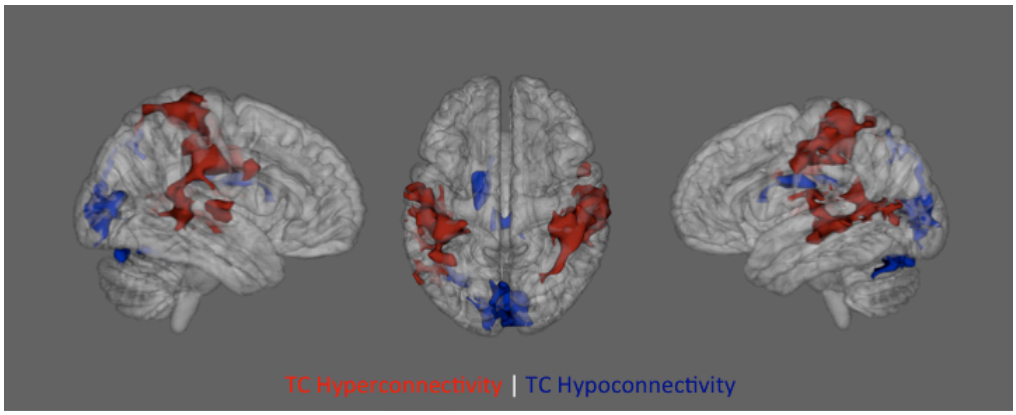


Fig 4.1 - Regions of TC hyperconnectivity (red) and TC hypoconnectivity (blue) for 22q11DS relative to CONT

4.4.b - Longitudinal Analyses

Significant [T2>T1] TC-hyperconnectivity was observed in the left hemisphere sensory cortex (precentral gyrus, postcentral gyrus, Heschl's gyrus, planum polare, superior temporal gyrus, central opercular cortex) (Figure 4.2). There was no evidence of significant changes in TC-hypoconnectivity over time.

Fig 4.2 - Regions of significant increase in TC hyperconnectivity from T1 to T2 for 22q11DS relative to CONT

4.4.c - Association with psychotic symptoms and prodromal risk status

We found evidence of a significant relationship between positive SIPS score and TC-hyperconnectivity in regions where 22q11DS showed a differential increase in TC-connectivity over time. Specifically, increased severity of prodromal psychotic symptoms in 22q11DS subjects was associated with increased strength of TC-connectivity in the left sensory cortex (Fig 4.3a). This pattern was replicated when investigating prodromal status categorically, showing that the 22q-HR subjects are driving the reported finding of TC-hyperconnectivity increases in the left sensory cortex (Figure 4.3b)

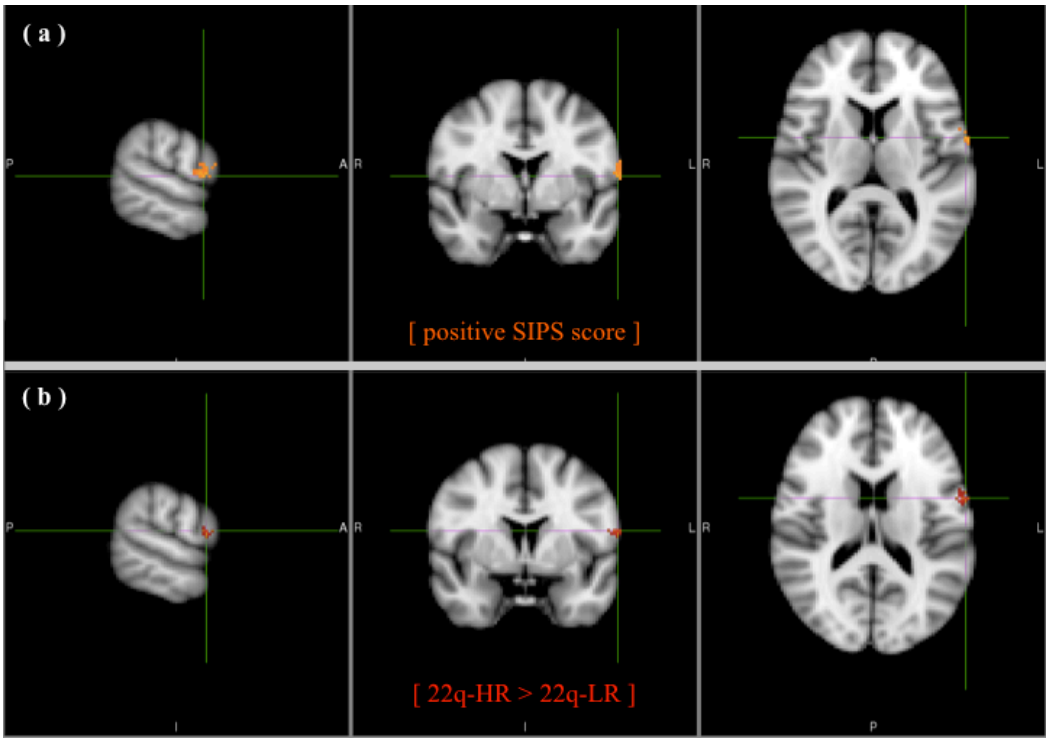


Fig 4.3 – (a) Regions in which positive psychotic symptoms are associated with TC-hyperconnectivity increases from T1 to T2, for 22q11DS subjects. (b) Regions in which there are significantly greater TC-hyperconnectivity increases from T1 to T2 for 22q-HR subjects vs 22q-LR subjects.

4.4.d - Diagnostic classifier performance on longitudinal TC-connectivity changes

The cross-validated diagnostic classifier was able to correctly partition the 22q11DS and CONT subjects with 100% accuracy ($p<0.05$) on the basis of the inter-subject variation in TC-connectivity changes, over a range of regularization parameters and model complexities. On average, the Minimum Deviance model identified during cross-validation consisted of 7 predictors (i.e., voxels of import) and the 1SE model consisted of 4 voxels of import (Figure 4.4).

A diagnostic classifier was also able to correctly partition the 22q-HR and 22q-LR subjects with 100% accuracy ($p<0.05$) on the basis of the inter-subject variation in TC-connectivity changes, over a range of regularization parameters and model complexities. On average, the Minimum Deviance model identified during cross-validation consisted of 6

predictors (i.e., voxels of import), and the 1SE model consisted of 4 voxels of import (Figure 4.4).

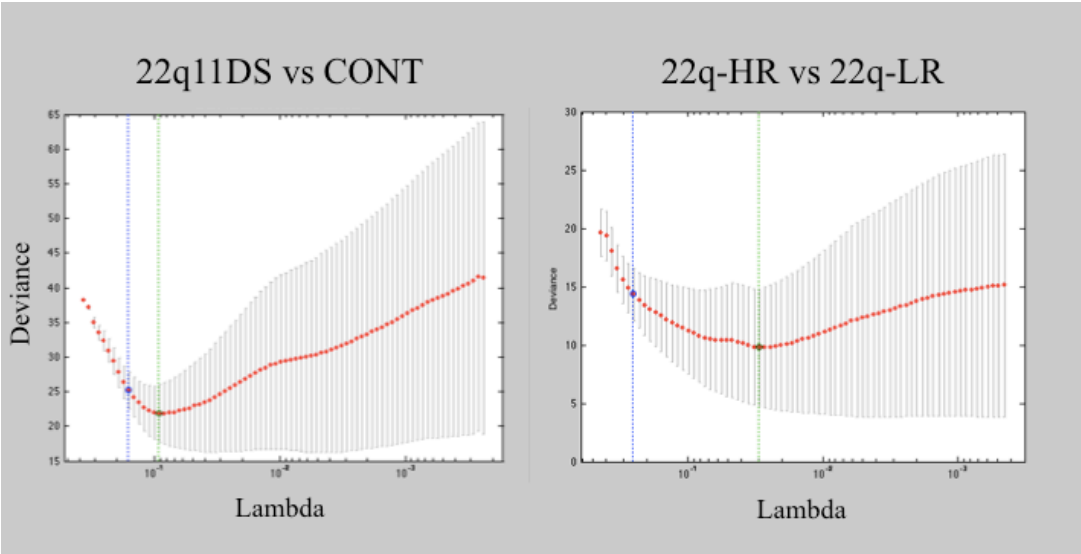


Fig 4.4 – Diagnostic classifier model deviance, as a function of regularization parameter (lambda). Very small values of lambda (moving right along x-axis) apply weaker penalties to the model parameters, yield more complex classifiers that tend to overfit the training data and therefore suffer during cross-validation. Larger values of lambda (moving left along x-axis) yield simpler classifiers that may generalize to testing data more easily, but may underfit the training data. Conventionally, the optimal classifiers, based on average cross-validation accuracy and model simplicity, occur at minimum deviance ("Min Dev", green lines) and no further than 1 Standard Error from Min Dev ("1SE", blue lines)

CHAPTER 5:

Conclusions and Future Directions

5.1 - Overview

In summation, these collected works have demonstrated that:

(1) The DMN is less robust in 22q11DS, and the reduction in DMN extent and strength presents commensurate with an individual's level of social cognition. These alterations become more pronounced with time.

(2) This characteristic within-network hypoconnectivity that we witness in the DMN in 22q11DS also extends to other cortical RSNs, largely responsible for higher cognition.

(3) These patterns of within-RSN hypoconnectivity can be used to distinguish amongst 22q11DS and controls. The DMN is the cortical RSN with the most diagnostic utility; It performs this partitioning most efficiently, can identify the severity of prodromal psychotic symptoms dimensionally, and can even separate 22q-HR from 22q-LR individuals categorically.

(4) Subcortical-cortical connectivity, anchored by the thalamus, is hyperactive in regions of sensory cortex and hypoactive in striatal, occipital and cerebellar regions, largely consistent with reports in idiopathic SZ and mouse models of 22q11DS.

(5) Longitudinal changes in patterns of TC-connectivity can predict the severity of psychotic symptom at a follow-up assessment, and can also be used to discriminate between the above clinical sub-groups of low and high-risk individuals.

5.2 - Limitations and Caveats

5.2.a. - Variation in processing pipelines necessitates the validation of results

As the optimal method for analysis of neuroimaging data is a constantly changing area of interest in the academic community, the consensus within the field about what constitutes best

practices with regards to data pre-processing has evolved over time. As such, I have strived to implement the most sound, up-to-date methodology, as it existed at the time of publication of the various articles included as part of this dissertation. However, there are differences in the preprocessing pipelines applied to the subject data in each chapter (and between our chosen methods and previous reports in the literature), and the reported results should ideally be replicated in an independent cohort (as was done in chapter 3) for confirmation of validity.

5.2.b - Internal comparability of results

This work has endeavored to characterize the alterations to cortical and subcortical networks that distinguish 22q11DS from controls, and most importantly, attempted to address a more subtle question: *'What specific RSN-related changes within 22q11DS, predispose an individual to developing a psychotic disorder?'* - However, as evidenced by the vast array of methodologies being tested in the literature, there is still considerable debate about the analysis technique with the most utility and efficacy at answering this question. Chapter 2 was primarily concerned with showing how alterations to DMN dynamics are associated with a behavioral biomarker related to psychosis risk. Chapter 3 showed that the connectivity of multiple RSNs could be used as salient biomarkers for the syndrome due to their within-network weaknesses. Chapter 4 also showed that diagnostic classifiers trained on changes in subcortical-cortical connectivity are sensitive to subtle differences between clinical groups. However, despite yielding largely convergent/consistent results, none of these methods are directly comparable due to the wide variability in the analysis approaches and preprocessing pipelines used. The small sample size available for our longitudinal analyses of TC-connectivity is of particular note, and

these findings, albeit exciting, should be interpreted with caution until they are validated in a larger cohort and replicated in an independent sample.

5.2.c - The pitfalls of sparse regression

As first referenced in the discussion section of chapter 3, the sparse methods utilized for the majority of classification analyses in this work are highly effective at generating simple models, but will not reliably identify the same predictors during multiple attempts at feature selection. Specifically, Lasso/L1-regularization penalties have been observed to force the majority of parameters in a model to zero, leaving only those predictors who are least correlated with one-another, and thus are optimal features for separating the data. While this stringent pruning is an apt approach to identifying a small set of salient predictors from within an RSN (in which a large degree of spatial and temporal correlation exists in neighboring voxels), L1-regularization will not necessarily choose the same predictor amongst a cluster of highly correlated voxels each time it is performed. As such, the feature selection is unlikely to be stable over multiple iterations, which could affect the generalizability of our network-based models to the larger population. Again, further research and validation of our results in an independent cohort is needed.

5.3 - Conclusions and Future Directions

Despite these promising steps taken towards the development of a classification algorithm that can identify subjects at risk for a debilitating psychotic disorder, the true utility of a universal classification algorithm predicated on network dysconnectivity will be measured by its ability to (i) generalize to a wider dataset and (ii) accurately distinguish psychosis risk from

the myriad other comorbidities known to plague this population – from autism, to ADHD and Depression (Drew et al., 2011; Schreiner et al., 2013). In regard to the first item, Elastic Net methods (Zou & Hastie, 2005; Ryali et al., 2010) that compromise between L1 & L2 regularization penalties, and thus provide a more stable set of predictors than those created by the pure L1-regularized logistic regression models developed herein, show great promise as tools for generating sparse-yet-reliable models of psychosis risk based on RSN dynamics. In regards to the second item, approaches based on an SVM-framework, that implement multiclass classification to differentiate between more than 2 groups, are promising candidates for future research (Allwein et al., 2000, Hsu & Lin, 2002).

Hopefully, this collected research represents a small but significant step towards a future in which we can identify a nascent psychiatric disorder and address it before the debilitating symptoms affect an individual's quality of life.

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