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Endophenotypes of Executive Control: A Reverse Genetic Approach

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Endophenotypes of Executive Control: A Reverse Genetic Approach

A dissertation submitted in partial satisfaction of the

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by

Rachel Karen Jonas

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ABSTRACT OF THE DISSERTATION

Endophenotypes of Executive Control: A Reverse Genetic Approach

by

Rachel Karen Jonas

Doctor of Philosophy in Neuroscience

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Professor Carrie E. Bearden, Chair

Neurogenetic disorders with distinct genetic etiologies and known psychiatric phenotypes present a compelling model to study the links between genotype and phenotype. In recent years, the study of quantitative endophenotypes has become increasingly relevant in garnering a comprehensive understanding of psychiatric dysfunction. Neurofibromatosis Type 1 (NF1) and 22q11.2 Deletion Syndrome (22q11DS) are two compelling examples of neurogenetic disorders that are highly penetrant for neuropsychiatric phenotypes, and are also characterized by prefrontal cognitive dysfunction. We investigated endophenotypes of executive control in these populations, using a reverse-genetic approach.

In Chapter 2, we measured risk-taking behavior using a child-friendly gambling task in patients with NF1 and controls (N=29 NF1, 23 controls). We used functional magnetic resonance imaging (fMRI) to investigate neural activity associated with risky decision making, as well as age-associated changes in these behavioral and neural processes. Behaviorally, patients with NF1

tended to made fewer risky decisions than controls. Neuroimaging analyses revealed significant hypoactivation of multiple brain regions involved in higher-order semantic processing and motivation (i.e., anterior cingulate, paracingulate, supramarginal, and angular gyri) in patients with NF1 relative to controls, in both decision-making and outcome phases of the task. We also observed atypical age-associated changes in neural activity in patients with NF1, such that during risk taking, neural activity tended to decrease with age in controls, whereas it tended to increase with age in patients with NF1. Findings suggest that developmental trajectories of neural activity during risky decision-making may be disrupted in youth with NF1.

In Chapter 3, we reviewed recent literature on the utility of studying endophenotypes of psychiatric illness in 22q11DS. We provided an overview of neuropsychiatric findings to date, which highlight the value of this syndrome in mapping the developmental trajectory of dimensional phenotypes that traverse multiple diagnostic categories. Potential sources of genetic variability that may contribute to the disorder's heterogeneous presentation were reviewed. We discussed the use of how animal models can readily be developed that recapitulate specific aspects of the syndrome. Future research directions involve translational models and potential for drug screenable targets in the context of this human model system.

In Chapter 4, we investigated executive function and its relationship with structural neuroanatomy in patients with 22q11DS and matched controls (N=43 22q11DS, 43 controls), along with cognitive measures that tap behavioral regulation and metacognitive aspects of executive function. Behaviorally, patients with 22q11DS were impaired on multiple executive function measures. Right orbitofrontal cortical thickness showed a differential relationship between real-world executive function in patients with 22q11DS and controls. We also observed a group difference in the relationship between behavioral regulation and metacognition measures

with thickness of ventral and dorsolateral prefrontal regions, respectively. Findings suggest that executive dysfunction characteristic of 22q11DS is underscored by altered prefrontal cortical structure.

In Chapter 5, we investigated gene expression levels and behavioral correlates in patients with 22q11DS and matched controls (N=56 22q11DS, 48 controls). Using real-time quantitative polymerase chain reaction (RT-qPCR), we measured gene expression levels of three genes that are hemizygous in 22q11DS (*COMT*, *DGCR8*, and *ZDHHC8*). We found decreased expression of all three genes in patients with 22q11DS. We found a positive relationship between age and *COMT* expression in patients with 22q11DS, but not in controls. We also investigated the relationship between gene expression levels and relevant cognitive measures related to executive function, and found interaction effects between group and IQ for *DGCR8* and *ZDHHC8*. Lastly, we found a relationship between working memory and *COMT* expression in patients with 22q11DS, but not in controls. Results suggest that the relationships between gene expression and cognitive function may differ between patients with 22q11DS and controls.

Overall, the collected work provides insight into the use of endophenotypes of executive control in neurogenetic disorders. In clinical psychiatry, current methods for diagnosing psychiatric illness rely on clinical symptoms. Our research is in line with the NIMH RDoC framework, which supports the notion of studying intermediate endophenotypes in order to better diagnose and treat patients with psychiatric illness. Having a comprehensive understanding of the building blocks of psychiatric disease can lead not only to better methods of diagnosis, but also to better treatment strategies.

The dissertation of Rachel Karen Jonas is approved.

Giovanni Coppola

Jamie D. Feusner

Adriana Galván

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Paul Thompson

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2017

I dedicate this dissertation to my parents, for encouraging me to pursue my joint interests in biology and psychology, and for telling me while I was in high school, "there's a new field called neuroscience that you should check out sometime."

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Acknowledgments

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Rob, my soon-to-be husband, is my top supporter. When I told him I wanted to move across the country to go to graduate school in LA, he was supportive from the first day. Soon after, he followed suit at the business school at UCLA, and now we're engaged to be married. Thank you for being so supportive of me and letting me follow my passions.

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review paper of 22q11DS reprinted from *Biological Psychiatry* (vol. 75, Jonas RK, Montojo CA, Bearden CE, 'The 22q11.2 Deletion Syndrome as a window into complex neuropsychiatric disorders over the lifespan' pp. 351-360, Copyright (2014), DOI: 10.1016/j.biopsych.2013.07.019. The work was co-authored by Caroline A. Montojo and Carrie E. Bearden, both of who have consented to the inclusion of this work in this thesis.

Chapter 4 consists primarily of a published manuscript reprinted from *Molecular Neuropsychiatry* (vol. 1, Jonas RK, Jalbrzikowski M, Montojo CA, Patel A, Kushan L, Chow CC, Vesagas TK, Bearden CE, Altered brain structure-function relationships underlie executive dysfunction in 22q11.2 Deletion Syndrome, pp. 235-246, Copyright (2015), DOI: 10.1159/000441979 with permission from Karger. The work was co-authored by Maria Jalbrzikowski, Caroline A. Montojo, Arati Patel, Leila Kushan, Carolyn C. Chow, Therese K. Vesagas, and Carrie E. Bearden, all of who have consented to the inclusion of this work in this thesis.

Chapter 5 consists primarily of a manuscript in preparation, entitled 'Gene expression in 22q11.2 Deletion Carriers'. The work was co-authored by Allison Furterer, Giovanni Coppola, and Carrie E. Bearden, all of who have consented to the inclusion of this work in this thesis.

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Academic Publications

1. **Jonas RK**, Roh E, Montojo CA, Pacheco LA, Rosser T, Silva AJ, Bearden CE (*in press*). Risky decision-making in Neurofibromatosis Type 1: An exploratory study.
2. Jalbrzikowski M, Ahmed KH, Patel A, **Jonas RK**, Kushan L, Chow C, Bearden, CE (2016). Categorical versus dimensional approaches to Autism-associated intermediate phenotypes in 22q11.2 Microdeletion Syndrome. *Biological Psychiatry: CNNI.* 2451-9022.
3. Bearden CE, Hellemann G, Rosser T, Montojo C, **Jonas R**, Enrique N, Pacheco L, Hussain S, Wu J, Ho J, McGough J, Sugar C, Silva, A (2016). A randomized placebo-controlled Lovastatin trial for Neurobehavioral function in Neurofibromatosis I. *Annals of Clinical and Translational Neurology.*
4. **Jonas RK**, Jalbrzikowski M, Montojo CA, Patel A, Kushan L, Chow CC, Vesagas TK, Bearden CE (2015). Altered brain structure-function relationships underlie executive dysfunction in 22q11.2 Deletion Syndrome. *Molecular Neuropsychiatry*. 1:235-246.
5. Montojo CA, Congdon E, Hwang L, Jalbrzikowski M, Kushan L, Vesagas TK, **Jonas RK**, Ventura J, Bilder RM, Bearden CE (2015). Neural mechanisms of response inhibition and impulsivity in 22q11.2 deletion carriers and idiopathic attention deficit hyperactivity disorder. *Neuroimage: Clinical*.
6. **Jonas RK**, Montojo CA, Bearden CE (2014). The 22q11.2 Deletion Syndrome as a window into complex neuropsychiatric disorders over the lifespan. *Biological Psychiatry* 75(5):351-60.
7. Montojo CA, Ibrahim A, Karlsgodt KH, Chow C, Hilton AE, **Jonas RK**, Vesagas TK, Bearden CE (2014). Disrupted working memory circuitry and psychotic symptoms in 22q11.2 deletion syndrome. *Neuroimage: Clinical* 4:392-402.
8. Jalbrzikowski M, **Jonas R**, Senturk D, Patel A, Chow C, Green MF, Bearden CE (2013). Structural abnormalities in cortical volume, thickness, and surface area in 22q11.2 microdeletion syndrome: Relationship with psychotic symptoms. *Neuroimage: Clinical* 14;3:405-15.

First Authored Conference Presentations

1. **Jonas RK**, Roh E, Montojo CA, Congdon EL, Pacheco LA, Rosser T, Silva AJ, Bearden CE (2016, July). Brain maturational trajectories underlying risky decision making in youth with

Neurofibromatosis Type 1 (NF1). Poster at the Federation of European Neuroscience Societies Forum, Copenhagen, Denmark.

2. **Jonas RK**, Gras EA, Fiksinski A, Bearden CE, Vorstman JA (2016, June). The relationship between proline and cortical neuroanatomy in 22q11.2 Deletion Syndrome. Poster at the Organization for Human Brain Mapping Meeting, Geneva, Switzerland.
3. **Jonas RK**, Roh E, Montojo CA, Congdon EL, Pacheco LA, Rosser T, Silva AJ, Bearden CE (2016, June). Neural maturational trajectories underlying risky decision making in youth with Neurofibromatosis Type 1. Poster at the Children's Tumor Foundation Conference, Austin, TX.
4. **Jonas RK**, Roh E, Montojo CA, Congdon EL, Pacheco LA, Rosser T, Silva AJ, Bearden CE (2015, September). Behavioral and fMRI measures of risky decision making in adolescents with Neurofibromatosis Type 1 (NF1). Poster at the Flux Congress Meeting, Leiden, Netherlands.
5. **Jonas RK**, Roh E, Montojo CA, Congdon EL, Pacheco LA, Rosser T, Silva AJ, Bearden CE (2015, June). Functional neuroimaging of risky decision making in adolescents with Neurofibromatosis Type 1 (NF1). Poster at the Organization for Human Brain Mapping Meeting, Honolulu, HI.
6. **Jonas RK,** Montojo CA, Congdon EL, Enrique NE, Rosser T, Silva AJ, Bearden CE (2014, November). Behavioral and neural measures of risky decision making in youth with Neurofibromatosis Type 1 (NF1). Poster at the Society for Neuroscience Meeting, Washington, DC.
7. **Jonas RK**, Jalbrzikowski M, Patel A, Kushan L, Montojo CA, Bearden CA (2014, September). Developmental neuroanatomic endophenotypes of executive function in 22q11.2 Deletion Syndrome. Poster at the Flux Congress Meeting, Hollywood, CA.
8. **Jonas RK**, Montojo CA, Congdon EL, Enrique NE, Bearden CE (2014, May). Risky decision making in children with Neurofibromatosis type 1 (Nf-1). Poster at the Society of Biological Psychiatry Meeting, New York, NY.
9. **Jonas RK**, Jalbrzikowski M, Patel A, Aggarwal S, Chow CC, Montojo CA, Bearden CE (2013, November). Examining neuroanatomical correlates of catechol-O-methyltransferase gene expression levels in patients with 22q11.2 Deletion Syndrome. Poster at the Society for Neuroscience meeting, San Diego, CA.
10. **Jonas RK**, Jalbrzikowski M, Patel A, Chow CC, Montojo CA, Bearden CE (2013, May). Abnormal behavioral and anatomical measures of dopaminergic regulation in patients with 22q11.2 Deletion Syndrome. Poster at the Society of Biological Psychiatry meeting, San Francisco, CA.
11. **Jonas RK**, Moody TD, Feusner JD, Chang S, Armstrong CC, McCracken J, Piancentini J, O'Neill J (2012, October). Correlations between symptom severity and activity in intrinsic connectivity networks in children with obsessive-compulsive disorder. Poster at the Society for Neuroscience meeting, New Orleans, LA.
12. **Jonas RK**, Gluck MA, Dorfman BJ, Istomina E, Henchcliffe C, Piboolnurak P, Nirenberg MJ (2011, November). Differences in hippocampal-dependent task performance in individuals with Parkinson's Disease-associated impulse control disorders and/or dopamine agonist withdrawal syndrome. Poster at the Society for Neuroscience meeting, Washington DC.
13. **Jonas RK**, Gluck MA, Levy-Gigi E, Piboolnurak P, Henchcliffe C, Dorfman BJ, Nirenberg MJ (2011, April). Identification of generalization learning deficits in Parkinson's Disease patients with impulse control disorders. Poster at the Cognitive Neuroscience Society meeting, San Francisco, CA.
14. **Jonas RK**, Gluck MA, Myers C, Ulasoglu C, Gurvit H (2010, November). Performance of Korsakoff patients on an acquired equivalent task. Poster presented at the Society for Neuroscience meeting, San Diego, CA.
15. **Jonas RK**, Keri S, Gluck MA (2010, April). Preference for positive versus negative-feedback learning in Parkinson's patients at risk for impulse control disorders. Poster at the Cognitive Neuroscience Society meeting, Montreal, QC.

CHAPTER 1: A Reverse genetic approach to understanding mechanisms of neuropsychiatric disorder and allied phenotypes

Rachel K. Jonas, Carrie E. Bearden

Although large-scale genomic investigations have recently provided insights into risk genes for developmental neuropsychiatric disorders (1-3), we continue to have a poor understanding of mechanism. The extensive heterogeneity of these disorders - at both a genetic and phenotypic level - presents a substantial challenge for our understanding of etiology (4). The investigation of individuals with a known, homogeneous genetic anomaly that is highly penetrant for the development of psychiatric illness provides a valuable alternative approach.

A substantial body of evidence has now accumulated that DNA copy number variation (CNVs) - stretches of DNA that are either deleted or duplicated in our genome (5) - play a major role in the etiology of neuropsychiatric disorders (6-8). One of the most common known recurrent CNVs is 22q11.2 Deletion Syndrome (22q11DS), a neurogenetic disorder whereby 30-60 known genes are hemizygously deleted (9-11). Patients have a greatly elevated risk of developing schizophrenia; 25-30% of this population will develop schizophrenia, as compared to 1% in the general population (12). In childhood these patients also have elevated rates of psychiatric disorders associated with dopaminergic dysfunction, notably ADHD (13; 14), and recent reports indicate a increased incidence of early-onset Parkinson's disease in adults (15). Patients with 22q11DS are hemizygous for genes related to dopaminergic metabolism, and this presumed dopaminergic dysregulation may be relevant to the development of psychiatric illness in this population (16; 17). Other genes within the locus are highly expressed during brain

development and critical for cortical circuit formation (18). As such, 22q11DS offers an attractive model to advance understanding of the neurobiological mechanisms underlying the development of schizophrenia and other developmental neuropsychiatric disorders, which may also be relevant to the broader population.

Single-gene mutations can also have vast effects on neurobehavioral phenotypes. Neurofibromatosis Type I (NF1) is a monogenic disorder whereby patients have a mutation on the *NF1* gene, which codes for a tumor-suppressor protein called neurofibromin (19). Patients exhibit elevated rates of attention-deficit hyperactivity disorder (ADHD) and executive dysfunction (20). Mouse models of the disorder have shed light on mechanisms underlying the characteristic neurobehavioral alterations, and have shown irregularities in GABA release and striatal dopamine metabolism (21); notably, one study found reduced levels of dopamine in the striatum of *Nf1* mutant mice, and reduced rearing in response to novel objects, suggesting a dampened response to novel stimuli (22). Treatment with drugs that increased dopaminergic levels (i.e. methylphenidate or L-DOPA) rescued the behavioral deficits seen in these mice (22).

Given this unique opportunity to link direct alterations in genetic makeup to behavioral and psychiatric phenotypes, we can use endophenotypes (quantitative traits that lie intermediate between genotype and phenotype) to help understand brain-related changes that may be mediating these relationships. Endophenotypes can refer to many intermediate traits including cellular changes, brain structure and function, or cognition (Figure 1).

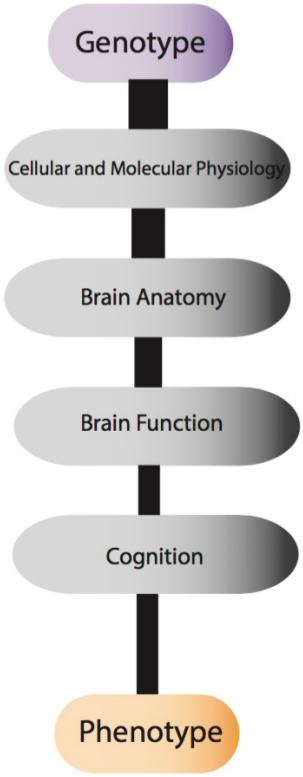

Figure 1. *Endophenotypes of psychiatric illness.*. Demonstration of endophenotypes to link genetic alterations to phenotypic traits. Levels illustrated here indicate how a known genetic etiology can inform pathophysiologic mechanisms relevant to neuropsychiatric phenotypes.

Endophenotypes of psychiatric illness are important to consider for several reasons. First, they can help us to understand the biological and physiological mechanisms that underlie normal psychiatric function as well as dysfunction. Second, certain endophenotypes are thought to precede behavioral symptoms. In several conditions, such as schizophrenia and Alzheimer's disease, changes in brain structure have been found years before the onset of disease (23; 24). Lastly, characterization of endophenotypes can help aid diagnosis and treatment for psychiatric disorders. The National Institute of Mental Health introduced the Research Domain Criteria (RDoC) initiative, which is a framework to study mental disorders by integrating various levels of information to help understand the basic dimensions of functioning underling human behavior

(25). Because of our current poor understanding of the underlying neurobiology of neuropsychiatric disorders, treatments are not targeted to etiologically defined symptoms. The discovery of endophenotypes may help us detect who is susceptible to psychiatric illness before clinical symptoms develop. Ultimately, we may be able to attenuate or even ameliorate symptoms of the disorder before they begin. In our case, we are taking a "genetics first" approach, by studying individuals with known rare genetic variants, and investigating intermediate endophenotypes on the path to psychiatric illness.

In this series of studies, we are taking a "genetics-first" approach, whereby we identify individuals with known genetic alterations and study intermediate endophenotypes on the path to psychiatric illness. We and others have proposed that the most informative solution to the challenge of bridging genes to complex behavior is via identification of highly penetrant syndromes with a single, known genetic cause, combined with use of valid animal models (Jonas 2014; La Mantia 2015). Within the context of executive control and dopaminergic dysregulation, we investigate cellular and molecular physiology (gene expression analyses), brain anatomy (structural MRI), brain function (functional MRI), and cognition (behavioral and cognitive tasks). Cutting across various systems levels will allow us to garner a fuller understanding of how genetics can impact psychiatric illness.

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CHAPTER 2: Risky decision-making in Neurofibromatosis Type: An exploratory study

Rachel K. Jonas, EunJi Roh, Caroline A. Montojo,
Laura A. Pacheco, Tena Rosser, Alcino J. Silva, Carrie E. Bearden

Abstract

Neurofibromatosis type 1 (NF1) is a monogenic disorder affecting cognitive function. About one third of children with NF1 have attentional disorders, and the cognitive phenotype is characterized by impairment in prefrontally-mediated functions. Mouse models of NF1 show irregularities in GABA release and striatal dopamine metabolism. We hypothesized that youth with NF1 would show abnormal behavior and neural activity on a task of risk-taking reliant on prefrontal-striatal circuits. Youth with NF1 (N=29) and demographically comparable typically developing (TD) controls (N=23), ages 8-19, were administered a developmentally sensitive gambling task, in which they chose between low-risk gambles with a high probability of obtaining a small reward, and high-risk gambles with a low probability of obtaining a large reward. We used functional magnetic resonance imaging (fMRI) to investigate neural activity associated with risky decision making, as well as age-associated changes in these behavioral and neural processes. Behaviorally, youth with NF1 tended to make fewer risky decisions than controls. Neuroimaging analyses revealed significantly reduced neural activity across multiple brain regions involved in higher-order semantic processing and motivation (i.e., anterior cingulate, paracingulate, supramarginal, and angular gyri) in patients with NF1 relative to controls, in both decision-making and outcome phases of the task. We also observed atypical age-associated changes in neural activity in patients with NF1, such that during risk taking,

neural activity tended to decrease with age in controls, whereas it tended to increase with age in patients with NF1. Findings suggest that developmental trajectories of neural activity during risky decision-making may be disrupted in youth with NF1.

Introduction

Neurofibromatosis type 1 (NF1; MIM 162200), a monogenic disorder caused by mutations in the neurofibromin gene on chromosome 17, is one of the most common single gene disorders affecting cognitive function (prevalence 1:3500) (1). Physical features include the formation of peripheral nerve sheath tumors, café-au-lait spots, and Lisch nodules (2). About one-third of children with NF1 meet diagnostic criteria for attention deficit hyperactivity disorder (ADHD), and the cognitive phenotype includes impairment in prefrontally-mediated functions that encompass attention, working memory, and inhibitory control (3).

The *Nf1* gene codes for the neurofibromin protein, which acts as a tumor suppressor that modulates the Ras signaling transduction pathway (4). Neurofibromin, a large cytoplasmic protein, is a negative regulator of Ras, and acts to keep it in its inactive, GDP-bound state. Mutations in the *Nf1* gene lead to compromised neurofibromin activity, and thus overactivity of Ras, resulting in dysregulation of cell growth and proliferation.

Mouse models of NF1 have uncovered Ras-signaling dependent increases in GABA release (5-7)) and deficits in plasticity that contribute to their learning and memory deficits. These mouse models have also shed light on irregularities in dopaminergic metabolism in this disorder (8). In NF1, tyrosine hydroxylase (TH), a precursor to dopamine, is reduced, leading to lower dopaminergic signaling (3). In addition to increases in GABA release in the striatum (7), Brown et al. (9) found reduced levels of dopamine in this structure in *Nf1* mutant mice, and reduced rearing in response to novel objects, suggesting a dampened response to novel stimuli.

In addition to treatments that target Ras signaling (10; 11), drugs that increased dopaminergic levels (i.e. methylphenidate or L-DOPA) rescued these behavioral deficits (9). Another study in a mouse model of NF1 found that deficiencies in dopaminergic signaling also appear to contribute to deficits in learning and memory (12). In humans, the stimulant methylphenidate (MPH) has been used to treat attentional deficits in patients with NF1, which acts by blocking dopamine reuptake, thus increasing extracellular dopamine (13; 14). Although it is still unknown how neurofibromin regulates dopamine homeostasis in the brain, a recent review of cognitive dysfunction in NF1 points towards dopamine as a possible molecular target for remediating cognitive and psychiatric symptoms in patients with NF1 (3). Recent evidence from animal models suggests that dopaminergic neurotransmission in the striatum plays a direct role in risky decision-making, in that it signals preference for making a risky or safe decision (15; 16). Further, individual differences in dopaminergic neurotransmission have been thought to underlie variability in risky decision-making in humans (17).

Based on these findings, we hypothesized that patients with NF1 would show abnormal behavior on a task of risk-taking shown to be reliant on the striatum and orbitofrontal cortex (OFC). The Cake Gambling Task (18) was developed as a child-friendly task to measure risky decision-making with varying amounts of potential reward. Participants are asked to choose between low-risk gambles with a high probability of receiving a small reward, and high-risk gambles with a low probability of obtaining a large reward. To date, this task has been used to investigate risky decision making in typical development; findings indicate that healthy youth tend to make riskier decisions as the potential reward is increased, and risk-taking in the low reward conditions tends to decrease with age (18). Functional MRI (fMRI) results revealed that risky decisions were associated with increased activation in the ventromedial prefrontal cortex

(vmPFC) and ventral striatum (VS), while cautious choices were associated with activation in the dorsolateral prefrontal cortex (dlPFC). Interestingly, this study also observed an adolescent-specific peak in vmPFC activation while making risky decisions, and an adolescent-specific peak in the VS when receiving reward feedback. The authors also found that older participants showed decreased activation in the dorsal anterior cingulate (ACC) during risky decision-making. Further, the tendency to make risky decisions was associated with decreased activation in the ACC and lateral OFC (19; 20).

To our knowledge, no studies of reward-based decision-making have been conducted in youth with NF1. Given the neurophysiological results in mouse models and the behavioral profile of NF1, we predicted that youth with NF1 would be more risk-averse than healthy individuals, particularly when the potential reward is high. Because of the increases in GABA-mediated inhibition previously documented in the prefrontal cortex of NF1 mouse models and associated hypoactivation of specific prefrontal structures in NF1 subjects (7), we hypothesized that patients with NF1 would not show the expected increases in neural activity in vmPFC during risky decision-making, nor increases in DLPFC activity during safe decision-making. Consequently, we also anticipated altered age-related trajectories during both risky and safe decision-making in patients with NF1. Additionally, we predicted that the relationship between individual risky decision-making and neural activity may differ in patients with NF1 versus controls. Lastly, we predicted that patients with NF1 wouldn't show an increase in VS activity when receiving positive feedback.

Methods and Materials

Participants

52 participants (29 patients with NF1 and 23 healthy controls, aged 8-19, were included in the study. Participants in the study were recruited from three primary sources: 1) The Children's Hospital Los Angeles Neurofibromatosis Clinic, a major NF1 referral center for the greater Los Angeles region; 2) local Children's Tumor Foundation and NF Network family educational symposia; 3) NF-related websites as well as www.clinicaltrials.gov. All aspects of the research study were granted IRB approval by the University of California, Los Angeles prior to the collection of any data. All participants underwent verbal and written consent after study procedures were fully explained, and their parents or guardians also completed written consent.

Patients with NF1 were screened and enrolled by a pediatric neurologist (T.R.), and had a confirmed diagnosis of Neurofibromatosis Type I, according to NIH criteria (21). Exclusion criteria for all participants included: substance or alcohol abuse and/or dependence in the last six months, insufficient fluency in English, intellectual disability (Full-Scale IQ $\leq$70, as determined by the Wechsler Abbreviated Scale of Intelligence; WASI (22); any major neurological or psychiatric condition; history of head injury with loss of consciousness; or any MRI contraindications. NF1 participants also could not have any intracranial pathology such as hydrocephalus or brain tumor, other than an asymptomatic optic pathway or other NF1-related glioma. In addition, major psychiatric disorders were exclusionary for controls, with the exception of ADHD, anxiety disorder, or a single past episode of depression. Demographic information for the sample is presented in Table 1.

Table 1. Demographic characteristics of study participants.

	NF1 (N=29)	Controls (N=23)	p value
Age (mean, SD, range)	11.93 (2.64), 8-16	12.78 (3.42), 8-19	0.338
Gender	14M, 15F	14M, 9F	0.366
Ethnicity (% Latino)	38%	35%	0.815
Full Scale IQ (mean, SD)	93.79 (2.95)	111.26 (3.34)	<0.001**
ADHD Diagnosis (%)	41%	4%	0.002**
Participant Education (years)	6.55	7.00	0.591
Highest Parental Education (years)	15.65	16.57	0.280

**Usable fMRI data were available on 36 participants (NF1: N=18, Control: N=18)*

Cognitive and Psychiatric Testing

Supervised clinical psychology doctoral students administered neurocognitive and psychiatric evaluations to study participants. IQ data were acquired with the WASI (22). Psychiatric diagnostic information was determined via parental interview using the Computerized Diagnostic Interview Schedule for Children (C-DISC) (23) and/or participant interview via the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (24)

Procedure

All participants first received extensive instructions in a quiet testing room, and performed 11 practice trials before their scan. They were told that they should attempt to win as many trials as possible, and that at the end of the scan they would receive a prize corresponding to their performance on two randomly selected outcomes. Participants all received the same

amount of money at the end of the scan; younger participants were given the option to choose a reward from a prize box.

Cake Task Description

The fMRI task was adapted from the Cake Gambling Task (18), a child-friendly gambling task in which subjects are asked to choose between a low- and a high-risk gamble, associated with varying probability of reward (Figure 1). These probabilities are represented visually, as a circle with six distinct "wedges", which are brown and pink (4:2 ratio). Two squares are located beneath the circle, in which the rewards associated with the colors are presented as stacks of coins. On each trial, subjects choose a color, and receive a reward based on the probability described above (66% for low-risk gambles and 33% for high-risk gambles). The reward value associated with the high-risk choice varies (4, 8, 12, or 16 coins) and remains constant for the low-risk choice (2 coins). There were 84 total trials, with 21 trials per condition, which were separated into two blocks.

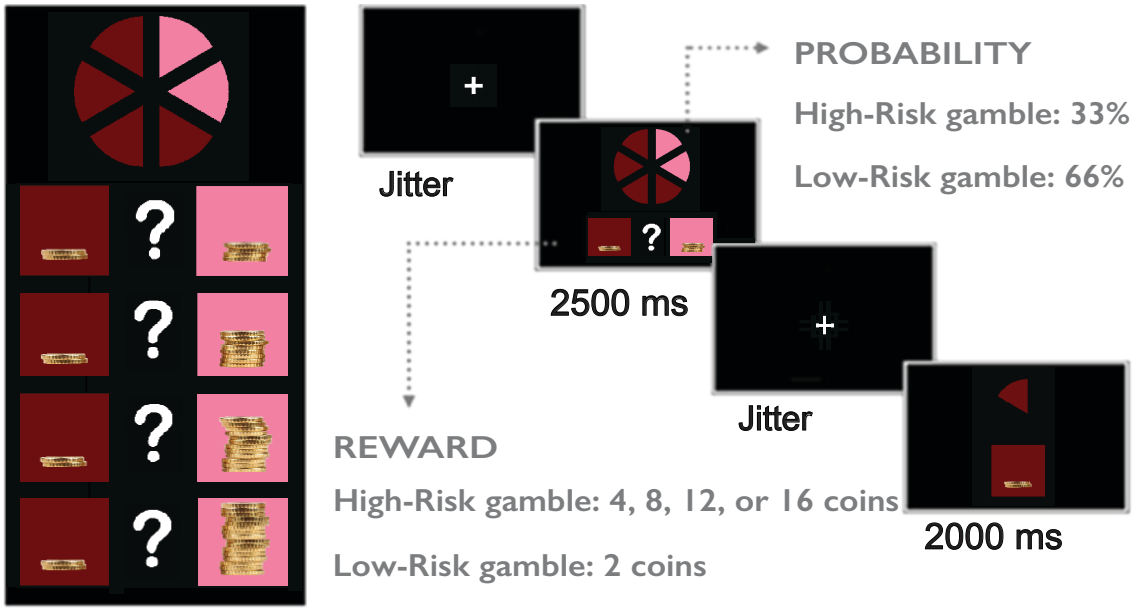


Figure 1. *The Cake Gambling Task.* Participants are asked to choose between low-risk gambles with a high probability of obtaining a small reward (2 coins), versus high-risk gambles with a smaller probability of obtaining a higher reward (4-16 coins). In total, there were 84 trials, with 21 trials per condition. The trials were presented in an event-related fashion, and the order was consistent among subjects.

fMRI Acquisition

Neuroimaging data were collected on a 3T Siemens Trio MRI scanner at the Center for Cognitive Neuroscience at the University of California, Los Angeles. Functional data were acquired using T2*-weighted echoplanar images. While participants completed the task, 400 functional T2* echoplanar images were collected with the following parameters: slice thickness = 3mm, 38 slices, TR = 2200ms, TE = 30ms, flip angle = 90° , matrix 64x64, FOV = 192 mm. As in Van Leijenhorst et al (2010) (25), two separate scans were acquired (seven minutes each), in order to check in with participants mid-scan. The two scans were subsequently combined for analysis (described below).

Each trial had the following structure: A jittered fixation cross was presented, varying between 300 and 5250 ms [using fsloptseq2; see http://surfer.nmr.mgh.har- vard.edu/optseq/ (26)], followed by the stimulus for 2500 ms. Participants were instructed to respond during the stimulus presentation using either their index finger (brown choice) or middle finger (pink choice). Another jitter crosshair appeared, and then response feedback was given for 2000 ms. For correct outcomes (win), participants were shown the color that they chose, and the amount of money earned. For incorrect outcomes (loss), they were shown a stack of coins with a grey cross through it.

Additionally, a T2-weighted matched-bandwidth high resolution anatomical scan, and MPRAGE were collected. The parameters for the MPRAGE were the following: TR = 2.3 s, TE = 2.91 ms, FOV = 256 mm, matrix = 240 x 256, flip angle = 9°, slice thickness = 1.20 mm, 160 slices. Foam inserts were placed around the participants' head to minimize head motion.

fMRI Preprocessing

fMRI analyses were performed using the FMRI Software Library (FSL) (www.fmrib.ox.ac.uk/fsl), version 5.0 (27). We excluded participants with translational motion that exceeded 4mm (NF1=11, Controls=5).

For participants that were retained in the analysis, images were first realigned to compensate for small head movements (28) Data were spatially smoothed using a 5-mm full-width-half-maximum Gaussian kernel. The data were filtered in the temporal domain using a nonlinear high-pass filter with a 66 second cutoff. The registration process first included registering the EPI images to the matched-bandwidth high-resolution scan, then to the structural MPRAGE image, and lastly into standard (Montreal Neurological Institute (MNI)) space, using nonlinear transformations. We ran each of the scans through a separate first-level analysis. These were then combined into a second-level analysis, before running group analyses. The two scans did not systemically differ from one another with regard to behavioral performance, nor patterns of neural activity (see Figure 2).

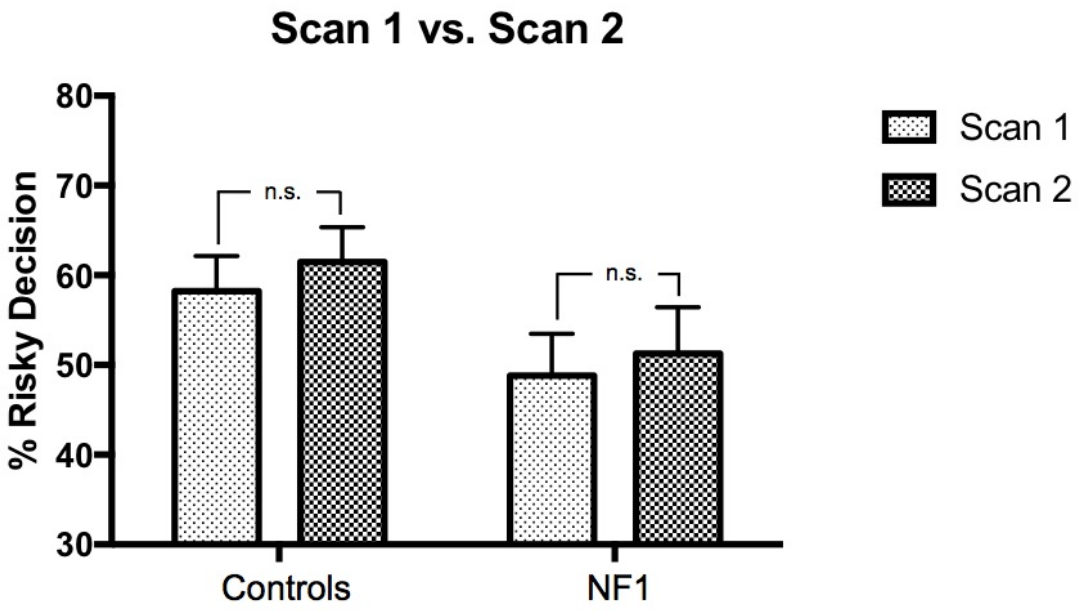


Figure 2. *Comparison of behavior across two separate scans within the Cake Task.* Percentage of risky decisions made across the two scans does not differ in patients with NF1 or controls.

Behavioral Analyses

Cognitive and clinical data were processed using SPSS software v. 23 (IBM). We compared demographic characteristics between groups using independent-sample t-tests for continuous variables, and chi-square tests for categorical variables. Primary analyses of behavioral response data assessed how risk-taking changes as potential reward increases, and the relationship of risky decision-making to age. We used repeated-measures ANOVA to compare overall risk-taking across conditions between groups, and a univariate ANOVA to compare risk-taking between groups within each separate condition (4, 8, 12, or 16 coins). Additionally, paired t-tests were used to compare risk taking for the lowest (4 coins) vs. highest reward condition (16 coins) within each group. Age and gender were included as covariates in all behavioral analyses. To explore the relationship between risk-taking and age we used Pearson

partial correlations controlling for gender. We also investigated post-feedback behavior, looking specifically at the tendency to make risky vs. safe decisions after receiving either a win or a loss. Separate univariate ANOVAs for post-win and post-loss were used to compare the percentage of trials in which the subject made a risky vs. safe choice, between groups. Secondary analyses investigated whether individual differences in IQ or diagnosis of ADHD may contribute to decision-making behavior.

fMRI Analyses

Standard model fitting was conducted for all subjects. After convolution with a canonical gamma hemodynamic response function, the following events were modeled: all risky decisions > baseline, all safe > baseline, all risky > all safe, all safe > all risky. We modeled both the decision-phase of the task (length determined as the reaction time between stimulus onset and participant response; <2500 ms), and the outcome-phase of the task (2000 ms). The six motion parameters as well as a motion outlier confound matrix produced by FSL motion outliers, designed to detect individual timepoints in the dataset that have been corrupted by excessive motion (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers),were included as covariates of no interest. Among participants included in the fMRI analyses, there were no differences in translational motion between patients with NF1 and controls ($p = 0.15$).

For group-level statistics, we investigated neural activity for the following contrasts: mean NF1 > baseline, mean control > baseline, NF1 > control, control > NF1, and age by group interactions in both directions. We investigated these for both the decision phase of the task (defined by the time between when the stimulus was first displayed, and when the participant gave a response) and the outcome phase of the task (during feedback; 2000 ms). We also ran a separate analysis, including the overall percentage of risky decision-making (defined as the

percentage of time participants chose the low probability option) as a covariate, in order to determine the relationship between neural activity and behavioral propensity to make risky decisions, both between and across groups.

Group-level statistics images were thresholded with a cluster-forming threshold of $z > 2.3$ and a cluster probability of $p < 0.05$, corrected for whole-brain multiple comparisons using Gaussian random field theory. Brain regions were identified using the Harvard-Oxford cortical and subcortical probabilistic atlases. For reporting of clusters, we used the cluster command in FSL, and calculated percent signal change in these regions. A list of clusters for each contrast is described in Tables 2-6). Figures were visualized using BrainNet Viewer, a MATLAB graphical user interface (29).

Table 2. Significant clusters for all risky > baseline; Control > NF1.

Cluster Index	Voxels	P value	Region	Z-Max	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)
8	1326	6.56e-07	Paracingulate Gyrus	3.95	2	52	14
7	833	9.67e-05	Cingulate Gyrus, anterior division	4.2	-6	20	34
6	795	0.000147	Lateral Occipital Cortex, superior division	4.64	-18	-72	54
5	755	0.00023	Frontal Pole	3.62	-34	46	36
4	639	0.00088	Temporal Occipital Fusiform Cortex	3.9	-24	-48	-16
3	511	0.00427	Lateral Occipital Cortex, superior division	4.21	10	-82	48
2	421	0.014	Orbitofrontal cortex	3.3	58	22	-6

1	359	0.0332	Inferior Frontal Gyrus, pars opercularis	3.72	-56	14	8

Table 3. Significant clusters for all safe > baseline; Control > NF1.

Cluster Index	Voxels	P value	Region	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)
2	2491	5.32E-11	Supramarginal Gyrus, anterior division	4.66	-44	-38	38
1	1137	4.83E-06	Lateral occipital cortex	3.85	30	-52	36

Table 4. Clusters for all risky > all safe; Age interaction NF1 > Control.

Cluster Index	Voxels	P value	Region	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)
2	2547	9.28E-11	Frontal pole	3.92	-38	16	50
1	411	0.022	Angular Gyrus	3.24	-60	-54	30

Table 5. Clusters for all risky > all safe; % Risky decision making interaction NF1 > Control.

Cluster Index	Voxels	P value	Region	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)
6	3016	9.93E-13	Cingulate Gyrus, posterior division	4.28	0	-46	38
5	1185	2.80E-06	Frontal Pole	3.82	12	56	28
4	795	0.000154	Cerebral white matter	3.52	14	8	22
3	628	0.00104	Lateral Occipital Cortex, superior division	3.6	40	-60	22
2	456	0.00901	Inferior Frontal Gyrus, pars triangularis	3.61	52	24	10
1	334	0.0485	Superior Frontal Gyrus	3.41	-20	22	44

Table 6. Clusters for all win > baseline; Controls only.

Cluster Index	Voxels	P value	Region	Z-MAX	Z-MAX X (mm)	Z-MAX Y (mm)	Z-MAX Z (mm)
2	723	0.000537	Left Putamen	5.23	-16	6	-8
1	704	0.000663	Right Putamen (Caudate, Nucleus Accumbens)	4.35	14	4	-12

Note: For each of these contrasts. we did not find any significant clusters in which patients with NF1 showed increased activation as compared to controls.

Results

Behavioral

Demographic characteristics

The total sample consisted of 52 participants (N=29 NF1, 23 controls). After excluding 16 subjects for excessive motion, as described above, fMRI data were available for analysis for 36 participants (N=18 NF1, 18 controls). As shown in Table 1, patients with NF1 were demographically matched with controls on age, gender, education, ethnicity, and parental education. Consistent with previous literature (3), patients with NF1 showed significantly decreased IQ as compared to controls ($p<0.001$**), and had higher rates of ADHD ($p=0.002$**).

Cake Task

Behavioral Results

Overall, patients with NF1 showed a non-significant tendency to make fewer risky decisions across all reward categories, as compared to controls ($p=0.101$; Figure 3a). This appears to be driven by high-reward categories (12 coins: $p=0.042$, 16 coins: $p=0.083$).

Relationship with Age

We did not find a behavioral relationship between risky decision-making and age in patients with NF1 or controls (see Figure 4).

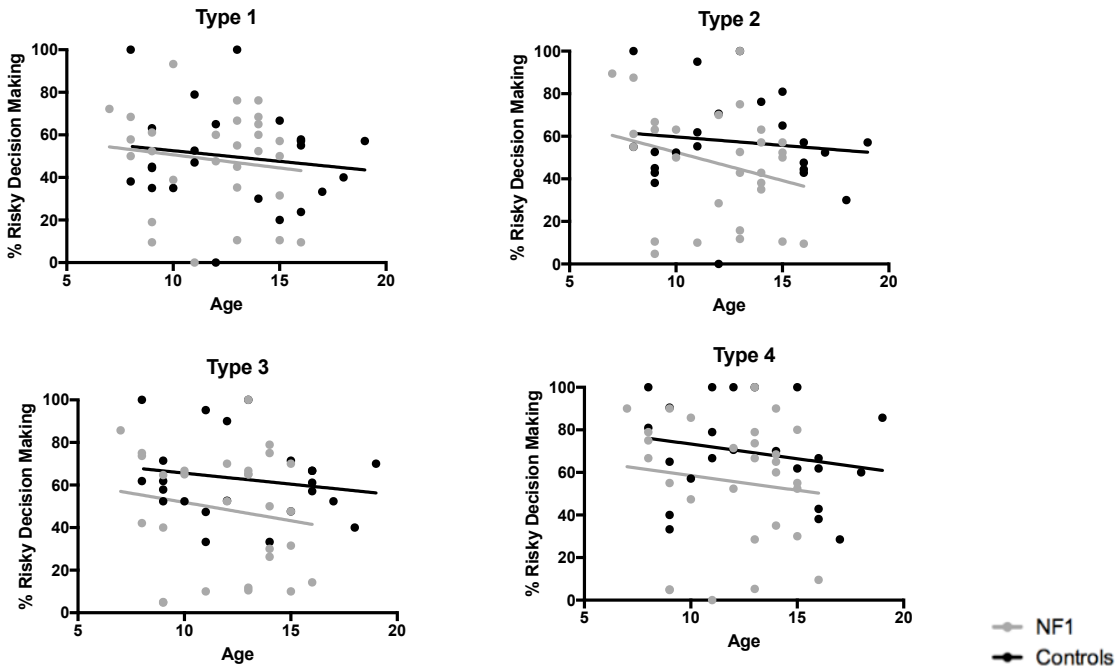


Figure 4. *Relationship between risky decision-making and age in NF1 and controls.* Correlation values for each trial type are the following: Type 1. NF1: r = -0.134, p = 0.497; Control: r = -0.144, p = 0.521. Type 2. NF1: r = -0.270, p = 0.164; Control: r = -0.122 p = 0.588. Type 3. NF1: r = -0.161, p = 0.412; Control: r = -0.182, p = 0.417. Type 4. NF1: r = -0.122, p = 0.538; Control: r = -0.206, p = 0.357.

Post-Feedback Behavior

Next we investigated how wins and losses affect subsequent trial behavior in patients with NF1 and controls. After receiving positive feedback (win), patients with NF1 were more

likely than controls to make a safe decision (p=0.043; Figure 3b). Patients and controls did not differ in behavioral responses after receiving negative feedback (loss).

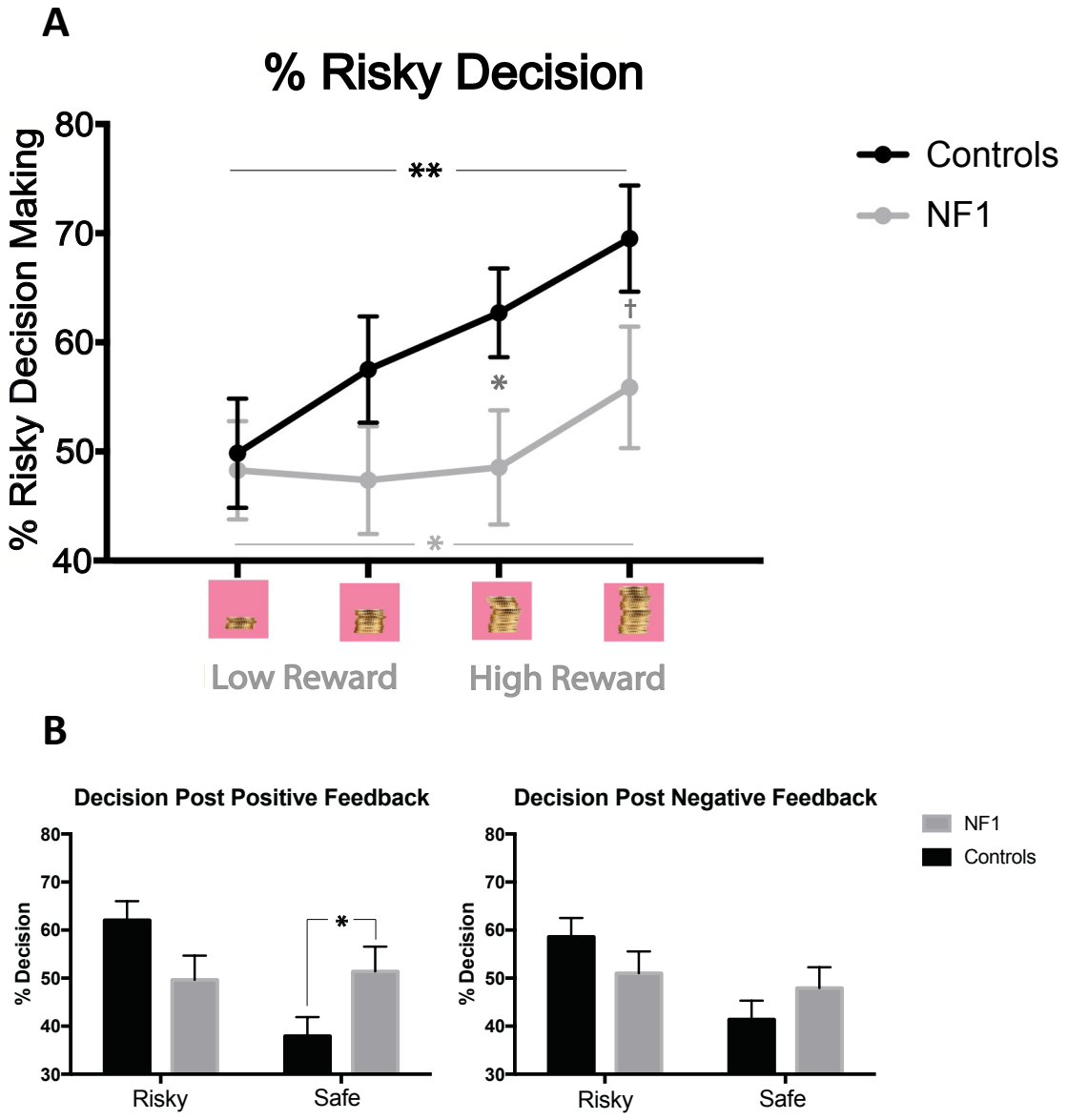
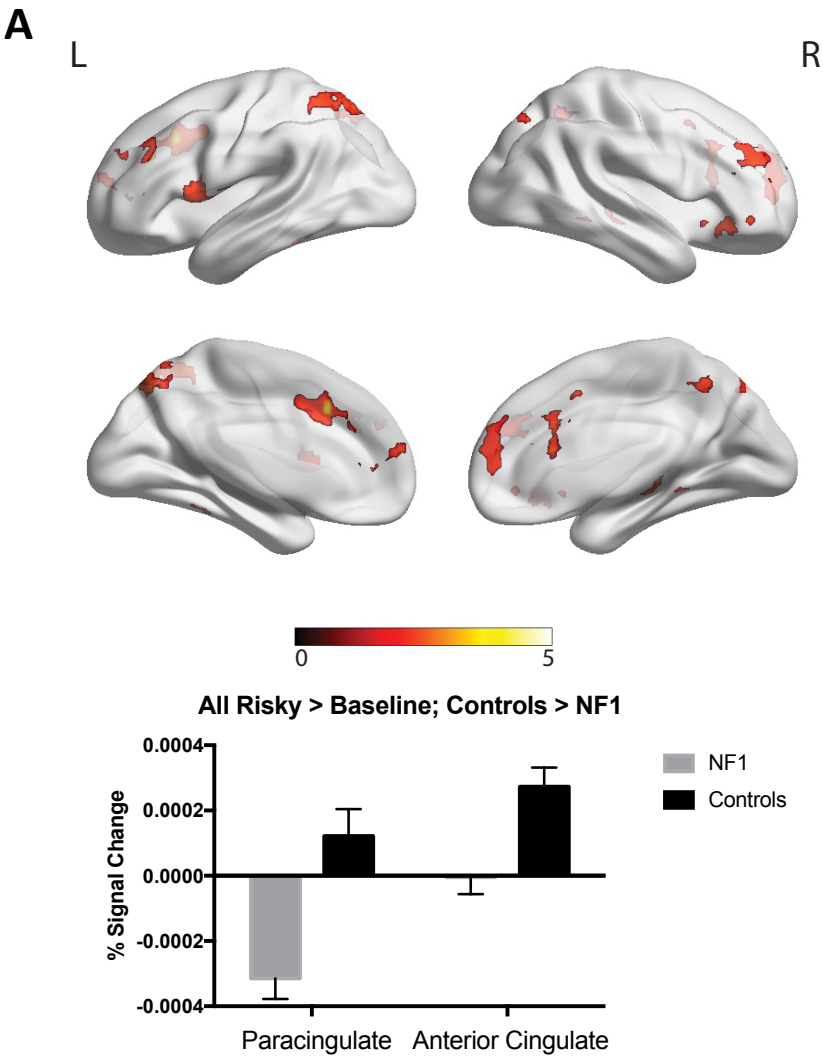


Figure 3. *A. Overall Performance.* Patients with NF1 showed reduced risky decision-making relative to controls in the 12 coin condition (p=0.042), and a trend towards reduced risky decision-making in the 16 coin condition (p=0.083). Both groups showed an increased tendency to make risky decisions with higher reward (p=0.003 in controls; p=0.013 in NF1 patients). *B. Post Feedback Behavior.* After receiving positive feedback (win), patients with NF1 were more likely than controls to make a safe decision (p=0.043). The groups did not differ on their responses after a loss.

fMRI Results

Decision Phase

When making risky decisions, patients with NF1 showed decreased neural activity as compared to controls in multiple regions, including the paracingulate cortex and anterior cingulate cortex (Figure 5a). Similarly, when making safe decisions, NF1 patients showed decreased activity relative to controls in the supramarginal gyrus and angular gyrus (Figure 5b).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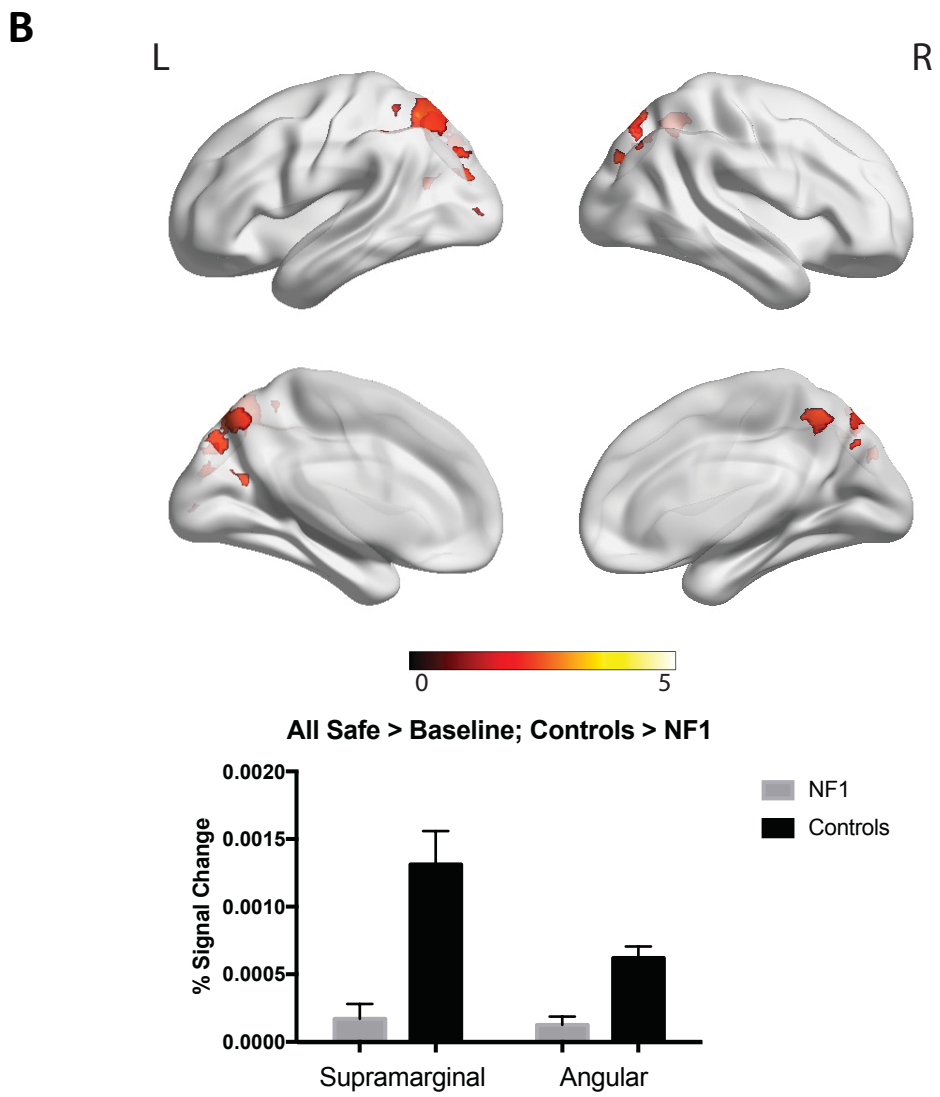

Figure 5. *fMRI results for neural activity during risky and safe decision making.* Clusters represent regions where controls show significantly increased activity as compared to patients with NF1. Graphs represent percent signal change. *A. Risky choice*. We found significantly increased activity in the paracingulate cortex (p=6.56e-07) and anterior cingulate cortex (p=9.67e-05) in controls relative to NF1 patients. *B. Safe choice*. Controls showed significantly increased activity in the supramarginal gyrus (p-5.32E-11) and angular gyrus (p=4.83E-06) relative to NF1 patients.

No regions were found in either of these contrasts in which patients with NF1 showed increased neural activity compared to controls. When investigating the effect of age, we found an

age by group interaction for risky decisions versus safe decisions in several regions, including the frontal pole and angular gyrus. Specifically, older controls showed decreased activity in these regions during risky decision-making, whereas patients with NF1 showed the opposite pattern (Figure 6).

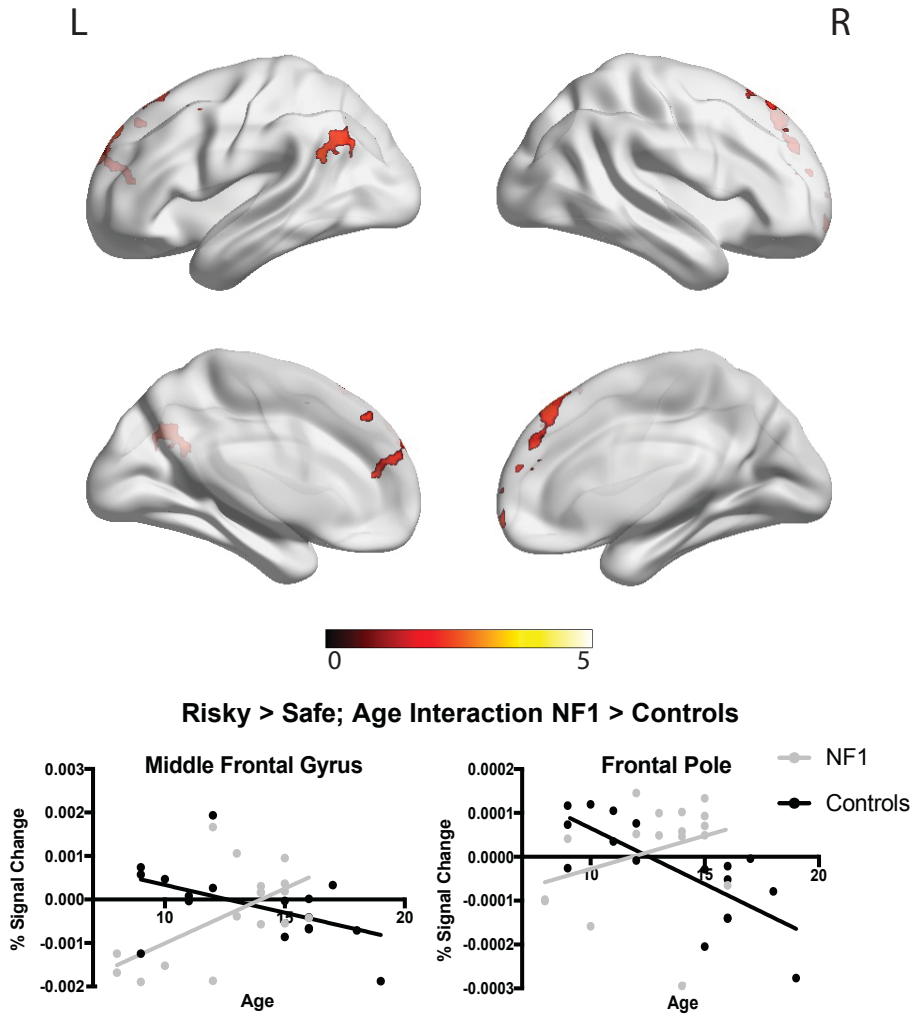

Figure 6. *fMRI results for neural activity during risky vs. safe decision making, and relationship with age.* Clusters represent regions that showed a significant group by age interaction effect for risky vs. safe decisions. We found a significant interaction in the middle frontal gyrus and frontal pole, such that controls showed a negative relationship between neural activity and age, whereas patients with NF1 showed increasing neural activity with increasing age. Graphs represent percent signal change.

When investigating the relationship between neural activity and risky decision-making (overall percentage of risky decisions made throughout the entire task), we found that controls with a higher tendency to make risky decisions showed decreased activity in the posterior cingulate cortex and frontal pole, whereas patients with NF1 showed increased activity in these regions in relation to risky decision making (Figure 7).

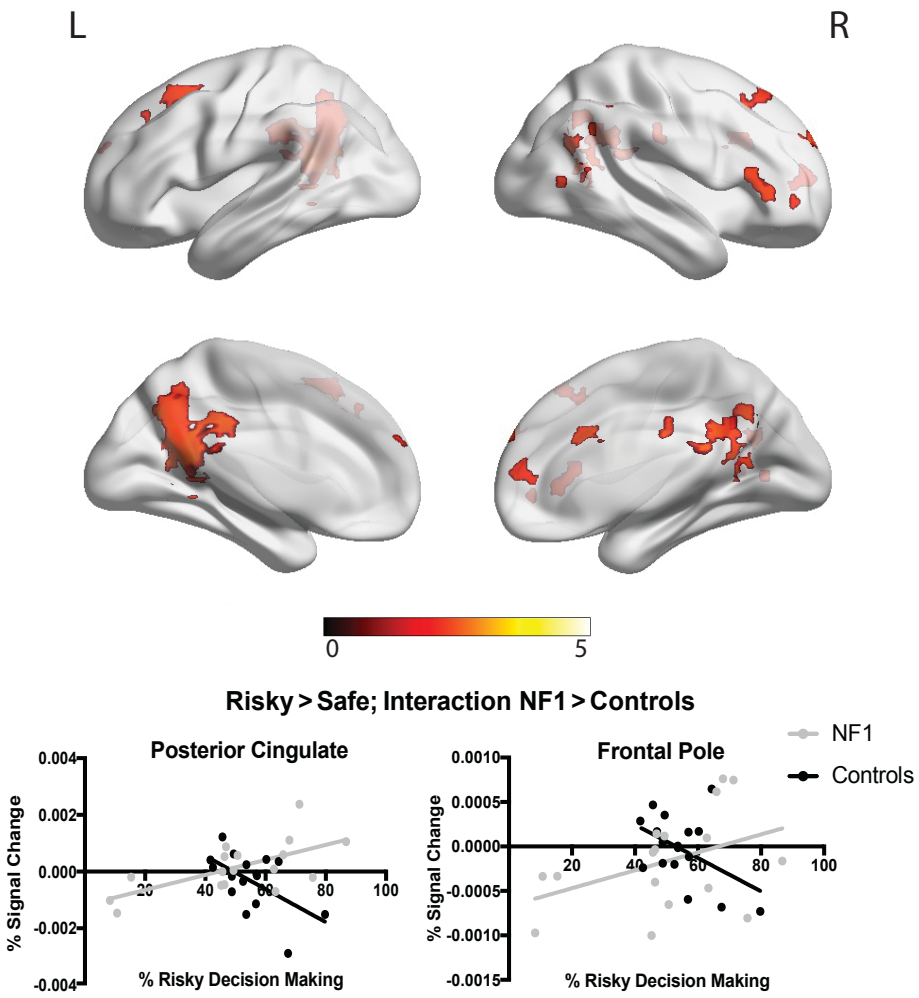

Figure 7. *fMRI results for neural activity during risky vs. safe decision making, and relationship with individual propensity to make risky decisions*. Clusters represent regions that showed a significant group interaction .Specifically, we found a significant interaction in the posterior cingulate cortex and frontal pole, such that controls showed a negative relationship between neural activity and risky decision-making, whereas patients with NF1 showed a positive relationship between neural activity and risky decision-making. Graphs represent percent signal change.

Outcome Phase

When receiving positive feedback (win), as compared to negative feedback (loss), controls showed increased activity in the nucleus accumbens, caudate, and putamen; in contrast, patients with NF1 did not show any differences in neural activity for win vs. loss (Figure 8).

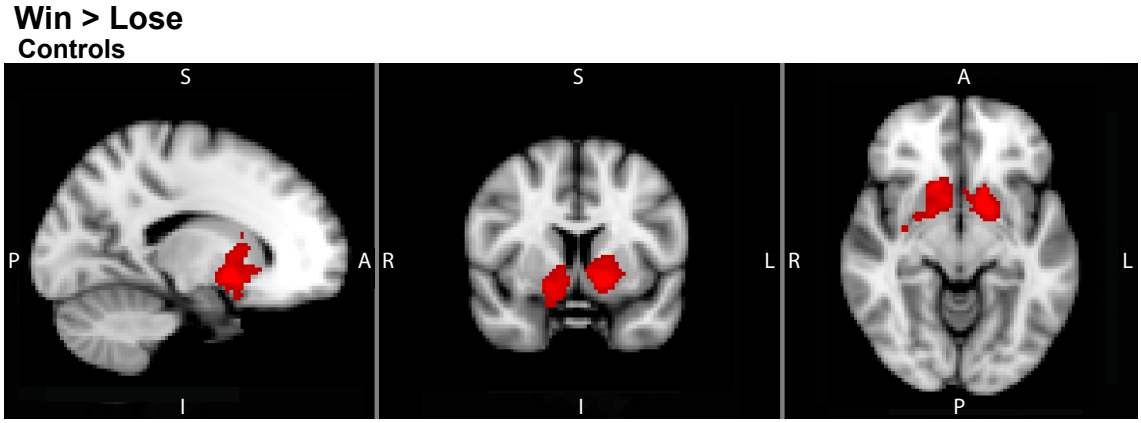

Figure 8. *fMRI results for neural activity during win vs. loss trials in controls*. Controls showed significantly increased activity in striatal regions (putamen, caudate, and nucleus accumbens) for win vs. loss. In contrast, patients with NF1 did not show any significantly increased neural activity during a win vs. loss.

Secondary Analyses

We conducted a secondary analysis in order to determine whether individual differences in IQ or ADHD diagnosis contributed to differences in risky decision-making. We did not find a significant relationship between IQ and risky decision-making in either patients with NF1 ($r = -0.05$, $p = 0.803$) or controls ($r = 0.35$, $p = 0.13$). Finally, there was no significant effect of ADHD status on risky decision-making in patients with NF1 ($p = 0.91$) or controls ($p = 0.67$).

Discussion

This is the first known study to investigate risky decision-making in patients with NF1, a highly relevant area of investigation given the high rates of ADHD, hypothesized dopaminergic

and GABAergic dysregulation in corticostriatal networks in this clinical population. Behaviorally, patients with NF1 tended to be less likely than controls to make risky decisions when the potential reward was high. They showed reduced neural activity in regions associated with reward, both during the decision-making and the outcome phases. Further, they did not show the typical age-related neural trajectories seen in controls, particularly in prefrontal regions.

Tendency towards decreased risky decision-making in patients with NF1

Our behavioral findings are suggestive of lower reward sensitivity in patients with NF1 as compared to controls, consistent with the notion that lower striatal dopamine levels are associated with decreased sensitivity to reward (30). In a mouse model of NF1, lower dopaminergic levels were found in the striatum (9), which may contribute to abnormalities in attentional and reward-based cognitive functions. The mice were given drugs that increased dopaminergic levels, which rescued the behavioral deficits, suggesting that abnormal dopaminergic homeostasis has a role in this behavior in NF1 patients (8). Relatedly, patients with Parkinson's disease, who have decreased dopamine levels in the striatum, show deficits on a variety of reward-based tasks, which are remediated with dopamine agonist therapy (31).

Neural activity during risky and cautious decision-making

When making risky (relative to safe) decisions, patients with NF1 showed decreased neural activity in the paracingulate and anterior cingulate cortex, whereas controls showed increased activity. Both of these regions have been implicated in reward processing (32; 33). When making a safe decision, controls showed increased supramarginal and angular gyrus activity, relative to patients with NF1. In a previous study of healthy individuals, risky decision-making was associated with increased regional activity in the medial PFC, whereas increased activity in dorsolateral PFC was observed during safe decision-making (25). The angular gyrus is

considered a functional hub within the default mode network, involved in self-referential mentation (34). It is also implicated in spatial representations of numbers (35). The decrease we observed in task-based neural activity within this region has also been observed in the resting state in patients with NF1 (36), a result consistent with increased GABA release in mouse models of this condition (5-7). Consistent with the directionality of our findings, prior fMRI studies have also found decreased neural activity in patients with NF1 relative to controls within the context of visual processing (36-38), and spatial working memory tasks (7).

The relationship between age and neural activity during risk-taking

When making risky decisions, patients with NF1 showed atypical age-related neural trajectories as compared to controls. In controls, age-associated decreases during risky decision-making were seen in the middle frontal gyrus and frontal pole, whereas patients with NF1 showed the opposite pattern. The prior study employing this task in healthy individuals found age-related decreases in neural activity in the anterior cingulate (25), which they attribute to a decreased need for cognitive control with increasing age. Frontal pole engagement has been linked the integration of higher-level cognitive processes (39), and specifically to processing effort and risk costs (40). The decreased activity we see in healthy individuals is consistent with the hypothesis of a reduced need for neural effort with increasing age. Patients with NF1 do not show this pattern, suggesting an altered maturational trajectory (41).

Individual differences in risk-taking and neural activity

In controls, a tendency to make risky decisions was associated with decreased activity in the frontal pole and posterior cingulate, whereas the opposite pattern was found in patients with NF1. The directionality of our findings in healthy individuals is consistent with that observed by Van Leijenhorst et al. (25). Decreased activity in the frontal pole (involved in cognitive control

and integration of higher-order functions) and the posterior cingulate (a functional hub of the default mode network) in individuals with high levels of risk-taking may reflect a lack of control and inhibition, resulting in riskier behavior. The positive relationship between risk-taking and neural activity in patients with NF1 suggests that deciding to make a risky decision may be a more cognitively demanding process for them.

Neural activity during positive outcome

When receiving positive feedback after making a decision, controls showed increased neural activity in the nucleus accumbens, caudate, and putamen, whereas patients with NF1 did not show this pattern. Similarly, Van Leijenhorst et al (25) found an adolescent-specific peak in the VS in response to a reward, which aligns with the average age of participants in our study. Consistent with this, reward anticipation is associated with activation of a cortico-basal ganglia circuit, known to be modulated by dopamine in the VS (42). The lack of increased striatal activity in patients with NF1 when winning a reward may reflect decreased reward sensitivity in this group, consistent with findings of enhanced GABAergic (7) and altered dopaminergic function in striatal regions in an NF1 mouse model (8).

Neuroimaging findings in idiopathic ADHD

Given the high rates of ADHD in patients with NF1 (41% of our patient sample; 58% of those with the "inattentive type", 42% with the "combined type", and none with the "hyperactive type"), it is worth noting general patterns from task-based functional neuroimaging studies of idiopathic ADHD. Several fMRI studies of executive function tasks have found globally decreased activation in patients with idiopathic ADHD compared to controls (43) . Smith et. al found decreased insula and inferior frontal gyrus activity in patients with ADHD during a response inhibition task (44), and Scheres et al found that patients with ADHD showed

decreased striatal activity during reward anticipation on a monetary incentive task (45). Our findings of hypoactivation during decision-making are consistent with the directionality of this prior work in idiopathic ADHD.

Limitations

Several limitations of the current study must be noted. First, the size of our sample with usable imaging data is relatively small, which reduces our statistical power. Given the prevalence of NF1 (1:3500), accruing large samples is challenging; our sample size is similar to prior neuroimaging studies in this patient population. Another limitation of our study is the high rate of motion experienced by participants in the scanner. Our participants are young, and many of the patients with NF1 have attentional problems, which may cause restlessness while in the scanner. Notably, however, there were no significant differences in motion in patients with NF1 vs. controls included in the analysis, indicating that our results are not an artifact of differential motion.

Conclusions

In the first study to investigate the functional neuroanatomy of risky decision-making in youth with NF1, we found a tendency toward more conservative decision making, concomitant with hypoactivity of brain regions critical for higher-order semantic processing and motivation, in patients with NF1 relative to typically developing controls. Future PET studies are warranted to investigate dopamine receptor occupancy in the brains of patients with NF1, and the relationship to reward-related behavior.

Further work should also investigate how differences in risk-taking develop across the lifespan, and how they may interact with clinical symptomatology of NF1. Additionally, given pre-clinical evidence for reduced striatal dopamine and enhanced GABAergic function in NF1,

studies that directly investigate the role of striatal dopaminergic and GABAergic function in reward processing in human patients with NF1 are warranted.

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CHAPTER 3: The 22q11.2 Deletion Syndrome as a Window into Complex Neuropsychiatric Disorders Over the Lifespan

Rachel K. Jonas, Caroline A. Montojo, Carrie E. Bearden

Abstract

Evidence is rapidly accumulating that rare, recurrent copy number variants (CNVs) represent large effect risk factors for neuropsychiatric disorders. 22q11.2 Deletion Syndrome (22q11DS; Velo-Cardio-Facial Syndrome (VCFS) or DiGeorge Syndrome) is the most common known contiguous gene deletion syndrome, and is associated with diverse neuropsychiatric disorders across the lifespan. One of the most intriguing aspects of the syndrome is the variability in clinical and cognitive presentation: children with 22q11DS have high prevalence of autism spectrum (ASD), attention deficit, and anxiety disorders, as well as psychotic-like features, and up to 30% of adolescents and adults develop schizophrenia-like psychosis. Recently, cases of early-onset Parkinson's Disease in adults have been reported, collectively suggesting a role for disrupted dopaminergic neurotransmission in the observed neuropsychiatric phenotypes. There is also some evidence that 22q11DS-associated ASD and schizophrenia represent two unrelated phenotypic manifestations, consistent with a neuropsychiatric pleiotropy model. This genetic lesion thus provides a unique model for the discovery of specific genomic risk and (potentially) protective factors for neuropsychiatric disease. Here we provide an overview of neuropsychiatric findings to date, which highlight the value of this syndrome in mapping the developmental trajectory of dimensional phenotypes that traverse multiple diagnostic categories. Potential sources of genetic variability that may contribute to the disorder's heterogeneous presentation are reviewed. Because of its known genetic etiology, animal models can readily be developed that recapitulate specific aspects of the syndrome.

Future research directions involve translational models and potential for drug screenable targets in the context of this human model system.

Introduction

22q11.2 Deletion Syndrome (22q11DS; OMIM #192430), also known as Velocardiofacial or DiGeorge Syndrome, is a neurogenetic disorder resulting from a hemizygous microdeletion of approximately 1.5 – 3 megabases (Mb) on the long arm of chromosome 22. With an estimated prevalence of 1/4000 live births, it represents one of the most common known recurrent copy number variants (CNVs). Its physical manifestations frequently include cleft palate, hypocalcemia, cardiac defects, and immune dysfunction (1; 2). 22q11DS is also associated with strikingly elevated risk for neuropsychiatric illness, particularly psychosis (3; 4); 25-30% of individuals with this syndrome develop schizophrenia or affective psychosis, making 22q11DS one of the greatest known risk factors for psychotic illness identified to date. Microdeletions of 22q11.2 account for up to 1–2% of schizophrenia cases and represent the only known recurrent CNV responsible for introducing new cases of schizophrenia in the population (5; 6). Moreover, non-psychotic psychiatric disorders and behavioral abnormalities are present from early childhood in 22q11DS (7; 8). This phenotypic variability implies that there may be distinct biological mechanisms that underlie the development of these psychiatric conditions. Several of the genes encoded in the deleted region are highly expressed in the brain, and known to affect early neuronal migration and cortical development (6; 9). As such, this syndrome provides a unique opportunity to connect genes to brain to behavior.

While about 85% of patients have approximately the same 3 Mb microdeletion, containing about 60 known genes (Figure 1), variability in the deletion size and breakpoint

locations, as well as the characteristics of the intact chromosome 3(1; 6), may play an important role in the observed phenotypic variability in individuals with 22q11DS.

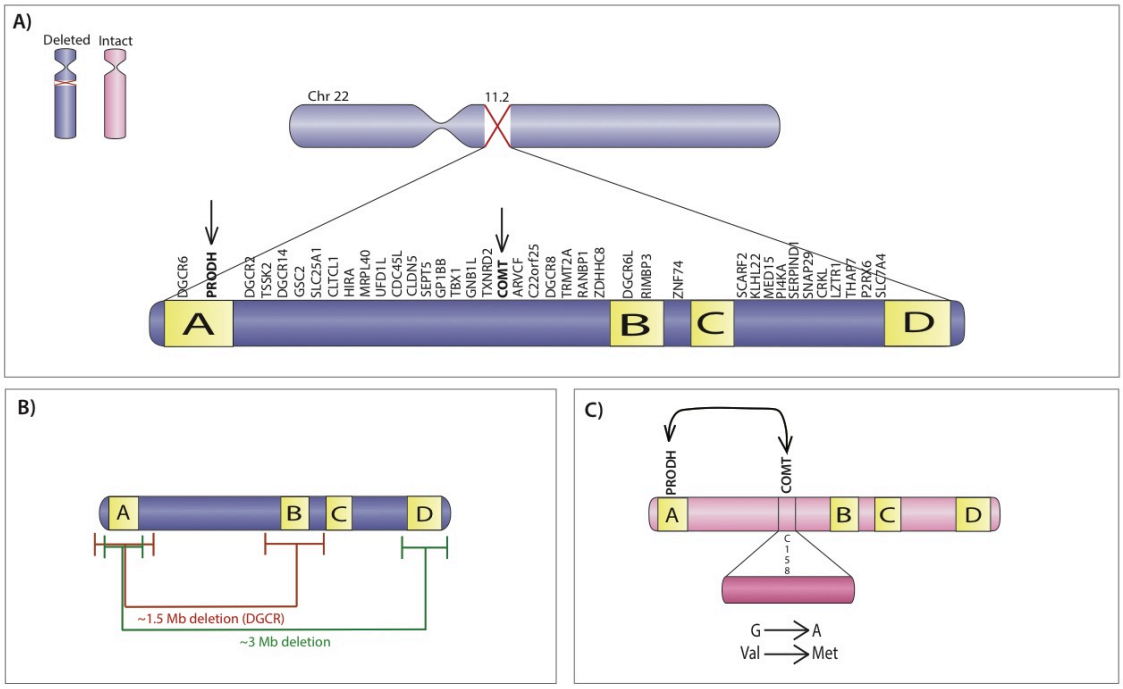
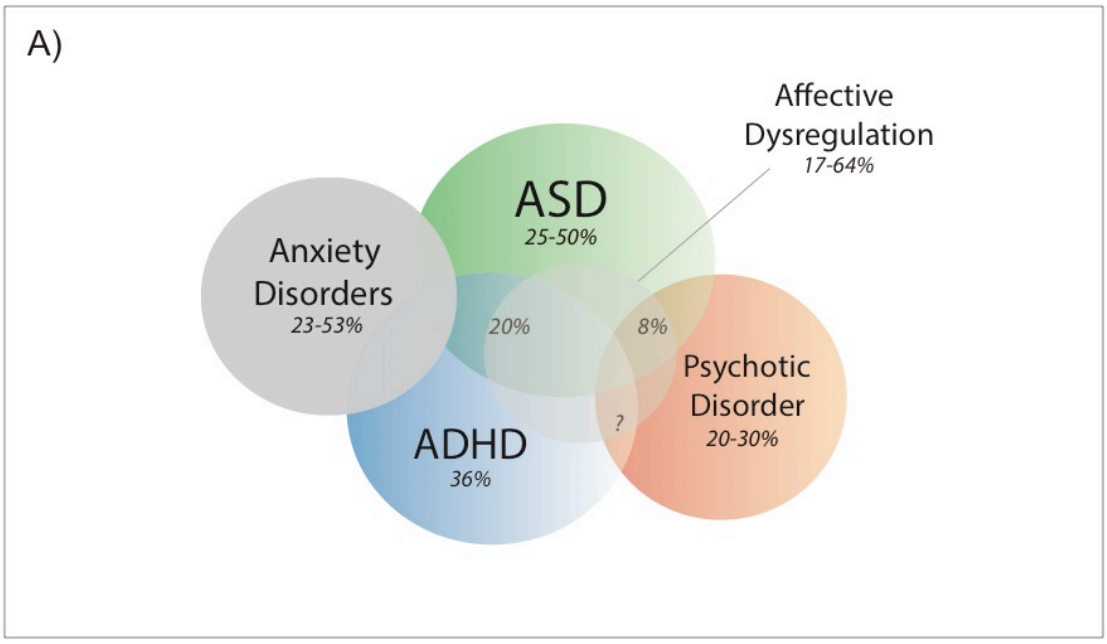


Figure 1. *Chromosome 22q and potential sources of genetic variability.* (A) Hemizygous 22q11.2 deletion (light purple) and intact chromosome (light pink). To the right, the deleted segment of Chromosome 22 is shown (dark purple). Deletion breakpoints most commonly occur within the four distinct blocks of LCRs that lie in the deletion interval (termed A, B, C, D) (yellow) and deleted genes in the 22q11.2 locus are labeled above the segment (102). The genes COMT and PRODH are highlighted with arrows to indicate specific examples of genetic variability that are discussed in this review. (B) Breakpoint variability. The deleted segment is illustrated with the two most common deletion lengths, 3Mb (green line) and 1.5Mb deletions (red line), though other atypical deletions have been reported. Error bars denote approximate variance in the deletion breakpoints for the 1.5 and 3Mb deletion lengths. The amount of variability in the deletion breakpoints may differ as a function of deletion size (102). The disorder is defined by a deletion in the DiGeorge critical region (DGCR), i.e. the region of the chromosome located between markers D22S36 and D22S788, which flank LCRs A and B. (C) Allelic variation within the intact chromosome and epistasis. The COMT Val^{158}Met gene variant on the intact chromosome is illustrated as an example of the potential role of allelic variation, involving substitution of a methionine (Met) for valine (Val). As an example of episatasis, PRODH and COMT are illustrated to show the interactive role of the two gene products. Additional sources of variability include, but are not limited to: unmasking of autosomal recessive mutations via hemizygous deletion, parent of origin effects, and epigenetic effects.

Here we first review current literature on neuropsychiatric phenotypes across the lifespan in 22q11DS, and potential sources of genetic variability that may contribute to the heterogeneous presentation of the disorder. Next, we describe candidate endophenotypes relevant to neuropsychiatric risk, which can be assayed in both humans and animal models, helping us to bridge the gap between genetic and phenotypic variation. Finally, we suggest key directions for future research, involving new computational modeling methods and *in vitro* disease models, which show great promise for elucidating the molecular mechanisms underlying variable neuropsychiatric phenotypes of 22q11DS. Detailed coverage of medical comorbidities of 22q11DS and genetic association findings relevant to this locus in idiopathic neuropsychiatric disorders are outside the scope of this review, but are reviewed in detail elsewhere (10-12) .

The Neuropsychiatric Phenotype of 22q11DS

The most specific neuropsychiatric phenotype associated with 22q11DS is schizophrenia, as this is the only psychiatric condition that appears to be found at much higher frequency among 22q11.2 microdeletion carriers relative to other neurogenetic and developmental disorders associated with intellectual disability (5; 6). Nevertheless, ⅓ to ½ of children with the deletion are diagnosed with attention-deficit/hyperactivity disorder (ADHD), anxiety disorders (most commonly specific and social phobia), mood disorder, and autism spectrum disorders [ASDs; (1; 2; 7; 8;13; 14); see Figure 2A]. Indeed, at any given age at least 60% of individuals with 22q11DS meet diagnostic criteria for at least one psychiatric diagnosis, regardless of ascertainment method (3; 4; 14). Notably, psychopathology in 22q11DS encompasses emotional, behavioral and social disruptions in domains that cut across traditional diagnostic categories. For example, affective dysregulation, assessed dimensionally, may be part of a 'core neuropsychiatric phenotype' of 22q11DS (5; 6; 14).


A)
Affective Dysregulation
17-64%
ASD
25-50%
Anxiety Disorders
23-53%
20%
8%
Psychotic Disorder
20-30%
?
ADHD
36%

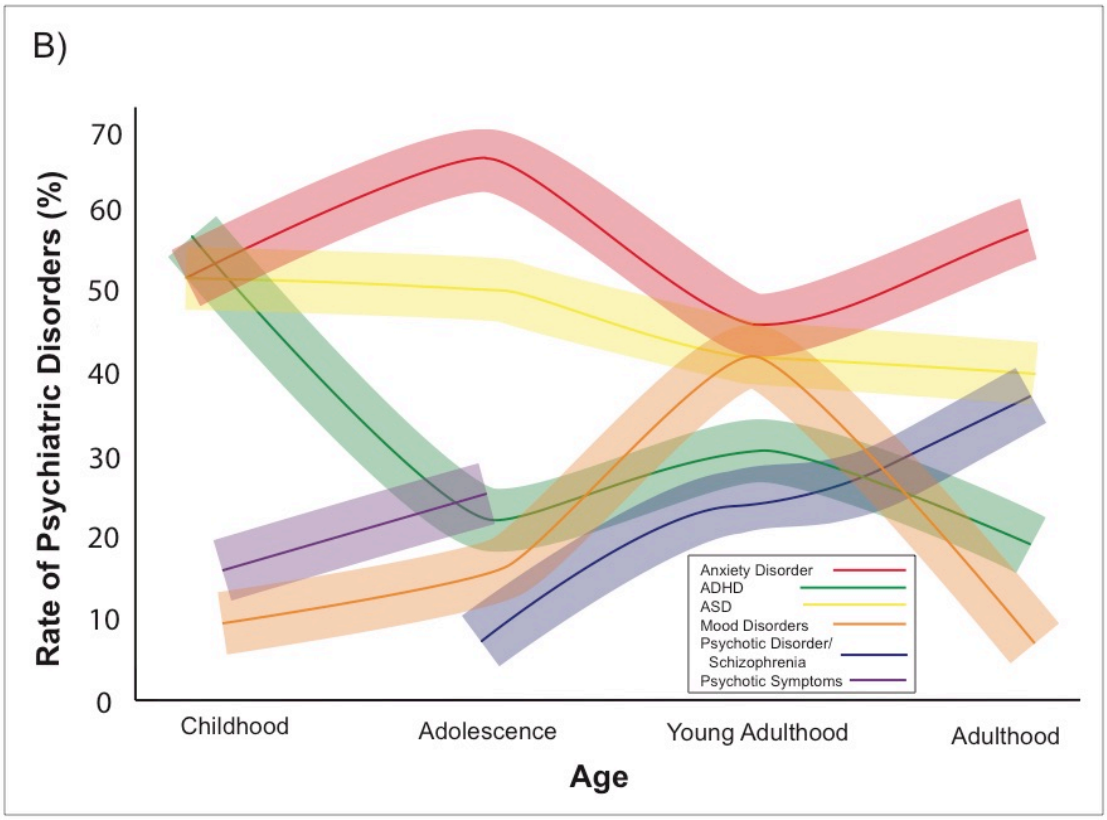

B)
Rate of Psychiatric Disorders (%)
70
60
50
40
30
20
10
0
Childhood
Adolescence
Young Adulthood
Adulthood
Age
Anxiety Disorder
ADHD
ASD
Mood Disorders
Psychotic Disorder/ Schizophrenia
Psychotic Symptoms

Figure 2. *Overlapping and Distinct Neuropsychiatric Phenotypes in 22q11DS.* (A) As a conceptual illustration of the variability of neuropsychiatric phenotypes in 22q11DS, we include autism spectrum disorder (ASD) (18; 103), attention-deficit/hyperactivity disorder (ADHD) (5), anxiety disorders, and psychosis (5), and estimated comorbidity rates across disorders based on existing literature (8; 103). It is important to note that comorbidity rates are not frequently reported in the literature; this is a critical issue for future research. Additionally, affective dysregulation is present in a substantial proportion of 22q11DS patients, regardless of diagnosis. (B) *Developmental trajectories of psychiatric disorders with 22q11DS*. As shown in the figure legend**,** each colored line portrays the estimated prevalence of a particular psychiatric disorder in 22q11DS patients throughout the lifespan. Shaded error bars for each line are illustrated to reflect variability across studies. Each percentage point on the line reflects data from published 22q11DS studies reporting on prevalence rates of anxiety disorder (5; 104), ADHD (5), ASD (18), mood disorder (5) psychotic disorder/schizophrenia (5; 77), and psychotic symptoms (17). In cross-sectional studies, rates of mood disorder (particularly depression) appear to peak in late adolescence and then decline, whereas rates of anxiety remain high through adulthood.

Some investigators have proposed that psychiatric diagnoses other than psychosis in 22q11DS represent nonspecific expressions of factors that affect brain development and function (6-8; 15); however, these distinct phenotypic manifestations may also represent genetic pleiotropy, in which the same genetic alteration can result in multiple physiological effects and phenotypic expressions. For instance, Vorstman et al. (8) found that, among patients diagnosed with schizophrenia in adulthood, only 8% had a probable ASD diagnosis in childhood, suggesting that ASD and schizophrenia may be distinct, pleiotropic manifestations of a 22q11.2 deletion. This phenomenon of distinct neuropsychiatric phenotypes is common to many CNVs that appear relevant to the etiology of idiopathic schizophrenia and autism (10-12; 15). It should be noted, however, that this study was conducted cross-sectionally, and thus the diagnosis of 'probable ASD' during childhood was made retrospectively (8). Prospective longitudinal studies

are needed to confirm the intriguing possibility that 22q11DS-associated ASD and schizophrenia represent examples of true neuropsychiatric pleiotropy.

Developmental Trajectories of Neuropsychiatric Phenotypes

Although attention deficits (dimensionally assessed) characterize the vast majority of children with 22q11DS, clinical diagnoses of ADHD are not particularly stable over time (14). Nevertheless, a four-year longitudinal study (16) recently found that persistence of ADHD into adolescence in 22q11DS is predicted by childhood variables previously documented in the non-22q11DS ADHD literature, including higher rates of familial ADHD and history of childhood depression. These findings suggest that genetic background (i.e. family history) may also play a role in the variable neuropsychiatric phenotypes of 22q11DS.

The largest study of lifetime psychiatric diagnoses to date in 22q11DS (N=172; ages 5-54 years) combined data from 22q11DS cohorts from Israel and Switzerland (5), and found remarkably similar prevalence and developmental trends across countries. ADHD and anxiety disorders were the most common diagnoses during childhood (although notably, this study did not report on rates of ASDs), whereas rates of psychosis and mood disorders increased dramatically during adolescence and young adulthood. Additionally, while the average age at onset of overt psychotic disorder in 22q11DS is 19 to 26 years (13), earlier manifestations of psychotic-like symptoms characterize almost 1/3 of 22q11DS adolescents (17; 18), and 17% of pre-adolescent children (Figure 2B), suggesting a continuum of psychotic symptom severity in 22q11DS. Moreover, the socio-behavioral correlates of psychotic symptoms in 22q11DS youth - increased social withdrawal, reduced adaptability, and higher anxiety/depression - appeared strikingly similar to those reported in prospective studies of familial risk for schizophrenia (19). Additionally, consistent with epidemiologic studies in the general population (20), cognitive

deterioration in adolescence is a dynamic phenotype that may be a potent predictor of psychosis in 22q11DS (21; 22).

Collectively, these findings implicate early social and cognitive abnormalities as risk factors for subsequent development of overt psychotic disorder in 22q11DS; however, longer-term longitudinal follow-up studies are required to better understand the clinical significance and persistence of early psychotic symptoms in 22q11DS youth.

Most children with 22q11DS now survive into adulthood, but little is known about adult functioning. A recent study found significant functional impairment in over 75% of adults with 22q11DS (23). Variability in adaptive functioning was mediated primarily by cognitive abilities and the presence of psychotic disorder. Identification of remediable factors associated with better functioning is a key question for future research.

Finally, one notable and understudied aspect of the 22q11DS phenotype in older adults is that of early-onset Parkinson's Disease. This phenotype has now been described in multiple case reports (24; 25), suggesting that dopaminergic disruption in 22q11DS may be relevant to the expression of both psychosis and Parkinson's Disease over the lifespan.

Collectively, these findings illustrate the substantial heterogeneity in the 22q11DS neuropsychiatric phenotype, which may be linked, at least in part, to underlying sources of genetic variability. The spectrum of associated psychopathology suggests a model of genetic pleiotropy, and additionally implies that schizophrenia and other neuropsychiatric disorders may share overlapping biological pathways (15; 26).

Sources of Genetic Variability

An initial approach to characterizing genetic variability in 22q11DS was to investigate the effect of the two most common deletion lengths, 1.5Mb and 3Mb, on clinical phenotypes.

Early studies did not find evidence for effects of deletion size on severity of syndromic features (27; 28), and it has been argued that the 1.5Mb region contains all of the key genes responsible for the development of the syndrome and associated psychiatric risk (28; 29). However, more recent studies using high-resolution tiling arrays have noted considerably more variation in deletion breakpoints than previously observed (26), suggesting that more precise mapping may yield new insights regarding phenotypic differences between patients with seemingly similar microdeletions.

Close to 90% of cases of 22q11DS arise from *de novo* mutations, whereas approximately 10% of cases are inherited in an autosomal dominant fashion (26; 29). In *de novo* cases, the microdeletion occurs due to mispairing of low copy repeats (LCRs) during meiosis (1; 30). LCRs, or segmental duplications, are found throughout the genome; the 22q11 region contains several large (60- to 600-kb) clusters of such LCRs (26; 31). These regions tend to predict genomic instability, and often cause breakages implicated in various genetic disorders, via non-allelic homologous recombination (see Table 1).

Table 1. *Glossary*

Breakpoint	A specific site of chromosomal breakage associated with a chromosomal abnormality.
Copy Number Variant (CNV)	A type of genomic variation in which segments of DNA of more than 1,000 base pairs are duplicated or deleted, as genomic risk factor for common complex brain disorders. 22q11.2 microdeletion and duplication are examples of specific CNVs.
Endophenotype	A state-independent biomarker or cognitive marker of an illness (present whether or not the illness is active) that is heritable and present in unaffected relatives of subjects that have the illness (146).
Epistasis	Interactions between genes in which the contribution of one gene to a phenotype depends on the genotype at another locus.
Haploinsufficiency	The situation in which one copy of a gene is incapable of providing sufficient protein production to ensure normal function.
Hemizygosity	A genetic condition where there is only one copy of a gene in an otherwise diploid cell or organism.
Low copy repeats (LCRs)	Highly homologous sequence elements within the eukaryotic genome arising from segmental duplication.
Long-term potentiation (LTP)	A long-lasting enhancement in signal transmission between two neurons that results from stimulating them synchronously.
Pleiotropy	The phenomenon whereby one genetic mutation results in multiple, independent phenotypes
Prepulse inhibition (PPI)	A quantitative trait, readily measurable in humans and in mice, involving reduced magnitude of the startle reflex that occurs when the subject is presented with a weak stimulus, or prepulse, immediately before the startling stimulus is presented.
Single nucleotide polymorphism (SNP)	Genetic variation in a DNA sequence that occurs when a single nucleotide - A, T, C, or G - in a genome is altered, which can affect function of the gene product.

Although the precise mechanisms are currently unknown, four possible genetic mechanisms which may play a role in the clinical heterogeneity of 22q11DS are: 1) breakpoint heterogeneity, which may impact gene expression via inclusion or exclusion of specific genes in LCR regions (see Figure 1); 2) Allelic variation within the intact 22q11.2 chromosome,

which may have substantial effects on amino acid translation as there is no compensating normal allele, potentially resulting in downstream effects on behavior (32); 3) Epistatic interactions (i.e., the phenomenon whereby one gene modifies the effects of another) within the intact chromosome (33); 4) Hemizygosity of microRNA (miRNA) genes. miRNAs are short, non-coding ribonucleic acid (RNA) molecules found in eukaryotic cells, and are an essential part of the cellular machinery for regulating gene expression and transcription. Hemizygosity of miRNA genes in the DiGeorge Critical Region (DGCR)- specifically, *Dgcr8*, *mir-185* and *mir-649*- results in insufficiency of mature miRNAs, which can dramatically affect the target gene protein function (34; 35).

Additionally, findings are mixed regarding the contribution of parent of origin for the 22q11.2 deletion to phenotypic variability. One study reported that 11 of 12 *de novo* cases with psychosis had a maternal origin of the deletion (5). While other studies have found quantitative endophenotypes – gray matter volume and language abilities - linked to maternal origin of the deletion (36; 37), another research group found no evidence for an effect of parental origin of the deletion on schizophrenia risk (38).

Recessive mutations in the intact chromosome, which may be unmasked by hemizygous deletion at 22q11.2, present another potential source of genetic variability. This mechanism has been shown to contribute to a variety of genetic disorders (39-41), although to our knowledge has not yet been investigated in 22q11DS.

Finally, epigenetic effects refer to inherited changes in phenotype or gene expression resulting from mechanisms other than alterations in the underlying DNA sequence, such as DNA methylation (42; 43). Epigenetic effects are not well understood in 22q11DS and may be

an important source of phenotypic variability requiring further study in large samples, and in animal models.

Endophenotypes in Mice and Men

Analysis of quantitative traits that lie intermediate between these levels of analysis, such as changes in molecular and cellular properties, brain structure and function, and cognition, may better elucidate the pathophysiologic mechanisms linking structural genetic variation to distal psychiatric phenotypes. Such "deep phenotyping" approaches in the context of a known genetic model such as 22q11DS allow us to map a relatively homogeneous biological pathway to the development of complex neuropsychiatric disorders.

Notably, the mouse genome contains a region on chromosome 16 that is homologous to the 22q11.2 region in humans, and thus genetic techniques for selective deletion and/or over-expression of genes within the syntenic region in mice allow us to pinpoint genes contributing to specific behavioral phenotypes. Below we provide examples across multiple levels of analysis, based on both human and animal studies.

Structural Neuroanatomy

Humans. Collectively, human studies suggest global brain volumetric reduction in 22q11DS, particularly in the parietal lobes (44; 45), as well as significant thinning of midline brain regions (46). Interestingly, there appears to be a rostro-caudal gradient of volumetric reduction in 22q11DS, with caudal regions such as the occipital lobe and cerebellum showing greater reductions, while the frontal lobe is relatively preserved, at least in children (44; 47) (Figure 3). This gradient is conserved subcortically, where the caudate is more reduced in posterior regions than anterior (48), as is the thalamus (49) and corpus callosum (50; 51).

Genetic influences are likely to play a role in this rostro-caudal gradient, particularly genes that encode neurodevelopmental morphogens involved in establishing the anterior-posterior axis (52). With increasing age, there appears to be differential reduction in fronto-temporal regions, which may also be relevant to increased vulnerability to psychosis onset in adolescence in 22q11DS.

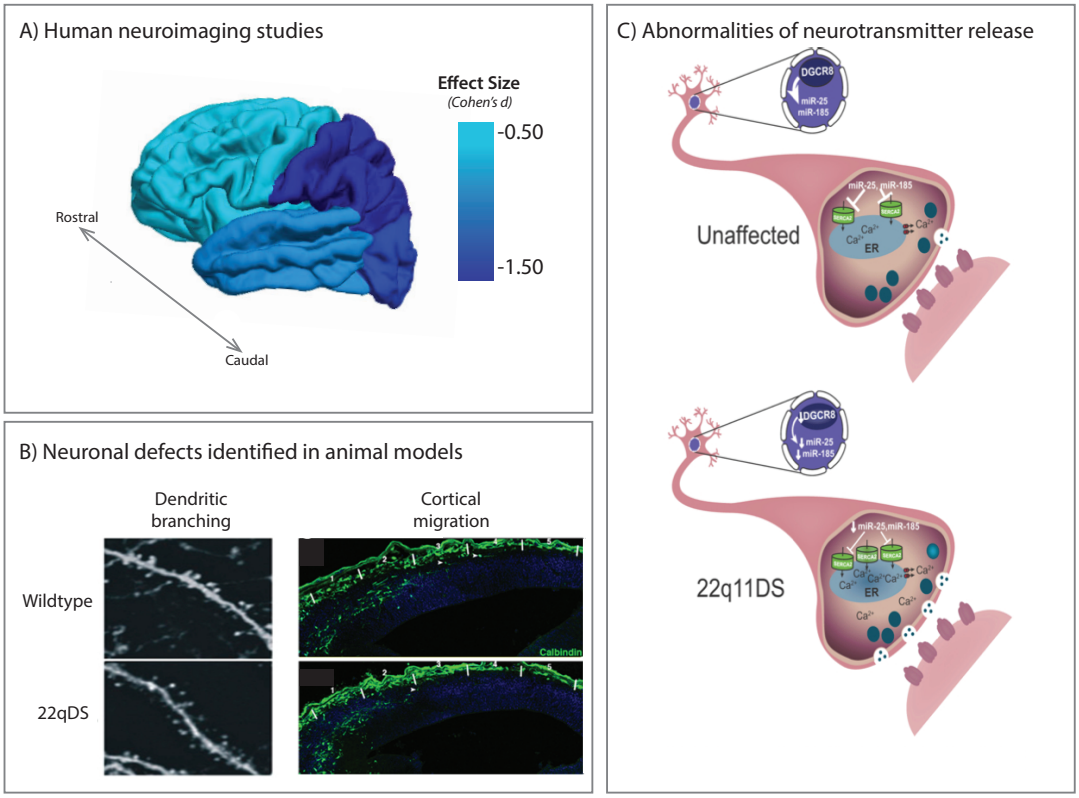


Figure 3. *Neuroanatomic Abnormalities in 22q11DS.* A) Effect sizes for lobar gray matter reduction in children with 22q11DS relative to typically developing controls, constructed from a meta-analysis of structural MRI studies (45). This effect tends to follow a rostral-caudal gradient. Although not displayed in the figure, effect sizes from subcortical and midline structures are variable, ranging from -0.86 (hippocampus) to -0.20 (amygdala). B) Irregular dendritic branching and abnormal interneuron cortical migration found in 22q11DS murine models (reprinted with permission from Fenelon et al, 2011 (105) and Meechan et al, 2009 (9)). The *Dgcr8* gene, within the 22q11.2 locus, is a key component of the microprocessor complex critical for miRNA production. As shown (left panel), *Dgcr8*$^{+/-}$ mice show reduced width of basal dendrites of pyramidal neurons in the medial prefrontal cortex (mPFC) compared to wildtype. Although basic synaptic transmission is normal in the mPFC of *Dgcr8*$^{+/-}$ mice, short-term synaptic plasticity is impaired, suggesting a neural substrate for cognitive impairment in 22q11DS. Right panel shows abnormal cortical migration of 22q11DS interneurons in the *LgDel* mouse model (9). While the frequency of calbindin-labeled interneurons did not differ between wildtype and *LgDel* mice, there is an abberant distribution, indicating disrupted interneuron migration, in the cortex of *LgDel* mice. C) Model of sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA2)-dependent mechanism of synaptic dysfunction in *Dgcr8*$^{+/-}$ mice, described in Earls et al., 2012 (58). SERCA2 upregulation leads to elevated endoplasmic reticulum Ca2+, increasing neurotransmitter release and increased long-term potentiation (LTP) in an age-dependent manner. MicroRNAs miR-25 and miR-185 are known regulators of SERCA2 and are absent in *Dgcr8*$^{+/-}$ mice; their restoration rescues LTP, suggesting that miRNA-dependent SERCA2 dysregulation may contribute to learning and neuropsychiatric phenotypes in 22q11DS.

Mice. Studies in mice suggest that anomalous cortical neurogenesis may underlie structural abnormalities observed in human MRI studies (Figure 3). Hemizygous deletion of the analogous 1.5 Mb region in mice disrupts proliferation of basal progenitors and interneuronal migration in the cerebral cortex (9), and leads to reduced dendritic spine density in the

hippocampus (34), suggesting that these phenomena may be partially responsible for observed cortical thinning in human 22q11DS patients. Nevertheless, studies in animal models cannot tell us whether specific neuroanatomic alterations are relevant to variable psychiatric outcomes.

Physiological Alterations and Synaptic Plasticity

Humans. Functional neuroimaging (fMRI) studies in humans with 22q11DS offer insights into how aforementioned structural changes manifest in terms of physiologic alterations. These studies have reported abnormal neural activity in tasks involving response inhibition (53) and working memory (54), specifically involving atypical parietal activation. Although differences in behavioral performance could contribute to the observed neurophysiologic differences, these findings offer preliminary evidence for atypical development of specific neural circuits critical for higher-order cognitive functions in 22q11DS.

Additionally, given that 22q11DS is associated with psychiatric disorders involving cortical dysconnectivity, the emerging field of resting state fMRI can offer important insights into the functional architecture of the resting brain in 22q11DS. Abnormalities in resting-state functional connectivity have been consistently implicated in idiopathic psychiatric illness (55); in particular, poorly synchronized 'long distance' connectivity in 22DS may serve as a biologically relevant intermediate phenotype for psychosis risk in 22q11DS (56). Functional variants in genes within the 22q11.2 locus have also been associated with schizophrenia risk, as well as relevant structural and functional neural connectivity defects, in the general population (57).

Mice. Intriguingly, studies in the *Df(16)A*$^{+/-}$ mouse model of 22q11DS have shown reduced hippocampal-prefrontal functional connectivity, suggesting a neuronal basis for long range connectivity defects in human 22q11DS patients. Additionally, altered synaptic plasticity, in the form of hippocampal long-term potentiation (LTP; see Table 1), has been found in mouse models of 22q11DS, a mechanism that may underlie task-based functional MRI abnormalities evident in human 22q11DS patients. This abnormal LTP phenotype is thought to result from haploinsufficiency of *Dgcr8*, a gene important for microRNA biogenesis; microRNA restoration rescued abnormal LTP levels in *Dgcr8*$^{+/-}$ mice (Figure 3). Interestingly, levels of SERCA2 were increased in brains of patients with idiopathic schizophrenia, providing a direct link between abnormalities in LTP and psychiatric illness (58).

Neurocognition and Behavior

Humans. Patients with 22q11DS exhibit a characteristic cognitive profile involving deficits in nonverbal learning, as well as social cognition, although there is substantial variability in IQ (59-61). Consistent with the literature on youth at familial high risk for psychosis (19; 62), executive function deficits also predict risk for subsequent development of psychotic symptoms in 22q11DS (63).

Notably, sensorimotor gating deficits, indexed by impairments in pre-pulse inhibition (PPI; see Table 1) have been consistently identified as an endophenotype of disorders characterized by poor inhibitory control of attention, including ASD and schizophrenia (64). Similarly, PPI was significantly reduced in 22q11DS patients relative to sibling controls, and lower PPI was associated with subsyndromal psychotic-like symptoms (65).

Mice. Through a series of studies, Hiroi and colleagues (66; 67) selectively knocked out or overexpressed various combinations of genes within the 22q11.2 homolog region, in order to

successfully pinpoint essential genes involved in PPI. Another study investigated mice carrying a multi-gene deletion (Df1+/-) that models 22q11DS, and subsequently used single-gene mutants to identify the causative genes involved in sensorimotor gating defects. Haploinsufficiency of two adjacent genes within the locus, *Tbx1* and *Gnb1l*, was found to cause the PPI phenotype, suggesting that these genes may be key contributors to the psychiatric phenotype of 22q11DS (68). In humans, the relevance of *TBX1* haploinsufficiency to the 22q11DS psychiatric presentation was further supported by the identification of a family in which the clinical manifestations of 22q11DS, including ASD, segregated with an inactivating mutation of *TBX1* (68).

COMT as a Model for Multi-Level Investigation in 22q11DS

Although haploinsufficiency for multiple genes in the 22q11.2 locus may contribute to the clinical phenotype, dopaminergic (DA) dysfunction is implicated in many of the associated neuropsychiatric phenotypes (69; 70). As such, the catechol-*O*-methyltransferase (*COMT*) gene within the DGCR has been a particular focus of investigation. *COMT* encodes a postsynaptic enzyme that modulates prefrontal cortical DA clearance (71). An evolutionarily recent, common polymorphism at codon 158 of the *COMT* gene involves substitution of a methionine (Met) for valine (Val). COMT enzyme activity in postmortem DLPFC is ~40% higher in human subjects with the *COMT*-Val allele than those with the *COMT*-Met allele (72). A similar effect on enzyme activity was confirmed in lymphocytes. This SNP has been widely studied in healthy individuals, and has been linked to differences in executive functioning and the development of psychiatric illness (73; 74). A recent meta-analysis indicated a significant effect of *COMT* genotype on prefrontal cortical function in the general population (75).

Given that *COMT* is hemizygously deleted in 22q11DS patients, genetic variation in the intact chromosome may have a more profound effect on phenotypic expression than that observed in non-deleted individuals. As such, many studies have investigated allelic variability in this gene in relation to phenotypic expression at multiple levels (Figure 4**)**. Overall, evidence for the contribution of *COMT* Val^{158}Met genotype to neuropsychiatric symptomatology in 22q11DS is mixed. While Gothelf and colleagues (76) found that the *COMT* Low-activity (Met) variant increased risk for the development of psychotic symptoms in 22q11DS youth, other groups found no effect of *COMT* genotype on psychosis spectrum phenomena (77) or neuropsychological performance (78). Discrepancies may be due to small and heterogeneous samples, highlighting the need for better-powered studies.

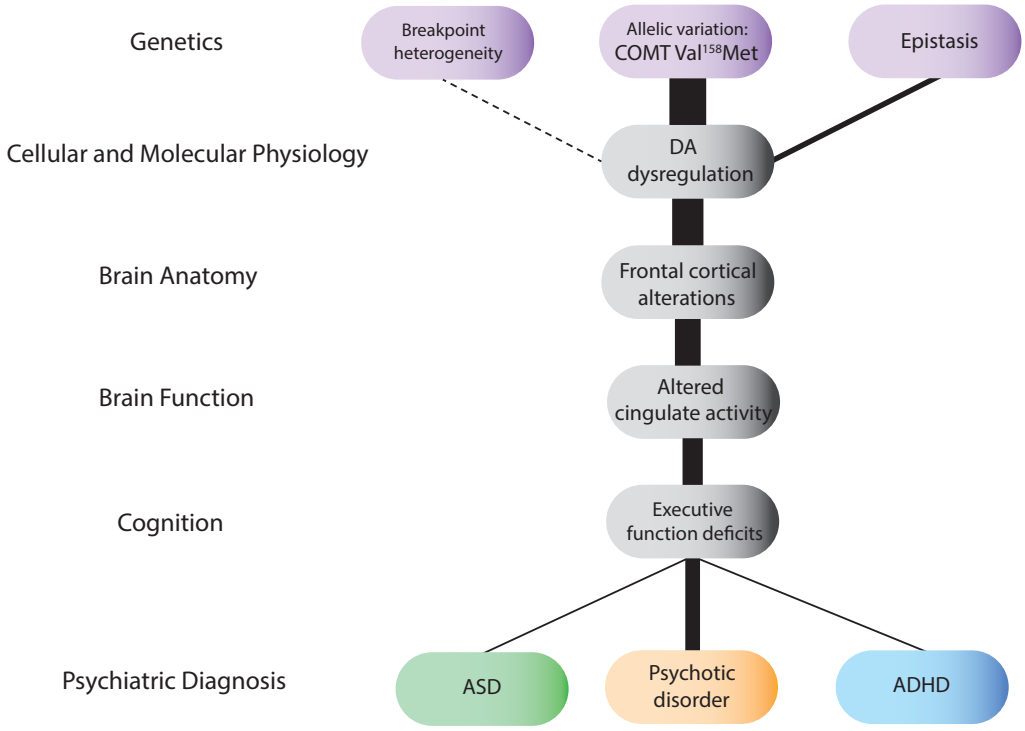


Figure 4. *Endophenotypes Relevant to Neuropsychiatric Disorders in 22q11DS***.** Levels illustrated here indicate how a known genetic etiology can inform pathophysiologic mechanisms relevant to neuropsychiatric phenotypes, using *COMT* Val158Met genotype as a particular example of how allelic variation in the intact chromosome may contribute to variable phenotypes. Potential sources of genetic variation are indicated in purple, intermediate sources of variability (e.g., endophenotypes) are indicated in gray, and varying phenotypic manifestations are indicated in the bottom row. The line thickness indicates the strength of associations between levels based on existing literature. Increased dopamine (DA) levels were found in patients with 22q11DS, and Met hemizygotes in particular show lower striatal binding potential as compared to Val hemizygotes (82). Studies of structural neuroanatomy have found that Met hemizygotes have smaller frontal lobe volume as compared to Val hemizygotes, which may be associated with inefficient breakdown of DA. One functional neuroimaging study found significantly increased cingulate activity during a Go/NoGo task in Met hemizygotes as compared to Val hemizygotes, implying that the Met subgroup of 22q11DS recruits additional cingulate activation for tasks that require attention and inhibition (53). Several studies have reported significant associations between cognition and *COMT* genotype such that Met hemizygotes shower better executive functioning (79), although not consistently (53; 81).

Variation in this SNP has more consistently been linked to differences in brain structure and cognition in 22q11DS patients (79-81). Using single photo emission computed tomography (SPECT) and a selective radiolabeled D_2 receptor antagonist, Boot and colleagues found that Met-hemizygous 22q11DS patients had significantly lower mean striatal Binding Potential (BP_{ND}) compared to Val hemizygotes, and presumably, higher levels of synaptic DA, thus providing initial evidence for a functional impact of allelic variation of genes within the 22q11.2 region (82; 83). Mouse models have helped to elucidate the direct effects of *COMT* depletion. For example, although baseline DA levels appear normal in *Comt*-deficient mice

(84), DA clearance from the extracellular space is twofold slower (71), suggesting that *COMT* hemizygosity may influence DA function primarily under conditions of increased DA release, such as times of increased stress.

Epistatic Interactions: *COMT* and *PRODH*

Epistasis is known to occur in at least one set of genes within the 22q11.2 locus, *COMT* and *PRODH* (85). *PRODH* encodes an enzyme that converts proline to glutamate in mitochondria, dysfunction of which has been linked to the development of psychiatric illness (86). *Prodh*-deficient mice show *Comt* upregulation in prefrontal cortex, perhaps as a feedback mechanism to increase DA transmission; moreover, brain function was most disrupted in mice having both increased proline and decreased *Comt* activity (87). As a result of the interactive role of these two gene products, it is likely that these genes participate in an epistatic relationship at the level of transcription and behavior.

Working memory appears mostly intact in mouse models with hemizygous deletion of specific genes in the 1.5 Mb deletion region (i.e., NoGo receptor, *Comt* (88) and *Prodh* (42); however, interfering pharmacologically with the epistatic interaction between *Comt* and *Prodh* unmasks an underlying dopamine dysfunction and reveals working memory deficits in *Prodh* mutant mice (42). Inhibition of *Comt* has also been shown to exacerbate other behaviors influenced by cortical DA, e.g. sensitivity to amphetamine and PPI (42).

Most 22q11DS patients are haploinsufficient for both *PRODH* and *COMT*, and thus may be unable to compensate for loss of PRODH (and subsequent increase in proline levels) by means of *COMT* up-regulation. 22q11DS patients who carry the low-activity *COMT*^{158}Met allele are more likely to have elevated serum proline levels and perform poorly on eye tracking

tasks (89; 90). These individuals may be less able to overcome dopaminergic dysregulation, thus placing them at greatest risk for psychotic symptom development.

Epistatic interactions between other genes within the intact chromosome, and/or with known transcription factors outside of the microdeletion region such as FGF1 (33), may also impact gene expression, and thus may offer clues about risk or resilience to various psychopathological phenotypes.

Biological Mechanisms of Psychotic Symptom Development

Studies in idiopathic schizophrenia have demonstrated a decline in cognitive abilities and other changes in behavior, as well as changes in brain morphology, which *precede* the onset of overt psychotic symptomology (91-93)**,** and thus may have utility as predictive biomarkers. Importantly, individuals with 22q11DS and schizophrenia do not differ from patients with idiopathic schizophrenia in terms of core clinical symptoms, including age at onset and course of illness (94), but may differ with regard to auxiliary features such as medication response; however, there is little empirical evidence for this to date. Further, neurocognitive and neuroanatomic features studied to date in 22q11DS overlap with those observed in idiopathic schizophrenia (94-96), although identified cognitive deficits appear more severe in 22q11DS-associated schizophrenia (97). Although the mechanisms underlying the development of psychotic symptoms in 22q11DS are not well understood at present, 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. A key advantage of studying a major mutational model like 22q11DS is that it can be diagnosed *in utero,* allowing for identification of at-risk individuals long before symptom onset; identification of predictive biomarkers early in life may ultimately lead to the development of novel treatment targets.

Environmental Influences

While the availability of a well-characterized genetic model presents an ideal opportunity to investigate genetic contributions to psychopathology, the emergence of psychopathology in 22q11DS is likely modulated by environmental factors. Factors such as perinatal infection, urban environment, cannabis use, and stressful life events are all known to increase risk for schizophrenia in the general population (98; 99). 22q11DS presents a highly sensitized background for the development of psychosis, and thus offers a valuable model in which to investigate role of stress in precipitating symptom onset (100), and/or exacerbations over time.

Moving Forward

Collectively, animal studies and candidate gene studies in humans implicate more than one gene within the 22q11.2 locus in the associated neurobehavioral phenotypes, suggesting an oligogenic basis. Evidence for the relevance of some of these genes to neuropsychiatric disorders in non-22q11DS individuals (Table 2) suggests that common variants within the 22q11.2 locus may contribute to broader disease risk.

Table 2. *Summary of evidence for the involvement of various sources of genetic variability on endophenotypic traits and behavior, organized by gene within the 22q11.2 locus**

Source of genetic variability	Gene(s) in 22q11.2 region	Effects on brain structure and function in 22q11DS	Effects on Psychiatric Phenotype in 22q11DS	Findings in Idiopathic Pychiatric Illness	Findings in Mouse Models
Haploinsufficiency/ Allelic Variation	*COMT*	SNP rs4680 low-activity (Met) allele associated with decreased frontal lobe volume in adults (53) and more robust decrease in PFC volume and Verbal IQ over time in adolescents (54); Met genotype also associated with better executive functioning in children (55; 56)	SNP rs4680 low-activity allele (Met) a risk factor for development of psychotic symptoms in youth (54); however, SNP rs4680 genotype was not associated with schizophrenia risk in adults (57); Low-activity allele (Met) associated with ADHD and (to a lesser extent) OCD (58)	SNP rs4680 high-activity allele (Val) is more often transmitted to probands with schizophrenia (59); however, (60) found no association of SNP rs4680 variants with schizophrenia; Relationship between this variant and ADHD diagnosis, although direction of association is mixed (61; 62); SNP rs4680 low-activity allele (Met) associated with diagnosis of OCD (63)	*Comt* deficiency resulted in region-specific changes in DA levels, particularly in PFC (64)
	PRODH	Inverse correlation between plasma proline level and IQ (65)	rs372055'T' allele carriers (22qDS subjects) had significantly higher BPRS scores than developmental disability controls (54)	Screened 6 SNPs: PRODH*1945 T->C, PRODH*1852 G->A showed significant association with schizophrenia (66)	Overexpression of *Prodh* led to increased PPI (67)
	PIK4CA		Screened 3 SNPs; significant association with SNP rs165793-G and schizophrenia (68; 69); No relationship between SNP rs165793-G and schizophrenia (70)	Significant association with SNP rs165793-G and schizophrenia (69)	
	GNB1L			SNPs rs5746832 and rs2269726 showed significant association with psychosis (71)	*Gnb1l*-deficient mice show PPI deficits (71)

	DGCR2			*DGCR2* protein expression elevated in DLPFC of schizophrenia patients; risk allele of a coding SNP associated with schizophrenia was associated with reduced expression of *DGCR2* (73)	
	TBX1			Inactivating mutations lead to increased risk of autism (72)	*Tbx1*-deficient mice show PPI deficits (72); Homozygous *Tbx1* mutants, the distribution of neural-crest-derived cells was disrupted, and the migration pathways of cranial nerves (IX & X) were abnormal (74)
	ZDHHC8			*ZDHHC8* rs175174 GG-genotype carries had gray matter reduction in frontal lobe and increased gray matter posterior volume compared to A-allele carriers (75); SNP rs175174 (A/G) showed significatnt association with schizophrenia (76)	*Zdhhc8*-deficient mice show decreased density of dendritic spines and glutamatergic synapses (77)

microRNA Disruption	*DGCR8*	Family-based association study of schizophrenia in 22q11DS identified statistically significant enrichment (12/72; 17%) of genes that are potential targets of miRNAs decreased in DGCR8 haploinsufficient mice (78)			Decrease in miRNA biogenesis in PFC and hippocampus; reduced dendritic complexity and impaired PPI (52); Increased LTP in mature mice (79); Abnormalities in dendritic spines and structural alterations in the hippocampus (80); Reduced cell proliferation and neurogenesis in adult hippocampus & impaired hippocampal-dependent learning, which could be rescued by IGF2 (81)
Epistasis	*COMT/PRODH*	Significant interaction between *COMT* genotype and proline level, with significantly decreased SPEM performance in children with high plasma proline levels and the low-activity *COMT* (Met) allele (82); Interaction between high plasma protein levels and *COMT* genotype on visual processing deficits (83)	Hyperprolinemic 22q11DS subjects with SNP rs4680 low-activity allele (Met) allele at increased risk for psychosis (OR = 2.8) (65)	Increased WM density in left IFL in patients with *COMT* high-activity (Val) allele and with one or two mutated PRODH alleles (84)	Epistatic interaction between *Prodh* and *Comt* at the level of transcription and behavior; *Prodh*-deficient mice show increased neurotransmitter release at glutamatergic synapses, upregulation of *Comt* mRNA in the PFC, associative learning deficits and increased sensitivity to

					psychomimetic drugs (85)

*numeric references correspond to reference list in Supplemental Material

SNP = single nucleotide polymorphism; SPEM=Smooth Pursuit Eye Movement; DA = dopamine; PFC = prefrontal cortex, DLPFC = dorsolateral prefrontal cortex, WM = white matter, IFL = inferior frontal lobe, miRNA = microRNA, LTP = long term potentiation; BPRS=Brief Psychiatric Rating Scale

Novel methods in functional genomics and systems biology can shed light on how genetic makeup in 22q11DS can translate into varying clinical phenotypes. For instance, next generation sequencing can now pinpoint, at a single base-pair level, the precise locations of deletion breakpoints. Advances in stem cell technology, such as the generation of induced pluripotent stem cells (iPSCs), offer incredible promise for modeling *in vivo* neuronal development. Specifically, fibroblasts and other tissues from human patients with 22q11DS can be reprogrammed and regenerated into neural progenitors and neurons, and investigated for properties of neuronal cytoarchitecture, electrophysiology, and synaptic transmission (57). Research using these *in vitro* models can lead to development of novel therapeutic agents, and could ultimately even prevent psychosis onset in both 22q11DS and in the broader population.

Finally, large-scale, prospective studies are warranted, paralleling those of behaviorally defined clinical high-risk studies (101), in order to determine clinical and neurobiological predictors of psychosis, as well as the role of environmental factors in contributing to psychosis risk. This is the first, critical step in developing targeted interventions that can be applied early in the course of illness, leading to improved outcomes.

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CHAPTER 4: Altered Brain Structure-function Relationships Underlie Executive Dysfunction in 22q11.2 Deletion Syndrome

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Abstract

22q11.2 Deletion Syndrome (22q11DS) is a neurogenetic disorder associated with elevated rates of developmental neuropsychiatric disorders and impaired executive function (EF). Disrupted brain structure-function relationships may underlie EF deficits in 22q11DS. We administered the Behavior Rating Inventory of Executive Function (BRIEF) to assess real-world EF in patients with 22q11DS and matched controls (N=86; age 6-17), along with cognitive measures that tap behavioral regulation and metacognition aspects of EF. Using FreeSurfer's whole-brain vertex cortical thickness pipeline, we investigated brain structure-EF relationships in patients with 22q11DS and controls. Behaviorally, patients with 22q11DS were impaired on multiple EF measures. Right orbitofrontal cortical thickness showed a differential relationship between real-world EF in patients with 22q11DS and controls. We also observed a group difference in the relationship between behavioral regulation and metacognition measures with thickness of ventral and dorsolateral prefrontal regions, respectively. Findings suggest that executive dysfunction characteristic of 22q11DS is underscored by altered prefrontal cortical structure.

Introduction

22q11.2 Deletion Syndrome (Velocardiofacial/DiGeorge syndrome; 22q11DS) is one of the most prevalent chromosomal deletion syndromes known, affecting nearly 1 in 1600 live births [1-3]. It results from a hemizygous 1.5-3 Megabase (Mb) deletion on the long arm of chromosome 22, and encompasses up to 60 known genes. Phenotypic expression is highly variable, ranging from congenital heart disease and palatal abnormalities, to developmental delays and cognitive dysfunction, particularly in the areas of executive function, attention and arithmetic ability [4-6]. Affected children also have greatly elevated rates of developmental neuropsychiatric disorders relative to the general population, particularly attention deficit hyperactivity disorder (ADHD; 37 %), anxiety disorder (36%) and autism spectrum disorders (ASD; 14-50%), as well as psychotic spectrum disorders (25-30%), typically diagnosed in adolescence or early adulthood [5,7-11]. Additionally, even those who do not meet full criteria for a clinical disorder exhibit dimensionally measured symptoms of impulsivity and inhibitory dyscontrol [12-14]. Although the precise mechanisms underlying the characteristic neurobehavioral manifestations of 22q11DS are not well understood, it is likely to result from reduced dosage of several genes involved in neurodevelopment within the 22q11.2 locus [15].

Executive functioning (EF) is a theoretical construct encompassing a range of higher-order cognitive processes such as motivation, planning, working memory, attention and response inhibition. EF deficits are characteristic of psychiatric disorders prevalent in 22q11DS, such as schizophrenia and ADHD [16,17]. Notably, variability in EF has also been linked to allelic variation in genes known to play a role in dopaminergic metabolism and regulation [18,19]. Given that patients with 22q11DS are hemizygous for catechol-O-methyltransferase (COMT), a gene involved in prefrontal dopamine metabolism [20], and also have elevated rates of

psychiatric conditions in which dopaminergic dysfunction is implicated [21,22], investigating dopamine-dependent frontally-mediated neurocognitive functions and underlying brain structural alterations in this syndrome may provide valuable insights into gene-brain-behavior relationships. By utilizing a Research Domain Criteria Project (RDoC) approach to investigate endophenotypic differences in 22q11DS, we have the ability to cut across traditionally-defined disorders and instead focus on dimensionally measured behavioral and neuroanatomic alterations that may underlie downstream psychiatric illnesses [23].

Patients with 22q11DS show alterations in brain morphology across cortical and subcortical regions, with prefrontal regions relatively preserved [24,25]. Specifically, relative to typically developing controls, 22q11DS patients show reduced cortical volume in occipito-parietal, temporal, and anterior cingulate cortices, and increases in cortical thickness in medial prefrontal regions as well as the insula [24,26-28]). While total brain volume is typically smaller or not different from controls [25,26,28], Jalbrzikowski et al, (2013) found increased thickness of the bilateral medial orbitofrontal, middle and inferior frontal cortices, in youth with 22q11DS compared to controls. Mouse models of 22q11DS have shown altered neuronal frequency in layers II/III of the medial prefrontal cortex, a characteristic that relates directly to performance on tasks of executive function [29]. The prefrontal cortex, particularly the orbitofrontal region, is late to mature in healthy individuals, which is thought to be attributable to later synaptic pruning and/or myelination [30,31]. Refinement of prefrontal neuronal connections may relate to increasing executive function capacities in early adulthood [32]. Specifically, dorsolateral regions of the prefrontal cortex (PFC) are believed to be primarily implicated in attentional and working memory functions [33], whereas ventromedial regions of the PFC are implicated in emotion regulation and impulse control [34]. Based on findings suggesting an abnormal

trajectory of prefrontal cortical development in 22q11DS [35-37] it is hypothesized that delayed prefrontal maturation in 22q11DS may be relevant to impairments in various aspects of executive function in this population.

The Behavior Rating Inventory of Executive Function (BRIEF) is a widely used and reliable parent-report measure of real-world EF for children and adolescents [38]. Elevated BRIEF scores have been observed in patients with 22q11DS, reflecting greater executive dysfunction [39]. This dysfunction was even more pronounced in patients with 22q11DS who met clinical criteria for a psychiatric diagnosis (ADHD, major depressive disorder, and/or phobia) [40,41].

Notably, neuroanatomic lesion studies have demonstrated a link between executive dysfunction, as assessed by the BRIEF, and prefrontal cortical pathology. In particular, Løvstad et al, 2012 found that lesions of the orbitofrontal cortex (OFC) were associated with elevated BRIEF scores (indicating greater executive dysfunction) [42], and Anderson and colleagues found that participants with *right* PFC lesions in particular showed greater day-to-day executive dysfunction, as measured by the BRIEF, as compared to those with lesions in the left PFC and controls [43].

It should be noted that real-world EF refers to everyday behaviors that rely on aspects of executive function (e.g., goal-directed behavior, attention, impulsivity, working memory) [38]. Laboratory tests that tap into real-world executive function remain limited in emulating natural, real-world experiences. For example, laboratory-based measures typically require simple responses to a single event, whereas daily life requires much more complex multitasking, which involve setting a series of goals and sub-goals, and making decisions about prioritization [44]; thus, it is important to measure both aspects of executive function.

The development of the frontal cortex in 22q11DS appears to be disrupted, based both on human studies and animal literature [29,36]. It is therefore plausible that abnormal prefrontal maturation may be related to characteristic impairments in executive function seen in this population [36]. In healthy youth, prefrontal regions are relatively late to mature, in that they continue to thin into late adolescence/early adulthood [30]; this pattern of increased thinning over this developmental period is associated with healthy cognitive development, particularly in relation to executive function [45]. Relatedly, youth with ADHD have delayed rates of cortical maturation, particularly in prefrontal regions [46]. Given evidence for similarly delayed maturation of prefrontal regions in 22q11DS, we anticipated that brain-behavior relationships observed in typically developing youth would be disrupted in those with the 22q11.2 deletion.

To our knowledge, no studies to date have examined the neuroanatomic substrates of laboratory and real-world executive dysfunction in 22q11DS, nor how these brain structure-function relationships differ from those observed in typically developing youth. Here, we compared the relationship between real-world EF, as measured by the BRIEF, with structural neuroanatomic variation in patients with 22q11DS and demographically comparable healthy controls. We hypothesized that patients with 22q11DS would show an altered relationship between EF and cortical thickness, particularly in prefrontal regions implicated in EF. In order to dissect the relationship between multiply determined real-world EF and prefrontal neuroanatomy, we then investigated neurocognitive measures related to two major subcomponents of EF (behavioral regulation and metacognition), and how they may differentially relate to neuroanatomy in patients with 22q11DS and controls. We anticipated that impulsivity functions related to the behavioral regulation aspects of EF would be preferentially related to ventral and medial prefrontal regions [47], whereas attentional functions related to

metacognition aspects of EF would be preferentially related to dorsal and lateral regions of the prefrontal cortex [48]. Moreover, we anticipated that these relationships would be altered in patients with 22q11DS relative to typically developing controls, given their known deficits in EF and hypothesized delayed neuromaturational trajectory in the prefrontal cortex.

Materials and Methods

Participants

Eighty-six participants, ages 6-17 years, participated in the study: 43 with a molecularly confirmed diagnosis of 22q11.2 deletion syndrome, and 43 typically developing controls (see Table 1). We recruited patients with molecularly confirmed 22q11.2 deletions from two sources: 1) the population of patients followed by the UCLA and Children's Hospital, Los Angeles (CHLA) Pediatric Genetics, Allergy/ Immunology and Craniofacial Clinics; and 2) local support groups (e.g. Velocardiofacial Education Foundation, 22q and You Support Network). Demographically comparable typically developing comparison subjects were recruited from the same communities as patients with 22q11DS, and were tested concurrently. This was accomplished by web-based advertisements about the research study, and by posting flyers and brochures at local schools, pediatric clinics, and other community sites. Exclusion criteria for all participants included: substance or alcohol abuse and/or dependence in the last six months and/or insufficient fluency in English. In addition, controls could not meet criteria for any major mental disorder, with the exception of ADHD or a single past episode of depression. Controls were also excluded if they had a neurological disorder, substance abuse/dependence, intellectual disability, and/or history of head injury with loss of consciousness,. This information was collected through administration of the Structured Clinical Interview for DSM-IV Axis I Disorders, with an additional developmental disorders module [49]. All interviews were conducted by psychology

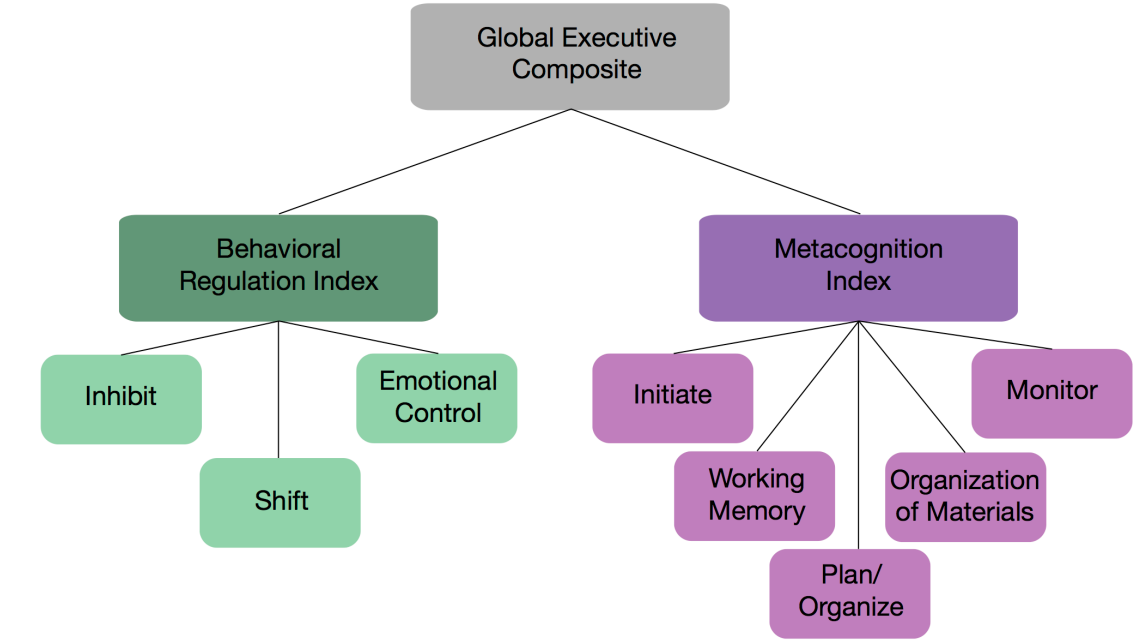
Ph.D. candidates who had undergone extensive training and reliability procedures under the supervision of the senior author (CEB), as described in detail elsewhere [24,50,51].

All participants underwent verbal and written consent after study procedures were fully explained, and their parents or guardians also completed written consent. The UCLA Institutional Review Board (IRB) approved all study procedures and informed consent documents.

BRIEF Assessment

The BRIEF is a well validated 86-item parent-report questionnaire for children ages 5-17, which assesses real-world executive function in the home and school environment [38]. Information is ascertained regarding how well a child is able to regulate his/her behavior, inhibit impulses, initiate projects, etc.; higher scores represent greater dysfunction. The Inhibit, Shift, and Emotional Control subscales sum to form the Behavioral Regulation Index, whereas the Initiate, Working Memory, Plan/Organize, Organization of Materials, and Monitor subscales sum to form the Metacognition Index. These indices comprise the Global Executive Composite (Figure 1a).

a)


Global Executive Composite
Behavioral Regulation Index
Metacognition Index
Inhibit
Emotional Control
Shift
Initiate
Monitor
Working Memory
Organization of Materials
Plan/ Organize

b)

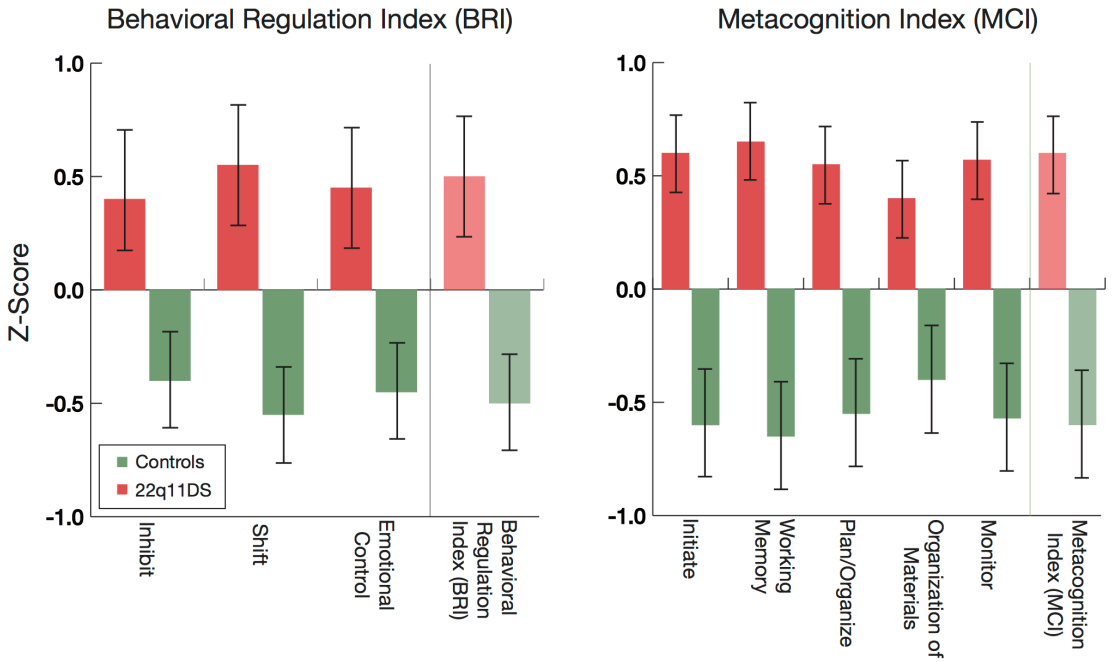

Behavioral Regulation Index (BRI)
Metacognition Index (MCI)
Z-Score
1.0
0.5
0.0
-0.5
-1.0
Controls
22q11DS
Inhibit
Shift
Emotional Control
Behavioral Regulation Index (BRI)
Initiate
Working Memory
Plan/Organize
Organization of Materials
Monitor
Metacognition Index (MCI)

Figure 1. (A) *BRIEF subscales.* (B) *Scores across BRIEF subscales in youth with 22q11DS and age matched typically developing controls*. Z scores were created based on normative data (Gioia, Isquith, Guy, & Kenworthy, 2000); higher scores indicate greater pathology. Patients with 22q11DS show greater dysfunction on all subscales (p<0.001, Bonferroni corrected).

Laboratory-Based Measures of Executive Function

The Continuous Performance Test – Identical Pairs (CPT-IP) [52] and Time Reproduction Task (TRT) (Barkley, 1998) were employed as laboratory-based measures of subcomponents of executive function. Both tasks were run on laptop computers in a quiet testing room.

The CPT-IP is a well-validated measure of sustained attention and working memory, in which sequential numeric stimuli are rapidly presented, and participants respond when two identical stimuli are presented in sequence. There are 450 total trials with varying numbers of digits. In target trials (20% total trials), two identical successive stimuli are shown, in catch trials (20% total trials), two *similar* successive stimuli are shown, and the other 60% of trials are random. We analyzed d-prime as our primary dependent variable, which is computed via the proportion of key presses on target trials to catch trials. Poor performance on this task has been shown to predict later development of psychotic spectrum disorder in at-risk individuals [53], and is a marker of genetic susceptibility to psychosis [54]. In patients with schizophrenia, impaired performance on this task is associated with structural and functional alterations in the medial prefrontal cortex, inferior frontal gyrus, anterior cingulate cortex, postcentral gyrus, and thalamus [55-57]. CPT impairment is also a hallmark symptom of ADHD [58], and children with ADHD have shown abnormal prefrontal neural activity during performance of this task [59]. Because the CPT-IP was added to our battery at a later date, and is not validated in children under 10, the sample size is reduced for this measure.

The TRT is a well-established measure of one's ability to accurately reproduce a time interval in which two light bulbs appear simultaneously on the screen. The light bulb on the left is illuminated on for 4, 8, 12, 16, or 20 seconds, and then turned off. The subject is asked to reproduce that time with the light bulb on the right, by holding down the space bar for the same amount of time. Our primary dependent variable for the task is average absolute accuracy across all trial types. Interval timing, which occurs on the order of seconds to minutes, has been shown to depend on dopaminergic function [60,61]; thus, deficits in this domain may be an important intermediate phenotype for development of psychiatric disorders in which dopaminergic dysfunction is implicated [62]. Functional neuroimaging studies, as well as studies in animal models, have shown that interval time reproduction relies on fronto-striatal-cerebellar neural circuitry [61,63-65].

MRI Data Acquisition

All scanning was carried out on an identical Siemens 3 Tesla Tim Trio MRI at the UCLA Brain Mapping Center or at the Center for Cognitive Neuroscience. Measures of brain structure were obtained with high-resolution structural MRI. Each scan began with a 10-minute acquisition of standard images used for determining regional anatomy, including a sagittal localizer image (TR/TE=500/10ms, 192x256 matrix), a high-resolution T2-weighted axial image (TR/TE=5000/33 ms, 128x128 matrix, FOV=200x200mm), and a sagittal 1 cubic mm T1-weighted image (MPRAGE, TR/TE = 2300/2.91, flip angle = 9 degrees; slice thickness = 1.20 mm, 240x256 acquisition matrix).

MRI Analysis

The FreeSurfer image analysis suite (version 5.0, http://surfer.nmr.mgh.harvard.edu) surface-based processing pipeline was used to derive measures of cortical thickness. FreeSurfer is a well-validated processing protocol that has been previously described in detail [66,67]. In short, the following steps were taken in the processing stream: motion correction, transformation of images to standard Talairach space, intensity normalization, removal of non-brain tissue, segmentation of white matter and subcortical structures, and final segmentation of cortical surfaces. Final segmentation is based on both a subject-independent probabilistic atlas and subject-specific measured values. Raters (RJ, MJ, AP) blind to diagnosis visually inspected the scans at several points along the processing pipeline and any errors were manually edited (see Supplementary Information). We focused on cortical thickness as our primary neuroanatomic measure of interest, due to the close link between changes in cortical thickness and cognitive development [68].

Statistical Analyses

Analyses of demographic and behavioral data were performed in SPSS software v. 21 (Chicago, IL). We conducted independent samples t-tests for continuous variables and chi-square tests for categorical variables.

For the analyses of behavioral measures, we conducted univariate ANCOVAs in SPSS with each behavioral measure as the dependent variable, diagnosis as the between-group factor, and age and gender as covariates. In secondary analyses, we additionally covaried for IQ in order to determine whether global cognitive abilities accounted for differences in EF task performance.

For the whole brain vertex-wise neuroanatomic analysis, all statistics were performed in FreeSurfer. A vertex refers to the spatial point of measurement resolution on the cortical surfaces derived in FreeSurfer. For each vertex, thickness measurements for each subject were mapped onto a common spherical coordinate system, and smoothed using a Gaussian kernel of 10 mm. In order to determine whether the relationship between regional cortical thickness and behavioral and neurocognitive measures differed between patients with 22q11DS and controls, we conducted whole-brain general linear model analyses to test for measure by group interactions at each vertex across the whole brain. In all analyses, we covaried for age and scanner location. For each analysis, we first investigated the main effect of gender, and if no main effect was found, it was not included in the final models. To control for multiple comparisons, cluster correction was completed using Monte Carlo simulation with 10,000 iterations (vertex-wise threshold of $p<0.05$), in order to determine the distribution of maximum cluster size under the null hypothesis, as described in Hagler et al., 2006 [69]. Right and left hemispheres were tested separately. We then conducted secondary analyses, which included adding IQ as a covariate into our models, and also investigated the main effect of psychiatric diagnoses (ADHD, anxiety disorder, and ASD) in three separate models so that we could investigate the main effects of each diagnosis separately.

Results

As shown in Table 1, patients with 22q11DS and controls were well matched in terms of age, gender, ethnicity and education. As expected, participants with 22q11DS had decreased IQ relative to controls. Further, patients with 22q11DS had elevated rates of neuropsychiatric disorders, consistent with rates observed in the literature [7]. We compared global measures of structural neuroanatomy in patients with 22q11DS and controls, and found no differences in

intracranial volume or overall cortical thickness. Finally, for all of the analyses described below, there was not a significant main effect of gender, and thus gender was not included as a covariate.

Table 1. Demographic characteristics of study participants.

	22q11DS (N=43)	Controls (N=43)	p value
Age (years)	11.42 (3.53)	10.74 (3.61)	0.384
Gender	23M, 20F	24M, 19F	0.829
Education (years)	5.16 (3.59)	4.77 (3.67)	0.615
Ethnicity (% Latino)	33%	40%	0.500
Highest Parental Education (years)[a]	15.81 (2.45)	15.56 (3.48)	0.713
Full Scale IQ	82.63 (13.87)	107.51 (21.25)	<0.001***
ADHD, N (%)[b]	18 (42%)	2 (5%)	<0.001***
Psychotic Disorder, N (%)	1 (2%)	n/a	
Autism Spectrum Disorder, N (%)	19 (23%)	n/a	
Anxiety Disorder, N (%)	21 (49%)	n/a	
No psychiatric diagnosis	9 (21%)		
Psychotropic medication (n, none/antipsychotic/antidepressant)	38/2/3		

a. Data unavailable for 6 participants
b. Comorbidities: ADHD only (5), ASD only (4), Anxiety Disorder only (5), ADHD + ASD (3), ADHD + Anxiety Disorder (3), Psychotic Disorder + Anxiety Disorder (1), ASD + Anxiety Disorder (6), ADHD + ASD + Anxiety Disorder (6)

*p<0.05
**p<0.01
***p<0.001

BRIEF Results

Behavioral**:** Results of group comparisons on the BRIEF are provided in Figure 1b. Patients with 22q11DS showed significantly elevated scores on each subscale, all of which survived Bonferroni correction for multiple comparisons ($p<0.001$). In order to minimize the number of comparisons in our neuroanatomic analyses, we used the Global Executive Composite (GEC) score as the primary dependent measure.

Relationship with Cortical Thickness: Whole brain vertex-wise analysis revealed a significant BRIEF by group interaction cluster in the right orbitofrontal cortex ($p<0.05$, Monte Carlo correction; Figure 2), which survived correction for multiple comparisons. In this region, increased cortical thickness was associated with more severe executive dysfunction in patients with 22q11DS, whereas greater cortical thickness was associated with better EF in controls.

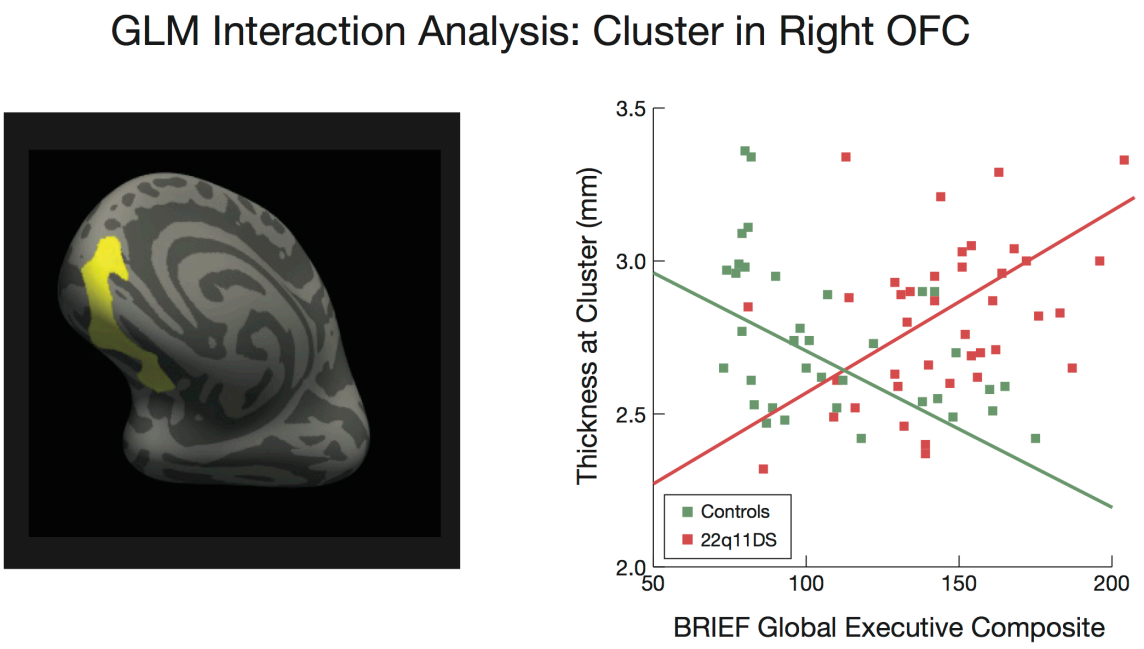


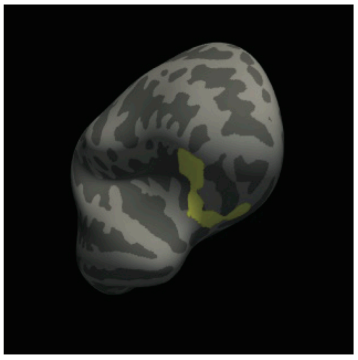
Figure 2. *Relationship between BRIEF Global Executive Composite (GEC) score and cortical thickness in 22q11DS vs. Controls*. Analysis was conducted using an unbiased, whole-brain approach. There was a significant interaction between group and BRIEF GEC score in the right orbitofrontal cortex (cluster size 65020.8 mm^2*; p*<.05, corrected), indicating that increased cortical thickness in the right OFC was associated with more severe real-world executive dysfunction (higher BRIEF score) in patients with 22q11DS, whereas thicker OFC was associated with *better* executive function (lower BRIEF score) in controls. The left panel shows the location of the cluster, and the right panel displays the relationship between mean thickness of the cluster and BRIEF scores in patients with 22q11DS and controls.

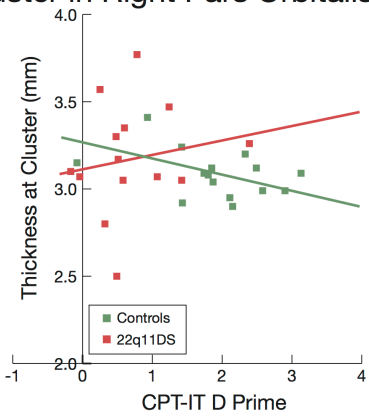
CPT Results

Behavioral: On the CPT-IP, patients with 22q11DS had significantly lower d-prime scores ($p<0.001$) than controls, indicating a higher proportion of hits on catch trials to hits on target trials, as well as a higher false alarm rate ($p=0.022$).

Relationship with Cortical Thickness: We found two significant CPT-IP by group clusters that survived correction for multiple comparisons, in the right pars orbitalis of the inferior frontal gyrus and the superior temporal gyrus ($p<0.05$, Monte Carlo correction; Figure 3a). In these regions, increased thickness was associated with better CPT-IP performance in patients with 22q11DS, whereas increased thickness was associated with worse performance in controls ($p<0.05$). Given our focus on prefrontal cognitive functions, follow-up analyses focus on the larger cluster in the pars orbitalis.

a) GLM Interaction Analysis: Cluster in Right Pars Orbitalis





b) GLM Interaction Analysis: Cluster in Right Precentral Gyrus

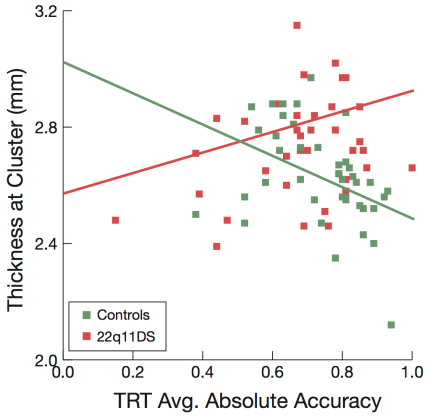


Figure 3. (A) *Relationship between performance on Continuous Performance Test – Identical Pairs (CPT-IP) and cortical thickness in 22q11DS vs. Controls.* Whole-brain vertex-wise analysis revealed a significant group by task interaction in the right pars orbitalis region of the inferior frontal gyrus (cluster size 919.46 mm^2, $p<.05$, corrected), indicating that the relationship between CPT-IP performance, as assessed by d-prime, and thickness in this region differs between patients with 22q11DS and controls. In patients with 22q11DS, increased cortical thickness in the inferior frontal gyrus was associated with higher d-prime scores, whereas in controls, increased cortical thickness in this region was associated with lower d-prime scores. (B) *Relationship between performance on the Time Reproduction Task (TRT) and cortical thickness in 22q11DS vs. Controls.* Whole-brain vertex-wise analyses revealed a significant group by task interaction in the right precentral gyrus (cluster size 1434.62 mm^2, $p<.05$, corrected), indicating that the relationship between TRT and thickness in this region significantly differs between patients with 22q11DS and controls. In patients with 22q11DS, increased cortical thickness in the precentral gyrus was associated with higher accuracy scores, whereas in controls, increased cortical thickness in this region was associated with lower accuracy scores.

TRT Results

Behavioral: Patients with 22q11DS showed significantly reduced consistency of responses on the TRT relative to controls (p=0.025), as well as a trend toward reduced accuracy (p=0.065).

Relationship with Cortical Thickness: We performed the same whole-brain GLM interaction analysis as described above, in order to determine whether the relationship between performance on the TRT and cortical thickness differs between patients with 22q11DS and controls. We found a significant cluster that survived correction for multiple comparisons in the

right precentral gyrus ($p<0.05$, Monte Carlo correction; Figure 3b). In this region, increased thickness was associated with better time reproduction performance in patients with 22q11DS, whereas increased thickness was associated with worse performance in controls.

Secondary Analyses: IQ and Psychiatric Disorders

Including IQ as a covariate, in addition to age, group differences on the BRIEF GEC and CPT-IP remained significant ($p<0.001$, $p=0.014$, respectively), whereas the trend-level group difference for TRT accuracy was no longer present.

Due to the high prevalence of psychiatric illness in 22q11DS, we investigated effects of ADHD, anxiety disorder, and ASD, and did not find a significant effect of any of these diagnoses in our analyses.

Discussion

Here we assessed brain-behavior relationships in patients with 22q11.2 deletion syndrome, a recurrent copy number variant associated with dysfunction in frontally-mediated cognitive functions [4], dopaminergic dysregulation [70], and high rates of developmental neuropsychiatric disorders associated with executive dysfunction [7]. We found that patients with 22q11DS have significant executive dysfunction, as measured both by real-world behaviors and neurocognitive probes of distinct sub-components of executive function, and that altered prefrontal neuroanatomic structure appears to underlie these deficits. Notably, in a mouse model of 22q11DS altered projection neuron frequency in layers II/III of the medial frontal cortex was observed, the severity of which predicted performance on a task of executive function involving reversal learning [29] .

Real-world Executive Function

We found that patients with 22q11DS show deficits on multiple indices of real-world EF, as measured by the BRIEF, and these appear to be underscored by abnormal prefrontal neuroanatomy. The Global Executive Composite score, which is comprised of both behavioral regulation and metacognition subscales, was significantly elevated in patients with 22q11DS relative to typically developing controls. Using an unbiased, whole brain approach, we found that the relationship between GEC scores and cortical thickness uniquely differed between patients with 22q11DS and controls in the right orbitofrontal cortex: in controls, increased right OFC thickness was associated with better EF, while in patients the opposite relationship was observed, adjusting for the effects of age. Notably, this finding is consistent with prior literature that found a relationship between right hemisphere OFC lesions and real-world EF, as measured by the BRIEF [42,43]. The OFC is critical for the formation of reward representation and decision-making [71,72], and is also involved in responding appropriately to social cues [73]. A recent structural MRI study found that OFC volume was correlated with one's tendency to conform to others' values [72]. The OFC is relatively late to mature in typically developing adolescents [30]. This maturational process may be altered in patients with 22q11DS [24,36], a possibility which warrants further investigation in prospective longitudinal studies.

Cognitive Tasks of Executive Function

In order to deconstruct these broad deficits in behavioral manifestations of EF, we took a targeted approach to examine how performance on specific cognitive tasks tapping different sub-components of EF relates to structural neuroanatomy in patients with 22q11DS relative to controls. Specifically, we were interested in how tasks indexing behavioral regulation and

metacognition aspects of EF may differentially relate to subregions of the prefrontal cortex between patients with 22q11DS and controls.

We investigated the CPT-IP, as a measure of behavioral regulation, and found that patients with 22q11DS had higher levels of impulsive responding, as indicated by increased false alarm rates and lower d-prime scores. Task performance was differentially related to cortical thickness in the right pars orbitalis of the inferior frontal gyrus (IFG), such that increased thickness in controls was associated with worse performance, whereas increased thickness in patients with 22q11DS was associated with better CPT-IP performance. The IFG, particularly in the right hemisphere, has been implicated in impulsivity, in both lesion and functional neuroimaging studies [74]. Our finding is consistent with a study of patients with idiopathic schizophrenia that found a positive relationship between CPT-IP performance and IFG gray matter density in patients, but not in controls [55]. This result is also in line with a study of healthy children and adolescents that found an association between performance on a task of executive function (Keep Track task) and thinner cortex in frontal regions, including the IFG [75].

Next, as a putative measure of metacognition (which is comprised of attention and working memory; [38,76]), we used the TRT to investigate accuracy in the reproduction of time intervals. We found that controls tended to be more accurate in time reproduction, and that this measure was differentially related to thickness of the right precentral gyrus; specifically, in controls increased thickness was associated with reduced accuracy, whereas in patients with 22q11DS increased thickness in this region was associated with better accuracy on the TRT. Interestingly, neural activity in the precentral gyrus has been linked to performance on attentional measures [77], and thinning of the precentral gyrus has been associated with poorer

outcome in children with idiopathic ADHD [78], indicating that the attentional aspect of time reproduction may be driving the observed relationship.

Taken together, the cluster differentially associated with CPT-IP in patients with 22q11DS was located in a more ventral region of the PFC, whereas the cluster associated with TRT was located in a more dorsal and lateral region, suggesting that altered brain structure–function relationships in distinct regions of PFC may underlie deficits in task performance in metacognitive vs. behavioral regulation aspects of EF in 22q11DS.

22q11DS presents an ideal context for a translational RDoC-based approach, given its well-characterized, homogeneous genetic etiology and phenotypic expression of neuropsychiatric symptoms that cut across multiple DSM categories [12,79]. Multi-level investigation of dimensionally measured traits relevant to EF deficits may better elucidate the pathophysiological mechanisms that underlie psychiatric phenotypes.

Altered Brain Structure – EF Relationships in 22q11DS

Prior neuroanatomic studies in 22q11DS have focused primarily on volume of cortical structures, but recent investigations have decomposed these differences into measures of cortical thickness and surface area, which are thought to be reliant on different genetic mechanisms, and reflect distinct cortical characteristics [24,80,81]. Here we focused on cortical thickness given prior studies indicating that it has a stronger relationship with global cognitive performance, relative to surface area measures [68].

Interestingly, all of the clusters in which we found an altered relationship between brain structure and EF performance in 22q11DS were located in the right hemisphere, suggesting there may be a differential hemispheric specialization of EF in 22q11DS. Of note, previous studies have found that lesions of the PFC in otherwise healthy individuals are related to executive

function, even more so in the right hemisphere than the left [43]. Children with right-sided prefrontal lesions have been shown to have worse performance than those with left prefrontal insults of similar severity on measures of executive function and attention [82,83], suggesting that the right PFC may play an essential role in the development of basic attentional skills. Anderson et al (2005) have hypothesized that the right prefrontal cortex may be particularly involved in mediating executive functions during childhood development, whereas the left hemisphere is differentially involved in language tasks [43].

Additionally, a previous study on a partially overlapping sample found that prefrontal cortical thickness in the medial PFC, inferior frontal gyrus, and middle frontal gyrus is increased in patients with 22q11DS relative to controls, and that this effect was stronger in the right hemisphere than the left [24]. This same study found that thickness of the right medial orbitofrontal cortex was associated with psychotic symptoms in patients with 22q11DS, further supporting the functional role of this region in the neurobehavioral presentation of this syndrome.

It has been hypothesized that reduced dosage of 22q11.2 genes may compromise projection neuron integrity (layers 2/3), specifically in frontal association cortices [29]. The precise mechanism is not fully understood, but it is plausible that abnormalities in cortical thickness may result from reduced dosage of genes involved in cortical development. These differences in cortical thickness between 22q11DS patients and controls are likely to play a role in the executive dysfunction characteristic of the disorder [84]. The relationship between cortical thickness and cognition in healthy individuals is complex, and a relatively thinner cortex may have different consequences, depending upon the developmental context [45,72,78,85]. Findings in healthy individuals are sometimes contradictory, depending on age range and function

investigated, but there is some evidence that thicker prefrontal cortex is associated with better executive function. In the case of real-word executive function, a thicker cortex is advantageous for healthy individuals, and the opposite is seen in patients with 22q11DS. Thus, while the precise mechanisms underlying the altered relationships between cortical thickness and various components of executive cognition in 22q11DS are not yet known, we can speculate that they are related to abnormal development of the prefrontal cortex.

It was particularly surprising that relationships with cortical thickness were in opposite directions for parent-reported vs. performance-based assessments of executive function. In particular, while we found that thinner orbitofrontal cortex was associated with better real-world EF in patients with 22q11DS, the opposite pattern was found for the relationship with performance-based cognitive measures. This implies that various subregions of the PFC may play different roles in EF in patients with 22q11DS and controls, and that real-world and cognitive aspects of EF may be related to distinct neuroanatomic intermediate phenotypes. Given the variability in findings of associations between cortical thickness and executive function across ages and specific domains, this inconsistency warrants further investigation in a larger, prospectively followed sample.

Study Limitations

Several limitations of this study should be noted. First, not all participants received all three measures of EF. Secondly, given the cross-sectional nature of the data, questions about distinct developmental trajectories of executive function processes in 22q11DS could not be addressed. Prospective longitudinal studies are now underway in order to examine trajectories of both behavioral and neuroanatomic changes.

We investigated the effects of three major psychiatric diagnoses in 22q11DS: ADHD,

anxiety disorder, and ASD. Given the relatively young sample included in the current study (age 6-18), only one of our patients had a psychotic disorder diagnosis at the time of assessment. While it is not yet known whether additional subjects in this cohort will develop a psychotic disorder, we are continuing to follow this cohort longitudinally; as such, baseline differences that are associated with subsequent development of psychotic illness can be investigated in future studies.

Future Directions

The genetic basis of these findings is unknown. In particular, given the role of dopamine in prefrontal 'tuning' [86], future studies should further explore the role of dopaminergic dysregulation in 22q11DS and how it may relate to these behavioral and neuroanatomic findings. It is widely theorized that the relationship between dopamine levels in the brain and PFC functioning follows an "inverted-U" pattern such levels that are too low or too high are maladaptive [87,88]. Investigating how genetic variation and resulting changes in gene expression may contribute to individual differences in brain structure, function and downstream behavior in both patients with 22q11.2 deletions and in animal models will help elucidate the neurobiological mechanisms relevant to behavioral pathology in 22q11DS and the broader population.

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CHAPTER 5: Gene Expression in 22q11.2 Deletion Carriers

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Abstract

22q11.2 Deletion Syndrome (22q11DS) is a neurogenetic disorder associated with elevated rates of developmental neuropsychiatric disorders and impaired cognitive function. Patients with 22q11DS are hemizygous for a number of genes thought to be related to psychiatric disease. Despite its well-characterized genetic etiology, the genetic basis for the variable phenotypic expression of this syndrome remains unknown. In this study, we analyze peripheral gene expression levels in three genes known to be relevant to neurobehavioral function within the 22q11.2 deletion region (*COMT*, *ZDHHC8*, *DGCR8*) in patients with 22q11DS (N=56) as compared to healthy, demographically matched controls (N=48). We used real-time quantitative polymerase chain reaction (RT-qPCR) to investigate group differences in expression levels of these genes, as well as relationships of gene expression to measures of global cognition, working memory and executive function. Results indicate significantly decreased expression of *COMT*, *DGCR8*, and *ZDHHC8* in patients with the typical 3 Megabase 22q11.2 deletion as compared to controls. We found that *COMT* expression was significantly positively correlated with age in patients with 22q11DS, but not controls; additionally, *COMT* expression was significantly associated with working memory in patients but not in controls. Higher *ZDHHC8* expression was

associated with better global cognition (IQ) in controls, but lower IQ in patients with 22q11DS (interaction: p=0.004). There was also a significant interaction between IQ and group for *DGCR8* expression (p=0.037), with results in the same direction as those for ZDHHC8. Future work in larger samples is warranted to investigate single nucleotide polymorphisms (SNPs) within these genes, and the relationship with function as well as with gene expression, using expression quantitative trait locus (eQTL) analyses.

Introduction

22q11.2 Deletion Syndrome (22q11DS) is a neurogenetic disorder with an estimated prevalence of 1 in 2,000 live births (1-3) that arises from a hemizygous deletion of approximately 60 known genes on the long arm of chromosome 22. Many of the genes within this locus are known to be involved in brain development and risk for psychiatric illness(4; 5). The psychiatric phenotype of 22q11DS can be quite variable, with elevated rates of developmental neuropsychiatric disorders, including autism spectrum, anxiety, and attention deficit hyperactivity disorder (ADHD), and psychotic disorder typically manifesting in adolescence or early adulthood (6-11). We do not yet understand the genetic contributions to phenotypic heterogeneity, and one possible source of variation is due to allelic variation in the intact chromosome (see more detail in Jonas et al, 2014 (12)).

To date, human studies of genes that lie within the 22q11.2 deletion region have primarily investigated single nucleotide polymorphism (SNP) variation in relation to behavior (13-15). Gene expression levels represent an intermediate trait that lies between genotype and phenotype, which may be more closely linked to behavior (16). While studying gene expression levels from brain tissue is ideal, this is not yet available in patients with 22q11DS. Whole-blood gene expression profiles have found many similarities with multiple CNS tissues (17), and the investigation of gene expression from leukocytes in blood is the most efficient way to measure these levels (18).

Real-time quantitative polymerase chain reaction (RT-qPCR) is considered the "gold standard" for investigation of gene expression analysis, when conducting a small-scale (less than 30 genes), hypothesis-driven study. As compared to microarray and RNA sequencing methods, RT-qPCR has some distinct advantages, as it allows for the widest dynamic range, lowest

quantification limits, least biased results, and the amount of starting material can be rather low (19).

Three key genes of interest located in the 22q11.2 deletion region are *COMT, DGCR8,* and *ZDHHC8*. Each of these genes have been linked to structural and behavioral abnormalities in mouse models of the deletion (20-22), with some evidence for association with neurobehavioral phenotypes in humans as well, as detailed below.

The *COMT* gene codes for the enzyme catechol-O-methyltransferase (COMT), which is responsible for breaking down catecholamines such as dopamine, particularly in the prefrontal cortex (PFC) (23). There is an evolutionarily recent, common functional polymorphism at codon 158 (rs4680) that results in a Val to Met substitution, leading to lower enzymatic activity, and presumably higher levels of prefrontal dopamine in carriers of the Met allele (23; 24). In healthy individuals this polymorphism has been extensively studied in relation to a wide range of phenotypes. Several studies show that COMT accounts for a small but significant proportion of variance in prefrontally-mediated cognitive function in humans (20) and in mice (25). The Met/Met genotype has been generally associated with better executive function and lower risk of schizophrenia, though the literature has been mixed (23; 26). This pattern is consistent with the "inverted-U" pattern of PFC function, supported experimentally by Mattay et al (27). Given that patients with 22q11DS are hemizygous for *COMT*, they are likely to have reduced enzymatic activity, which may lead to reduced clearance of prefrontal dopamine. Further, there is some evidence that 22q11DS patients who are hemizygous for the Met allele (Met/-) have impaired executive function, as compared to those with the Val/- genotype, suggesting an interaction effect between group and COMT allele (14; 28). Thus, while SNP variation has been studied extensively, with mixed findings, the relationship of COMT gene expression levels to behavior

has not been investigated in 22q11DS. Developmental changes in *COMT* expression also warrant investigation, and Chen et al. (29) found increased *COMT* enzyme activity with increasing age, which they propose may be related to decreased dopamine levels post-adolescence.

DGCR8 (DiGeorge Syndrome Critical Region 8) codes for a subunit of the microprocessor complex, which mediates the biogenesis of microRNAs (miRNAs), and therefore global gene regulation (30; 31). DGRC8 heterozygous null mutant (*Dgcr8*$^{+/-}$*)* mice show behavioral abnormalities in sensorimotor gating, spatial working memory deficits and hyperactivity (21), as well as altered neuronal morphology (32). Further, decreases in *DGCR8* expression levels in 22q11DS have been linked to miRNA dysregulation (33). miRNA dysregulation has recently been linked to schizophrenia in both 22q11DS (34; 35) and more broadly (36; 37). Recently, a small whole-genome sequencing study of 22q11DS patients found that rare, damaging variants impacting protein-coding genes were more common in 22q11DS patients with schizophrenia; notably, restricting analysis to genes affected by *DGCR8* tended to amplify the group difference for 22q11DS patients with versus without schizophrenia (38). Their findings provide support for a miRNA hypothesis for schizophrenia.

ZDHHC8 (Zinc Finger, DHHC-Type Containing 8) codes for a four transmembrane protein that functions as a palmitoytransferase; a SNP within this gene (rs175174) has been linked to higher rates of schizophrenia (39). *Zdhhc8*-deficient mice show decreased density of dendritic spines and glutamatergic synapses, as well as decreases in exploratory activity and novelty-seeking behaviors (22). Recently, a study showed that structural neuronal defects in a mouse model of 22q11DS were rescued by overexpression of *Zdhhc8* (40), suggesting that this gene plays a critical role in the pathophysiology of 22q11DS.

Given that patients with 22q11DS are hemizygous for these three key neurodevelopmental genes, we sought to understand how gene expression may be affected in these individuals, using RT-qPCR. Allelic variation in *COMT* has repeatedly been linked to executive function, and animal model research on *DGCR8* and *ZDHHC8* suggest a potential role for these genes in executive control. Given this background, we wished to investigate how expression of these genes may relate to global cognition, executive function and working memory. We also explored the effects of age on expression levels.

Methods and Materials

Participants

Fifty-six patients with a molecularly confirmed diagnosis of 22q11.2 deletion syndrome, and 43 typically developing controls participated in the study (Table 1). Patients were recruited via 1) the population of patients followed by the UCLA and Children's Hospital, Los Angeles (CHLA) Pediatric Genetics, Allergy/ Immunology and Craniofacial Clinics; and 2) local support groups (e.g. Velocardiofacial Education Foundation, 22q and You Support Network). Demographically comparable healthy control subjects were recruited from the same communities as patients with 22q11DS, and were tested concurrently. This was accomplished via web-based advertisements about the research study, and by posting flyers and brochures at local schools, pediatric clinics, and other community sites. Exclusion criteria for participants included: substance or alcohol abuse and/or dependence in the last six months and/or insufficient fluency in English. Additionally, healthy controls could not meet criteria for any major mental disorder, with the exception of ADHD or a single past episode of depression. Controls were also excluded if they had a neurological disorder, substance abuse/dependence, intellectual disability, and/or history of head injury with loss of consciousness. This information was collected through

administration of the Structured Clinical Interview for DSM-IV Axis I Disorders with an additional developmental disorders module (41). All interviews were conducted by psychology Ph.D. candidates who had undergone extensive training and reliability procedures, as described in detail elsewhere (42-44).

All participants underwent verbal and written consent after study procedures were fully explained, and their parents or guardians also completed written consent. The UCLA Institutional Review Board (IRB) approved all study procedures. Demographic information for the sample is presented in Table 1. All patients with 22q11DS underwent multiplex ligation-dependent probe amplification (MLPA) analyses to determine specific breakpoint locations; all had the typical 3Mb 22q11.2 deletion (LCR A-D; (45)).

Table 1. Demographic characteristics of study participants.

	22q11DS (N=56)	Controls (N=48)	p value
Age (mean, SD, range)	13.16 (5.28),	13.10 (4.95)	0.95
Gender	28M, 28F	30M, 18F	0.20
Education (years)	6.25 (4.29)	7.13 (4.92)	0.34
Highest Parental Education (years)	15.73 (2.51)	15.72 (2.83)	0.99
Full Scale IQ (mean, SD)	79.59 (16.67)	108.36 (19.21)	<0.001**
ADHD, N (%)	21 (38%)	2 (4%)	<0.001**
Psychotic Disorder, N (%)	5 (9%)	N/A	N/A
ASD, N (%)	26 (46%)	N/A	N/A
Anxiety Disorder, N (%)	26 (46%)	N/A	N/A

Cognitive and Psychiatric Assessment

Supervised clinical psychology doctoral students administered neurocognitive and psychiatric evaluations to participants. IQ data were acquired with the WASI (46). The Behavior Rating Inventory of Executive Function (BRIEF) (47), and University of Maryland letter–number sequencing (LNS) task (48) were also administered to investigate executive function and working memory, respectively. Psychiatric diagnostic information was determined via parental interview using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (41).

Gene Expression

Peripheral blood samples were drawn in two PAXgene tubes, and were stored at 4°C. RNA was extracted using the PAXgene blood RNA kit (PreAnalytix GmbH, QIAGEN, Germany). We assessed RNA quantity using Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE), and quality with Agilent Bioanalyzer Nanochips.

Gene expression was assayed with RT-qPCR using TaqMan assays, as described in Coppola et al, 2006 (49). Total RNA was converted into cDNA by SuperScript II kit (Invitrogen). The reactions were performed with a TaqMan Master Mix (BioRad), in a 25 µl volume. Assays were performed in triplicate, and analyzed using an Roche Lightcycler. qPCR analyses were carried out using the 2(-Delta Delta C(T)) method (2-ΔΔCt). Our genes of interest were *COMT*, *DGCR8* and *ZDHHC8*, and we used *GAPDH* as a reference gene.

Statistical Analyses

Analyses of demographic and behavioral data were performed in SPSS software v. 21 (Chicago, IL). We conducted independent samples t-tests for continuous variables and chi-square tests for categorical variables. For the analyses of relative gene expression differences between patients with 22q11DS and controls, we conducted separate univariate ANCOVAs with gene expression level as the dependent variable, diagnosis as the between-group factor, and age, gender, and RT-qPCR batch as covariates.

We investigated the relationship between expression levels of the three genes with age, IQ, BRIEF global executive composite score, and LNS. We ran within-group partial correlations, controlling for age, gender, and RT-qPCR batch. We also ran univariate ANCOVAs controlling

for age, gender, and RT-qPCR batch to investigate measure by group interactions. Because of the exploratory nature of this study, we did not control for multiple comparisons.

Results

Gene Expression

RT-qPCR analyses reveal significant differences in gene expression between patients with 22q11DS and controls *in COMT, DGCR8*, and *ZDHHC8* (all p<0.001**). As hypothesized, for each gene, expression is decreased in patients with 22q11DS as compared to controls (Figure 1).

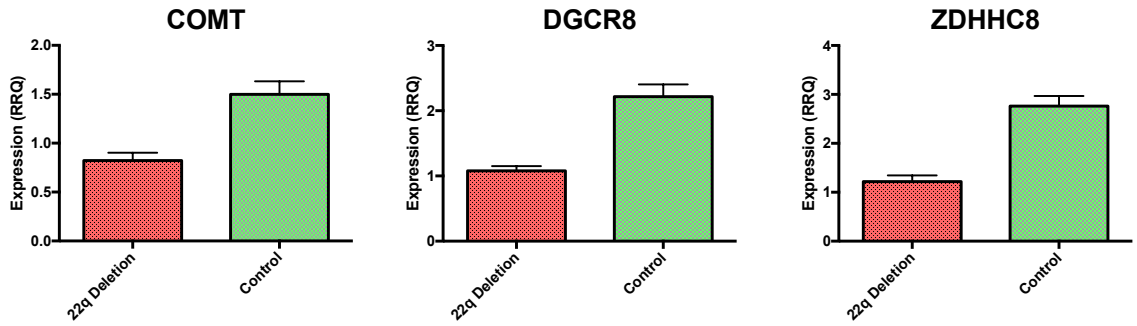

Figure 1. *Gene expression in 22q11DS.* RT-qPCR gene expression results for *COMT, DGCR8,* and *ZDHHC8*. All differences are significant (p<0.001**).

COMT: Relationship with age and cognitive measures

We found a positive relationship between *COMT* expression and age in patients with 22q11DS (r = 0.338, p = 0.016), but not in controls (r = -0.009, p=0.952) (Figure 2). We also found an inverse relationship between *COMT* expression levels in patients with 22q11DS and LNS (r=-0.331, p=0.026), and no relationship in healthy individuals. We found no relationships of COMT expression with IQ or BRIEF scores.

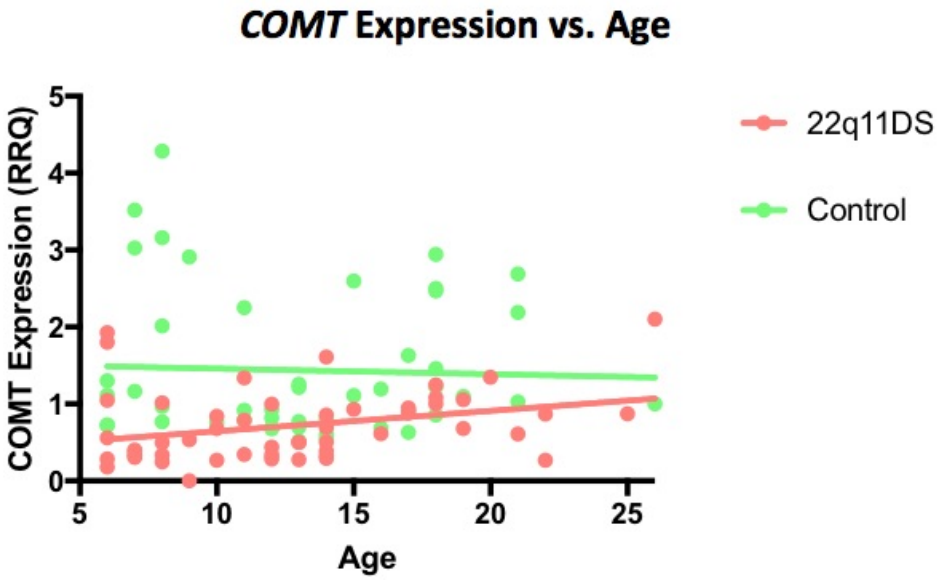

Figure 2. COMT *expression and age.* There is a positive relationship between *COMT* expression and age in patients with 22q11DS (r=0.338, p=0.016*), but not in healthy controls (r=0.009, p=0.962).

DGCR8: Relationship with age and cognitive measures

For *DGCR8* expression, we found a significant group interaction with IQ (p=0.037), suggesting that *DGCR8* levels may affect general intelligence differently in patients with 22q11DS versus controls; within-group correlations between DGCR8 and IQ were not significant, however. We did not find any relationships between *DGCR8* expression and age, LNS, or BRIEF score.

ZDHHC8: Relationship with age and cognitive measures

We found a positive relationship between *ZDHHC8* expression and IQ in controls (r = 0.377, p = 0.018), and a trending negative relationship in patients with 22q11DS (r = -0.248, p =

0.086). There was a significant IQ by group interaction (p=0.004) (Figure 3). We found no relationships between *ZDHHC8* and LNS or BRIEF, nor with age, in either group. A complete list of results is below (Table 2).

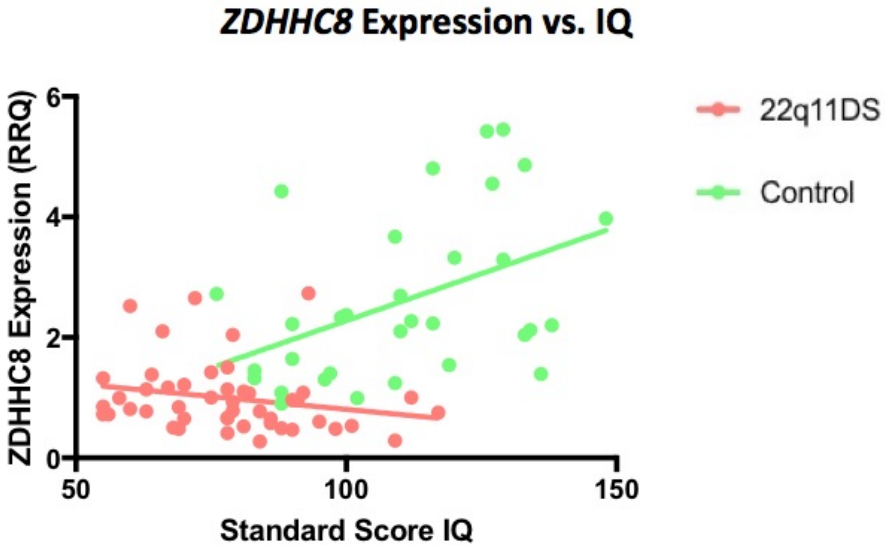

Figure 3. ZDHHC8 *expression and IQ*. There is a positive relationship between *ZDHHC8* expression and IQ in healthy controls (r=0.337, p=0.018*), and a trending negative relationship in patients with 22q11DS (r=-0.248, p=0.086). There is a significant IQ by group interaction (p=0.004**).

Table 2. Relationship between gene expression levels and variables of interest.

	22q11DS	Controls	Interaction
COMT			
Age	r=0.338, p=0.016	r=-0.009, p=0.952	p=0.171
IQ	r=-0.108, p=0.459	r=-0.118, p=0.451	p=0.278
LNS	r=-0.331, p=0.026	r=-0.005, p=0.975	p=0.799
BRIEF	r=0.158, p=0.295	r=-0.233, p=0.192	p=0.178
DGCR8			
Age	r=-0.050, p=0.729	r=-0.042, p=0.782	p=0.946
IQ	r=-0.213, p=0.142	r=0.238, p=0.125	p=0.037
LNS	r=-0.280, p=0.059	r=0.083, p=0.598	p=0.233
BRIEF	r=0.029, p=0.848	r=-0.001, p=0.998	p=0.480
ZDHHC8			
Age	r=-0.090, p=0.532	r=-0.037, p=0.818	p=0.852
IQ	r=-0.248, p=0.086	r=0.377, p=0.018	p=0.004
LNS	r=-0.123, p=0.422	r=0.230, p=0.160	p=0.115
BRIEF	r=0.158, p=0.295	r=-0.147, p=0.447	p=0.065

Discussion

In this study, we sought to measure peripheral gene expression levels of three genes located within the 22q11.2 deletion region, and patients with 22q11DS and controls, and to relate those to measures of cognition. Overall, we found decreased expression of *COMT*, *DGCR8*, and *ZDHHC8* in patients with 22q11DS, as compared to controls, using RT-qPCR. These findings are in line with a previous gene expression study in 22q11DS conducted using microarray

techniques (50). Further, we found that age was associated with *COMT* expression in patients with 22q11DS, and found a differential effect of *ZDHHC8* expression on IQ in controls vs. patients with 22q11DS.

COMT expression levels and relation to neurobehavioral function

We found a positive relationship between age and *COMT* expression levels in patients with 22q11DS, but not in controls. A previous study measured *COMT* activity in postmortem brains of non-deleted individuals, and found increased activity associated with older age (23). The authors postulated that an increase in *COMT* activity is related to a decrease in dopaminergic functioning post-adolescence. Investigation in a larger sample and over a wider age range is warranted to determine age-related trajectories in healthy individuals. We also found a correlation between *COMT* expression levels in patients with 22q11DS and working memory, assessed via LNS ($r=-0.331$; $p=0.026$), such that patients with high *COMT* expression had worse performance on this task. Future work is warranted to determine how SNP variation within COMT corresponds with expression levels and working memory function.

Relationship between *DGCR8* expression levels and IQ

We found a significant interaction for *DGCR8* expression levels when investigating the relationship between group and IQ ($p=0.037$). Although within-group correlations were not significant, they were in opposite directions; 22q11DS patients showed a negative relationship of DGCR8 expression with IQ, whereas this relationship was positive in controls. Patients with 22q11DS have previously been shown to have decreased *DGCR8* expression levels as well as miRNA dysregulation (33), and *Dgcr8*$^{+/-}$ knockout mice have shown behavioral abnormalities in

working memory, hyperactivity, and sensorimotor gating (21). Earls et al (51) found changes in synaptic plasticity as a function of *Dgcr8* expression in mice, a mechanism that may play a role in the underlying physiology underscoring our findings.

Association between *ZDHHC8* expression and IQ in healthy individuals

We found a positive relationship between *ZDHHC8* expression and IQ in healthy individuals, and a trending negative relationship in patients with 22q11DS. *ZDHHC8* codes for an enzyme that acts as a palmitoyltransferase, and SNPs in this gene have been linked to schizophrenia(39). *Zdhhc8* knockout mice show impairments in exploratory behaviors and novelty seeking activity (22). A recent paper showed that abnormalities in long-term dendritic spine stability seen in mouse models of 22q11DS are rescued with overexpression of *Zdhhc8*, indicating a major role for this gene in structural plasticity, which may play a larger role in cognitive function in 22q11DS (40). Specifically, Our results, although preliminary, suggest that *ZDHHC8* may play a role in general intelligence, and that this relationship may differ between patients with 22q11DS and controls. These results warrant further investigation and replication in a larger sample.

Future Directions

In future studies we plan to investigate SNPs in the genes discussed above, in order to determine how allelic variation may impact cognitive function in patients with 22q11DS. In addition to linking the relationship between genotype and function, it is important to consider how allelic variation may relate to gene expression levels.

The link between genotype and gene expression varies across genes and tissue types. Expression quantitative trait locus (eQTL) studies have tried to elucidate these relationships

across healthy individuals and in various psychiatric populations (52). These studies investigate statistical relationships between SNPs and gene expression, revealing loci in the genome whereby there is a linear relationship between expression and allele (53).The first eQTL study of the human brain was published by Myers et al (54), whereby their whole-genome analysis revealed that 21% of their cortically expressed transcripts correlate with genotype. To date, no studies have investigated eQTL in patients with 22q11DS. Online databases, such as http://www.gtexportal.org indicate that many of the SNPs within the genes discussed in this study have a significant effect on gene expression levels in non-blood tissue.

Large-scale genetic studies are warranted to more thoroughly examine gene expression in patients with 22q11DS. The International 22q11.2 Brain Behavior Consortium is a new multi-site initiative to collect genomic and phenotypic information to characterize 1000 individuals with 22q11DS (7). These larger-scale studies will allow for more comprehensive analyses of SNP and sequence variation in relation to both gene expression and quantitative behavioral traits, as well as incorporation of categorical psychiatric diagnoses.

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CHAPTER 6: Conclusions

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Taken together, these studies demonstrate the value of studying endophenotypes of psychiatric disease, using a reverse genetic approach. Our first chapter provides an overview of the utility of studying measurable endophenotypes to link genetic variation to psychiatric phenotypes . The use of studying rare genetic disorders with known genetic alterations that confer a high risk of disease is particularly valuable, and can provide a window into mechanisms underlying idiopathic psychiatric illness.

In Chapter 2, we investigated behavior and neural activity during a task of risky decision-making in patients with NF1 and controls. Behaviorally, youth with NF1 tended to make fewer risky decisions than controls when potential reward was high. Neuroimaging analyses revealed significantly reduced neural activity across multiple brain regions involved in higher-order semantic processing and motivation in patients with NF1 relative to controls, in both decision-making and outcome phases of the task. We also observed atypical age-associated changes in neural activity in patients with NF1, such that during risk taking, neural activity tended to decrease with age in controls, whereas it tended to increase with age in patients with NF1. Findings of this study suggest that developmental trajectories of neural activity during risky decision-making may be disrupted in youth with NF1.

In Chapter 3, we reviewed recent literature on the utility of studying endophenotypes of psychiatric illness in the context of 22q11.2 deletion syndrome, a recurrent copy number variant associated with particularly elevated risk for neuropsychiatric disorder (1). One of the most intriguing aspects of the syndrome is the variability in clinical and cognitive presentation: children with 22q11DS have high prevalence of autism spectrum (ASD), attention deficit, and

anxiety disorders, as well as psychotic-like features, and up to 30% of adolescents and adults develop schizophrenia-like psychosis. This genetic lesion thus provides a unique model for the discovery of specific genomic risk and (potentially) protective factors for neuropsychiatric disease. We provided an overview of neuropsychiatric findings to date, which highlight the value of this syndrome in mapping the developmental trajectory of dimensional phenotypes that traverse multiple diagnostic categories. Potential sources of genetic variability that may contribute to the disorder's heterogeneous presentation are reviewed. Because of its known genetic etiology, animal models can readily be developed that recapitulate specific aspects of the syndrome. Future research directions involve translational models and potential for drug screenable targets in the context of this human model system.

In Chapter 4, we investigated components of real-world executive function in patients with 22q11DS and healthy controls, and the relationship with structural neuroanatomy in the prefrontal cortex (2). We administered the Behavior Rating Inventory of Executive Function (BRIEF), an ecologically valid measure of executive function, in patients with 22q11DS and matched typically developing controls, along with cognitive measures that tap behavioral regulation and metacognition aspects of executive function. Using FreeSurfer's whole-brain vertex cortical thickness pipeline, we investigated brain structure-executive function relationships in patients with 22q11DS and controls. Behaviorally, patients with 22q11DS were impaired on multiple executive function measures. Right orbitofrontal cortical thickness showed a differential relationship between real-world executive function in patients with 22q11DS and controls. This finding was notable given prior literature linking structural lesions in the right prefrontal cortex with executive dysfunction, also measured by the BRIEF (3). We also observed a group difference in the relationship between behavioral regulation and metacognition measures

with thickness of ventral and dorsolateral prefrontal regions, respectively. Findings suggest that executive dysfunction characteristic of 22q11DS is underscored by altered prefrontal cortical structure.

In Chapter 5, we investigated peripheral expression of genes hemizygously affected by the 22q11.2 deletion. Using real-time quantitative polymerase chain reaction (RT-qPCR), we found decreased expression of *COMT*, *DGCR8*, and *ZDHHC8* in patients with 22q11DS as compared to demographically matched controls. We found that increased age was associated with higher *COMT* expression in patients with 22q11DS, but not in controls. We also found a relationship between *COMT* expression and performance on a working memory task in patients with 22q11DS, but not in controls. Next, when looking at *DGCR8* expression levels, we found a significant group interaction with IQ, indicating that *DGCR8* levels may play differing roles in general intelligence in patients with 22q11DS versus controls. Lastly, we found that increased IQ was associated with higher *ZDHHC8* expression in controls, but not in patients with 22q11DS, also suggesting a role of *ZDHHC8* in general intelligence that may differ between patients with 22q11DS and controls. In future work, we plan to investigate single nucleotide polymorphisms (SNPs) within these genes, and the relationship with function as well as with gene expression, using expression quantitative trait locus (eQTL) analyses.

In summation, this collective body of work highlights the importance of studying endophenotypes of psychiatric illness. Current methods of diagnosis in psychiatry rely on clinical symptoms. The RDoC framework supports the notion of studying intermediate endophenotypes in order to better diagnose and treat patients with psychiatric illness (4). Stratifying patients with psychiatric illness into distinct genetic categories could improve intervention and treatment strategies that are based upon their unique etiologies and underlying

physiology (5). If we can get to the root of the building blocks of psychiatric disease by better understanding the intermediate traits that connect genotype to the distal clinical phenotypes, we can not only provide better, more mechanistically informed diagnoses, but ultimately utilize precision medicine techniques to better treat psychiatric illness.

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