DEVELOPMENTAL GENETIC ASPECTS OF SCHIZOPHRENIA, NEUREGULIN-1, AND COGNITION

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Submitted to the Graduate Faculty of

the Kenneth P. Dietrich School of Arts and Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2013

UNIVERSITY OF PITTSBURGH

KENNETH P. DIETRICH SCHOOL OF ARTS AND SCIENCES

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Recent work suggests potentially promising relationships between sequence variation in Neuregulin-1 (NRG1) and both schizophrenia and neurocognitive function. Cognitive deficits are very common in schizophrenia and have been shown to be familial in nature. Based on these findings, we hypothesized that cognitive deficits may be related to variation in NRG1 in an age-dependent manner during adolescence and adulthood, thus providing a possible mechanism by which NRG1 could act as a late neurodevelopmental susceptibility gene for schizophrenia.

This question was examined using individuals from 39 multigenerational multiplex families, including 58 affected participants and 361 unaffected relatives aged 15-85 years. Participants were genotyped for 36 NRG1 single nucleotide polymorphisms (SNPs) previously associated with schizophrenia. Participants completed diagnostic interviews and a computerized neurocognitive battery that assessed eight cognitive domains.

Pedigree-based variance component analyses were performed to estimate the main effects of age and individual SNPs, and the interactions between age and SNPs in predicting cognition for each domain. There were multiple nominally significant NRG1 x age interactions across several domains and markers, although few remained significant after modified Bonferroni correction. Overall, this study suggests a potential role of NRG1 x age interactions in cognitive performance within multiplex families with schizophrenia, especially within the domains of

Emotional Processing, Abstraction/Mental Flexibility, Attention, and Sensorimotor Dexterity and the NRG1 markers SNP8NRG221132, SNP8NRG221533/rs35753505, rs776401, and rs1473438 that warrants further investigation.

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PREFACE

I would like to thank my committee members for all of their assistance and guidance throughout this project.

1.0 INTRODUCTION

Schizophrenia is a psychotic disorder whose acute phases are characterized by positive symptoms, including hallucinations, delusions, and disorganized speech and behavior. In chronic phases, patients frequently experience negative symptoms, including affective flattening, avolition, and alogia. The course of the disorder is persistent for the majority of patients, with full remission to premorbid functioning being relatively uncommon. Clinical age of onset peaks in young adulthood for the majority of people who develop the disorder, with onset during childhood or after the age of 40 being relatively rare. Men and women are diagnosed equally; however, males' age of onset peaks 5-10 years earlier than women's.

A major question regarding schizophrenia concerns these developmental aspects, in particular its age of onset. The literature is quite consistent, finding that the age of greatest risk of disorder onset is in the post-pubertal, late adolescent-young adulthood age range. Most work on these questions has evaluated early developmental hypotheses that posit that schizophrenia-specific factors are present very early in life, possibly in utero, but that they are not expressed until later normative developmental processes occur and "release" them (Murray & Lewis, 1987; Pogue-Geile, 1991; Weinberger, 1987). Late developmental hypotheses, on the other hand, suggest that schizophrenia-specific neuronal changes reflecting genetic or environmental factors occur in adolescence or early adulthood, closer to the actual age of onset of the disorder (Feinberg, 1982a; Pogue-Geile, 1991). Both hypotheses emphasize the importance of such

possible late genetic effects on schizophrenia, but few studies have focused on these effects in the young adult period.

The importance of genes in the etiology of schizophrenia is well accepted; however, relatively little is known about how schizophrenia susceptibility genes' effects change through the lifespan to contribute to changes in brain function and clinical onset. Ideally, prospective longitudinal studies of gene expression in multiple brain regions could be done up to the onset of schizophrenia. Given that such a study would currently be impractical and unethically invasive, other avenues must be pursued. An alternate method would be to perform expression assays in post-mortem samples, but these analyses usually occur many decades after clinical onset. The limitations of ideal and other currently available study designs make it necessary to develop alternate methods to address the question of late developmental changes in schizophrenia's genetic effects.

Given these constraints, investigating changes in genetic effects during the peak age of onset of schizophrenia could employ individuals with elevated genetic liability to schizophrenia, such as relatives of patients with this disorder. In the absence of gene expression data from relevant brain regions, a characteristic or trait that is affected by the genetic liability to schizophrenia should be used. Neurocognitive functions are excellent candidate traits given their strong genetic correlations with schizophrenia. Although a prospective study of cognition among relatives of schizophrenia patients before, during, and after the peak age of onset would be ideal, cross-sectional measurements among a relative sample that has a broad age range is more economical. Finally, a gene that is associated with schizophrenia and known to have strong developmental effects should be used in such an analysis.

The current study employed a multigenerational, multiplex family sample, which theoretically increases the frequency of schizophrenia risk alleles. The participants were assessed for cognitive function, an endophenotype of schizophrenia that is strongly familially related to the disorder, and the age range of relatives was broad and included the peak age of onset of schizophrenia. We investigated variants of the gene neuregulin-1 (NRG1), which has been associated with schizophrenia and has both developmental and cognitive effects. Evaluating the relationships between NRG1 and cognition in the relatives of individuals with schizophrenia from a developmental perspective aims to address questions of how particular gene variants may contribute to liability to the disorder through their impact on cognition in an age-dependent manner, with a specific focus on the period that is the peak of age of onset for the disorder. Each of these study components will be discussed in greater detail below.

1.1 DEVELOPMENT & SCHIZOPHRENIA

1.1.1 Age of Onset in Schizophrenia

Although there are difficulties in determining the onset of schizophrenia and significant differences in how this event is defined between studies (e.g., age of first hospitalization, age of psychotic disorder diagnosis, age of first positive psychotic symptom, age of first symptom of mental illness), most studies report different age of onset patterns between males and females, despite a relatively equal prevalence for both sexes (Pogue-Geile, 1997). Appearance of first psychotic symptom peaks at 20-24 years of age for males with risk generally decreasing with age, while the major peak for females is at 20-24 years of age with a second risk period in the peri-menopausal period (Pogue-Geile, 1997; Rajji, Ismail, & Mulsant, 2009). The median age of first diagnosed psychotic episode is earlier for males than females, however, with males' onset

being typically between the ages of 18 and 25, and females' being between 20 and 30 years of age (American Psychiatric Association, 2000). The age of first hospitalization closely follows this pattern (Pogue-Geile, 1997). The presence of a non-uniform, non-linear pattern of onset suggests that this disorder is likely related to important developmental factors during the post-pubertal, young adult age range. Such factors may be related to changes in gene expression, environmental exposures, or a combination of gene-environment elements that result in an overt change in functioning and/or symptom presence (Pogue-Geile, 1997).

1.1.2 Early Developmental Models

The ways in which genetic and/or environmental changes that occur throughout development may lead to schizophrenia are currently unknown, but two main developmental models have been proposed. The early developmental model hypothesizes that schizophrenia-specific changes in gene expression or environmental insults occur early but do not result in overt abnormality until later in life when the schizophrenia-causing abnormality is released by a nonspecific factor during the course of otherwise normal development (Murray & Lewis, 1987; Pogue-Geile, 1991; Weinberger, 1987). In other words, the maturation of brain systems associated with adolescence and adulthood release abnormalities that have been present as early as conception in patients who go on to develop this disorder.

Much of the initial support for this hypothesis comes from studies that have assessed pre- and perinatal developmental factors in this disorder. Retrospective studies of patients with schizophrenia have found multiple differences between children who go onto develop the disorder compared to those that do not. Specific impairments included reduced premorbid IQ, as well as problems with cognition and language, social and emotional development, and motor performance (Murray et al., 2004; Tarbox & Pogue-Geile, 2008). In addition, a significant subset

of patients with schizophrenia has minor physical abnormalities that suggest anomalies in very early development, despite causing little or no functional problems (Compton & Walker, 2009). Such features include abnormalities in craniofacial traits, eyes and ears, hands and feet, and torso (Compton & Walker, 2009). There is also evidence of abnormal cerebral dominance that may be related to a significantly higher proportion of left-handed patients compared to healthy control samples (Murray & Lewis, 1987).

Many studies have also shown a significant increase of obstetric complications in patients with schizophrenia. Specifically, meta-analyses of studies that assessed prenatal complications or infection, abnormal growth and development of the fetus during pregnancy, and problems during labor and delivery estimated that the odds ratio of developing schizophrenia after exposure to obstetric complications is 2.0 (Rapoport, Addington, Frangou, & Psych, 2005).

Despite the evidence for the early developmental model, it cannot exclude the causal role of genetic and environmental insults that occur after birth and does not explain a significant percentage of patients who do not have physical abnormalities or other birth-related risk factors. In addition, although such early developmental problems are more common in patients with schizophrenia, they are generally non-specific. For example, hypoxia increases the risk of numerous negative outcomes other than schizophrenia, including epilepsy, cerebral palsy, and infant mortality. It is not known how such non-specific events may lead to schizophrenia at different neurodevelopmental phases (Schmidt-Kastner, van Os, Steinbusch, & Schmitz, 2006), which is a weakness of the early developmental models.

1.1.3 Late Developmental Models

In contrast, late developmental hypotheses suggest that abnormalities in maturational processes lead to psychosis, preceding the development of overt symptoms by a much shorter time period

(Pogue-Geile, 1991). As mentioned, many studies suggest that there are premorbid indicators for a subset of patients with schizophrenia that suggest a neurodevelopmental abnormality early in life; however, there is also a significant subset of patients with schizophrenia whose premorbid functioning is average or above average (Feinberg, 1982a, 1982b). In addition, there are no early risk factors or lesions that are specific to schizophrenia or found in all patients, suggesting that late genetic and environmental factors may influence the development of this disorder.

One potential biological mechanism that may support the late developmental models is the pattern of synaptic pruning that occurs in healthy compared to schizophrenia groups. The density of synaptic connections between neurons peaks at approximately age 2, with a significant decline in synaptic density beginning at this time and continuing until late adolescence. This decline is followed by a relatively constant level of synaptic density until late life (Huttenlocher, 1979). Studies have found rather consistent evidence of extreme synaptic pruning in patients with schizophrenia compared to healthy individuals, suggesting that an abnormality in this process may result in conversion to psychosis (MacDonald & Chafee, 2006; Saugstad, 1989). Importantly, this excessive synaptic loss is present in adolescence, suggesting that it is not secondary to medication effects or the chronic nature of the disorder (Bennett, 2008). Moreover, the eliminated synapses are mostly glutamatergic in nature, which is interesting in light of the hypothesis that glutamatergic systems are disrupted in schizophrenia.

In addition to a loss of synapses during adolescence, there is a corresponding loss of dendritic spines that normally occurs during this time, without a corresponding loss in overall neurons. Spines are located on the dendrites of most major neuron types in the brain, including those found in the cortex, striatum, and cerebellum. They typically receive excitatory input, as they generally express glutamatergic receptors, including both N-methyl-D-aspartic acid

(NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor types. Just as patients with schizophrenia seem to have increased synaptic loss, they also have excessive spine loss, particularly in the dorsolateral prefrontal cortex (DLPFC) (Bennett, 2008). Recent neuroimaging studies provide evidence that this loss also occurs during adolescence and is not secondary to the effects of the disorder or its treatment (Bennett, 2008). The neuropathological observation that patients have increased pruning of synapses and loss of dendritic spines during early adolescence makes the hypothesis that schizophrenia is a disorder related to such changes very appealing (Jaaro-Peled et al., 2009).

It may also be that late acting genes are triggered by environmental stressors during young adulthood. One environmental factor that may increase the risk of schizophrenia is the experience of trauma during the typical risk period. Studies estimate that 50-60% of the general population has been exposed to trauma, while traumatic exposure is significantly increased among patients with schizophrenia, in the range of 75-98% of cases (Lysaker, Outcalt, & Ringer, 2010). Importantly, this range includes studies of trauma experienced before and/or after the onset of the disorder. In a study that assessed the rate of significant life events in the month preceding the onset of the disorder, it was found that 46% of the patient sample had experienced at least one of these events (Brown & Birley, 1968). These data suggest that exposure to trauma or stressful life events may play a role in timing the onset of the disorder, in addition to a large literature that suggests that stress can exacerbate symptoms of the disorder after it has developed. Although trauma and stressful life events are non-specific, stress hormones impact the normal structural remodeling of the brain and can lead to dendritic atrophy, reduced neurogenesis, and potentially, synaptic loss in the prefrontal cortex (PFC) and hippocampal regions (McEwen, 2010; Radley & Morrison, 2005). Given the ongoing maturation of the PFC, it is not surprising

that this period of time is also thought to be one in which the brain is particularly vulnerable to environmental stresses, such as stress related to negative life events (Harris et al., 2009). Such effects may be due to the effects of late gene-environment interactions, in which environmental factors influence the phenotype of interest differently depending on genetic background of the individual.

In summary, both the early and late developmental hypotheses highlight the importance of genetic and environmental risk factors, as well as changes during the late adolescent-young adulthood period. There appears to be some support for both hypotheses and intermediate models are also plausible.

1.2 SCHIZOPHRENIA & COGNITION

Although there is significant symptom heterogeneity among patients, one relatively common problem in schizophrenia is cognitive dysfunction. Cognitive deficits are not unique to this disorder and are seen in other psychiatric and neurological disorders; however, the patterns of deficits in schizophrenia are somewhat distinct from other illnesses (Buchanan et al., 2005). Overall, patients have measurable deficits in nearly every cognitive domain compared to healthy individuals, but there is evidence that attention, memory, and executive function are more significantly impaired than verbal and visuospatial abilities (Buchanan et al., 2005; Goldberg & Green, 2002). The cognitive impairment seen in schizophrenia is so common and impacts patient functioning to such a great extent that some have suggested that it is one of the core impairments in this disorder (Murray et al., 2004).

1.2.1 Cognition as an Endophenotype

In addition to being considered a core feature of schizophrenia, cognitive deficits have also been shown to be state independent, that is, they are present in both the acute and chronic phases of the illness (Heydebrand, 2006). Such deficits are also heritable, with similar deficits being commonly detected in individuals at high-risk for developing the disorder and the unaffected biological relatives of patients (Simon et al., 2007; Snitz, MacDonald, & Carter, 2006; Thompson, Watson, Steinhauer, Goldstein, & Pogue-Geile, 2005). Although generally less impaired than patients, the unaffected relatives of patients typically demonstrate small-medium impairments in performance across a wide range of cognitive tasks and abilities (Snitz et al., 2006), with the largest deficits seen on tasks that require attention and working memory, memory, and executive functioning abilities (Snitz et al., 2006). Such dysfunctions are found regardless of relative type (e.g., parent, sibling, or child of a patient with schizophrenia) or presence of schizotypal features in relatives. This suggests that cognitive deficits may be an endophenotype of schizophrenia (Goldberg & Green, 2002). Endophenotypes are traits that can be useful in identifying genetic factors that increase the risk of a disorder (Braff, Freedman, Schork, & Gottesman, 2007; Braff, Schork, & Gottesman, 2007; Gottesman & Gould, 2003).

In assessing the relationship between cognition and schizophrenia, genetic correlations (R_g) are typically used to estimate the proportion of variance that is shared between two traits due to common genetic effects. In other words, this measure reflects pleiotropic effects in which genes influence both schizophrenia risk and different domains of cognitive function (Hare et al., Unpublished data). A recent study (Toulopoulou et al., 2007) found significant genetic correlations between schizophrenia and Wechsler Adult Intelligence Scale estimates among a sample of monozygotic and dizygotic twins who were concordant or discordant for

schizophrenia and a sample of healthy control individuals. The full scale intelligence quotient, verbal comprehension, perceptual organization, and working memory genetic correlations were all significant and ranged from -0.34 to -0.79. Processing speed was not significant in this sample.

In the only other known study of genetic correlations between schizophrenia and cognition, Hare et al. (unpublished data) found that abstraction/mental flexibility, attention, language, spatial processing, facial memory, and emotion processing were all significantly different from zero, with genetic correlations ranging from -0.34 to -0.56 in a multigenerational, multiplex family sample of schizophrenia. Spatial and verbal memory domains were not significant in this sample.

1.2.1.1 Heritability of Neurocognitive Function. A large literature has consistently shown that most cognitive domains have some genetic basis. A number of studies have documented heritability estimates for multiple cognitive areas in families with one or more members with schizophrenia (Glahn et al., 2007; Greenwood et al., 2007; Husted, Lim, Chow, Greenwood, & Bassett, 2009; Toulopoulou et al., 2007; Tuulio-Henriksson et al., 2002; Yokley et al., 2012), with the most commonly assessed domains being: intelligence; attention and working memory; emotional processing; memory; executive functioning; processing speed; sensorimotor dexterity; and spatial processing. Of studies that included affected individuals in their heritability estimates, the majority found significant genetic effects for individual tasks, with heritability in the range of 0.09-0.77 across all tasks and domains. Here, intelligence showed the highest heritability across two studies (Husted et al., 2009; Toulopoulou et al., 2007), in the range of 0.70-0.74, while at least one task in the processing speed, executive function, and attention/working memory domains had very high heritability, in the range of 0.74-0.78 (Glahn et al., 2007; Husted et al.,

2009). Only one known study (Glahn et al., 2007) has estimated heritability in schizophrenia families where the affected member was excluded from the estimate, finding that most tasks have significant genetic effects in the range of 0.00-0.79 across all tasks and domains. Here, processing speed, attention/working memory, and verbal memory all had at least one task showing very high heritability, in the range of 0.75-0.79 (Glahn et al., 2007).

Together, the findings regarding cognitive dysfunction as an endophenotype of schizophrenia, the heritability of cognition, and genetic correlations between schizophrenia and cognition provide strong evidence that neurocognitive function is a good measure of schizophrenia's genetic effects, especially in the domains of attention/working memory, executive function, and memory.

1.3 COGNITIVE DEVELOPMENT THROUGH THE LIFESPAN

Overall, ability in all areas of cognition increases from birth until young adulthood. The order of structural brain development mimics the pattern of increasing brain activation that is believed to underlie improvements in complex cognitive functions. Postnatal brain development occurs in a precise order, beginning with the sensorimotor regions and progressing throughout the brain ending with the DLPFC. Subcortical structure development is relatively complete by late childhood, while cortical development continues into early adulthood, thus full brain development is not completed until mid-adulthood (Craik & Bialystok, 2006).

Brain structure and function are impacted by the onset of puberty in humans, and animal studies provide evidence that hormone changes related to pubertal onset further develop the brain, leading to plasticity and reorganization (Blakemore, Burnett, & Dahl, 2010). The human PFC also continues to mature during this period, including the aforementioned synaptic pruning,

loss of grey matter, and generally linear increase in white matter volume related to continued myelination. Although not much evidence is available about the relationship that puberty itself has with cognitive function in humans (Blakemore et al., 2010; Luna & Sweeney, 2001), longitudinal studies show significant improvements from early to late adolescence (Crown, 1993), likely related to the continued brain maturation (Luna & Sweeney, 2001). Executive function improvements (including planning, reasoning, verbal fluency, and flexible problem solving) are especially noteworthy in this developmental period (Luna & Sweeney, 2001).

Overall, the brain is refined during adolescence through processes of synaptic pruning and continued myelination (Luna & Sweeney, 2001). The temporal relationship between continued maturation of brain systems in adolescence and the typical age of onset of schizophrenia adds further support to late developmental hypotheses, as abnormalities in this stage of life may lead to variability in complex cognitive functioning and schizophrenia onset (Feinberg, 1982a; Luna & Sweeney, 2001).

In midlife, general knowledge does not decline, but retrieval of stored information is reduced, leading some abilities to be maintained, while others decline (Lachman, 2004). Crystallized intelligence, or experience-related tacit knowledge, increases through the lifespan and is maintained; however, fluid intelligence, or logical reasoning and problem-solving, declines in the mid-20s and after (Craik & Bialystok, 2006). Such changes in cognition are likely related to the pattern of further maturation and decline of the brain. The frontal cortex is the last to fully mature, yet it is also the first to be impaired by the aging process (Craik & Bialystok, 2006), thus although cognitive function increases significantly early in life, it plateaus and/or begins to decline near the time of highest risk of schizophrenia. Overall, the development and decline of complex cognitive functions are associated with dynamic processes across the

lifespan. The consistent findings regarding the time of highest risk for schizophrenia and continued cognitive and brain maturation makes the late adolescent-early adulthood stage an interesting period in which to assess these relationships.

1.4 OVERVIEW OF SCHIZOPHRENIA GENETICS

Although studies have identified potentially important non-shared environmental contributions to the risk of developing schizophrenia, studies of families, twins, and adoptees have consistently estimated high heritability (h^2) for the disorder, in the range of 0.80-0.85. These findings suggest that genetic variation is the most important factor overall (Cardno & Gottesman, 2000; Sullivan, Kendler, & Neale, 2003). Despite strong genetic features, the genetic risk for schizophrenia is believed to be transmitted in a complex, polygenic manner (Gottesman & Shields, 1967), which has been confirmed by recent linkage and association studies that have implicated over 130 potential susceptibility genes in the pathogenesis of the disorder, each with small effect sizes and inconsistent replication attempts (Carter, 2006).

1.5 NEUREGULIN-1 & SCHIZOPHRENIA

1.5.1 NRG1 Genetic Associations with Schizophrenia

As mentioned, the genetic effects on schizophrenia are well accepted, but studies have generally found small effect sizes for individual variants and have been plagued by inconsistent replication attempts. Two recent meta-analyses reassessed inconsistencies among the more than 20 genome-wide linkage studies of this disorder, with chromosomal regions 8p and 22q being the only loci identified by both studies (Badner & Gershon, 2002; Lewis et al., 2003). Similar inconsistencies

are found among association studies of schizophrenia, with NRG1, dysbindin (DTNBP1), regulator of G-protein signaling 4 (RGS4), and metabotropic glutamate receptor 3 (GRM3) being among the most commonly replicated genes (see Harrison & Weinberger, 2005 for a review). On the other hand, recently completed genome-wide linkage (Holmans et al., 2009) and association (Purcell et al., 2009; Ripke et al., 2012; Shi et al., 2009; Stefansson et al., 2009) studies have not found significant results for NRG1.

NRG1 was originally identified as a candidate gene for schizophrenia by a fine mapping linkage study of Icelandic multiplex families with the disorder (Stefansson et al., 2002) after previous studies had identified the 8p region as potentially possessing schizophrenia-related genes (Pulver et al., 1995). Over 60 replication attempts of this association between schizophrenia and NRG1 have been conducted using different designs and different ethnic and geographical samples, with more than half finding evidence of an association (Allen et al., 2008). Overall, estimates of relative risk lie between 1.0 and 2.2 for individual NRG1 variants and haplotypes (Tosato, Dazzan, & Collier, 2005).

The combination of findings from genetic linkage and association studies suggest that the 8p chromosomal region may harbor a schizophrenia risk gene, and that NRG1 may be this susceptibility gene. The known biological functions of NRG1 overlap with a number of the dysfunctions believed to be a part of the pathogenesis of the disorder, making this gene both a positional and functional candidate for schizophrenia (Harrison & Law, 2006).

1.5.2 NRG1's Structure & Function

Positionally, NRG1 lies at 8p22-p11, encompassing 1.3 million bases and including at least 21 alternatively spliced exons (Steinthorsdottir et al., 2004), although only 0.3% of the gene codes for protein (Scolnick, Petryshen, & Sklar, 2006). NRG1 encodes 15-20 proteins from the

transcription of 6 promoters with significant alternative splicing (Law et al., 2006; Talmage, 2008). In total, there are six known splicing isoforms, but much is still being learned about their distributions in the human nervous system and their developmental expression patterns.

This gene is highly polymorphic, with nearly 350 SNPs required to "tag" the gene. Most variants associated with schizophrenia are noncoding, and are instead intronic or upstream of the transcription site. Thus, NRG1 variants associated with the disorder may impact disorder risk by their regulatory roles, such as impacting the stability of the mRNA and/or through alternative splicing (Law et al., 2006; Mei & Xiong, 2008).

Functionally, NRG1 is a ligand for the ErbB receptor family, which are receptor tyrosine kinases (Scolnick et al., 2006; Wolpowitz et al., 2000). NRG1 is generally released from the presynaptic cell and binds to and modifies postsynaptic ErbB receptors. NRG1 has multiple roles in the development and organization of the human nervous system, as well as roles in its continued function throughout life, through its relationship with this receptor system. It acts as a pleiotropic growth factor (D. Li, Collier, & He, 2006) with more than 12 known functions. The multiple isoforms of NRG1 produce the wide diversity in this gene's functions over the lifespan via the formation of different proteins (Law, Shannon Weickert, Hyde, Kleinman, & Harrison, 2004; Rapoport et al., 2005).

Many of the processes that NRG1 is involved in are thought to be altered in schizophrenia, either by direct or indirect means, leading to multiple possibilities by which NRG1 may be involved in the pathogenesis of this disorder (Corfas, Roy, & Buxbaum, 2004). Of particular interest in the current study are the functions of NRG1 that also have important developmental implications, including control of neuronal migration and differentiation, synaptogenesis and modulation of synaptic transmission, hormonal control of puberty, regulation

of NMDA receptors (NMDAR), and the modulation of long-term potentiation (LTP) (Harrison & Law, 2006; Jaaro-Peled et al., 2009).

1.5.2.1 Neuronal Migration & Differentiation. NRG1 has multiple roles in pre- and perinatal development. Specifically, it aids in the processes of radial and tangential migration and axon guidance in the cortex by stimulating neurite outgrowth and radial glia cell growth (Jaaro-Peled et al., 2009; Mei & Xiong, 2008). In addition, it promotes myelination via its roles in oligodendrocyte differentiation and development (Jaaro-Peled et al., 2009; Mei & Xiong, 2008).

When compared to control groups, patients with schizophrenia have consistently been found to have decreased brain volume and enlarged ventricles (R. E. Gur et al., 2000; Honea, Crow, Passingham, & Mackay, 2005), significant differences in neuronal density and migration (Sei et al., 2007; Weinberger & Marenco, 2003), and reduced oligodendrocyte levels and subsequent disturbances in myelination (Corfas et al., 2004), suggesting a possible role for NRG1 in these atypically occurring processes.

1.5.2.2 Synaptogenesis & Modulation of Synaptic Transmission. A recent study (Fazzari et al., 2010) found that NRG1-ErbB4 signaling promotes both inhibitory and excitatory synaptogenesis in animals. NRG1's role in the synapse also extends beyond synaptogenesis to synaptic maintenance and maturation. Increased NRG1, and resultant over-expression of ErbB4, selectively increases AMPA receptor synaptic currents (Bennett, 2008; B. Li, Woo, Mei, & Malinow, 2007); dendritic spine size, development, and maturation (Barros et al., 2009); and dendritic arborization (Krivosheya et al., 2008). Prevention of NRG1-ErbB4 signaling has been shown to result in the opposite effects.

Overall, NRG1 may impact the formation, maturation, and/or stability of synapses in important brain regions directly (Fischbach 2007), as aberrant synaptic connections seen in schizophrenia may be due to problems with dendritic arborization, activity-dependent dendritic spine plasticity, myelination, and/or pruning (Jaaro-Peled et al., 2009). Although it is not known how altered expression of NRG1 results in schizophrenia symptoms, it is possible that abnormal expression of NRG1 and subsequent changes in synapse formation, maturation, stability, and transmission (Fischbach, 2007; Krivosheya et al., 2008) could play a part in the disorder's pathogenesis.

1.5.2.3 Hormonal Control of Puberty. NRG1-ErbB signaling has also been shown to play an important part in the neuroendocrine regulation of puberty and hormone production and release in animal studies. Specifically, transgenic mice expressing a dominant-negative form of ErbB4 receptors in hypothalamic astrocytes show delayed pubertal onset and reduced fertility due to abnormally low release of luteinizing hormone-releasing hormone, one of the neuropeptides that regulates sexual development (Prevot et al., 2003). The release of this hormone generally initiates puberty, thus NRG1 signaling problems may lead to abnormal initiation and regulation of hormone levels, which may increase schizophrenia risk due to disruption of neurodevelopmental processes (Corfas et al., 2004), and is particularly interesting in light of the typical post-pubertal onset patterns of this disorder.

1.5.2.4 Regulation of NMDAR and Modulation of LTP. Although the focus of much of the research on the pathophysiology of schizophrenia has been on dopamine receptors, the role of other neurotransmitters cannot be ignored. NRG1 is known to regulate both the expression and plasticity of multiple neurotransmitter receptor systems, including NMDAR and others (Corfas et al., 2004; Hashimoto et al., 2004; MacDonald & Chafee, 2006), that are thought to be

altered in schizophrenia. Of particular interest in this study is that NRG1 is believed to play a role in the maturation of NMDAR, which are tetrameric and composed of multiple subunit types, including NR1, NR2A-NR2D, and NR3A-NR3B (Kristiansen, Huerta, Beneyto, & Meador-Woodruff, 2007; Meador-Woodruff & Kleinman, 2002). NRG1 is believed to promote the maturation of this receptor system via its roles in regulating receptor subunit composition. Specifically, NRG1 facilitates a change from receptor structures that are primarily comprised of NR2B receptor subunits to those with a higher proportion of NR2C subunits. This is interesting because glutamate-dopamine connections are known to mature during puberty in multiple brain regions, including the PFC. This maturation is believed to be triggered by the levels of dopamine receptors as well as the mature composition of NMDAR that are reached at this developmental stage.

The maturational process of NMDAR may also be related to psychosis in that some (but not all) studies (Farber, 2003; Olney & Farber, 1995a, 1995b) have shown that the administration of NMDAR antagonists induces psychosis and reversible morphological changes to neurons in the animal retrosplenial cortex in a selective manner—only causing such changes in animals that are in or have passed through puberty at the time of drug administration. Consistent with these animal findings is that pre-pubertal humans rarely develop psychotic symptoms after administration of NMDA antagonists used for anesthetic purposes, such ketamine, while adults commonly do (Farber, 2003). This further suggests that these circuits are not activated until the post-pubertal developmental stage and may be related to NMDAR subunit composition.

In the adult brain, NRG1-ErbB4 signaling is believed to modulate plasticity (Jaaro-Peled et al., 2009). Upon NRG1 binding with ErbB4, ErbB4 becomes hyperphosphorylated, which is believed to lead to hypophosphorylation of activated NMDAR via the shared connections ErbB

and NMDA receptors have with postsynaptic density 95 (PSD95). PSD95 is an electron-dense area on the tip of dendritic spines and is where most glutamatergic receptors are localized. Increased NRG1 release has been found to result in sustained increases in the intensity of ErbB4-PSD95 interactions and results in increased ErbB4 expression on dendritic spines (Bennett, 2008, 2009; B. Li et al., 2007). Thus, increased NRG1-ErbB4 signaling may result in a prolonged state of NMDAR hypofunction, as has been found in the PFC of patients with schizophrenia (Hahn et al., 2006) and is thought to be related to changes in expression of NRG1, rather than a specific mutation or polymorphism (Fischbach, 2006; Gu, Jiang, Fu, Ip, & Yan, 2005).

In addition, LTP is one of the mechanisms that is believed to underlie neuronal plasticity and is currently the main experimental model of learning and memory. NMDAR-dependent LTP involves the strengthening of connections between cells that leads to long-term improvements in synaptic efficacy through changes to pre- and postsynaptic cells (Lau & Zukin, 2007). Although the way in which NRG1 controls LTP is unknown, current evidence suggests that both low and high levels of NRG1-ErbB4 signaling impair synaptic plasticity and the induction and maintenance of LTP (Mei & Xiong, 2008). In their recent review, Mei and Xiong (2008) suggest that this phenomenon may be due to basal NRG1 signaling activity, ErbB4 receptor levels, and neuron activity that all combine to drive NRG1 expression. Further, they suggest that the NRG1-ErbB4 signaling effects lie downstream of the NMDAR. Evidence from recent studies supports the idea that NRG1 prevents LTP and depotentiates this process in the hippocampus by promoting AMPA receptor internalization; however, this effect is not seen in other brain regions, including the PFC (Buonanno, 2010). Thus, the impact of NRG1-ErbB4 signaling on plasticity likely varies by brain region, and abnormally high expression of NRG1 may result in cognitive dysfunction like that seen in schizophrenia.

1.5.3 NRG1 Expression Patterns

Although there have been promising linkage and association findings from studies of molecular genetics in schizophrenia, these polymorphisms are not necessarily related to protein structure, function, or specific model of pathogenesis. This may suggest that the expression of these genes, especially those with neurodevelopmental roles, needs to be better understood. With the exception of somatic mutations that are acquired over the lifespan, DNA sequence does not change; however, the level that individual genes are expressed, that is, the amount of functional protein or RNA product produced by the gene, does vary over the lifespan, with different patterns for different genes and tissues.

1.5.3.1 NRG1 Expression & Age in the Healthy Brain. Animal studies have found a wide distribution of NRG1 expression throughout the brain and nervous system during embryonic development, with a more selective expression pattern with aging (Addington et al., 2007). In the human brain, NRG1 mRNA expression is highest during prenatal and early postnatal development and then significantly decreases to a near stable level with continued aging, suggesting that NRG1 has a continual presence and function in the adult brain, including schizophrenia-related regions (Buonanno, 2010; Pankonin, Sohi, Kamholz, & Loeb, 2009).

As previously mentioned, there are multiple splicing isoforms: three major (Types I-III) and three minor isoform families (Types IV-VI), with promoter use and splicing patterns being related to developmental stage, tissue, and cell type (Talmage, 2008). Although different NRG1 isoforms are believed to play different roles in neurodevelopment, not much is known about whether specific NRG1 isoforms impact schizophrenia risk more than others (Hashimoto et al., 2004). Decreased overall NRG1 expression has been shown to be related to a significant reduction in the number of functional NMDAR in animals (Esper, Pankonin, & Loeb, 2006);

however, it is thought that increased expression, specifically of Types I or IV, would lead to a decrease in NMDAR signaling and thus a hypofunctional state (Law et al., 2006).

In a recent study in which NRG1 mRNA and protein were localized in the healthy adult human brain, widespread expression was found in multiple regions and cell types thought to be involved in the pathogenesis of schizophrenia (Law et al., 2004). Specifically, NRG1 mRNA was detected in the DLPFC, cingulate cortex, thalamus, amygdala, hippocampal formation, cerebellum, and multiple brainstem nuclei in pyramidal neurons, some interneurons, Purkinje, and Golgi cells. NRG1 protein was detected in the hippocampal formation, DLPFC, brainstem nuclei, and cerebellum in pyramidal, Purkinje, and white matter cells.

In a recent study of age-related NRG1 expression in Brodmann Area 10 of healthy human PFC, Colantuoni et al. (2008) found that there is a significant reduction of NRG1 expression at the end of the schizophrenia risk period. Specifically, expression significantly decreased in early adulthood (ages 18-30) followed by a mostly constant expression pattern throughout late adulthood (ages 31-67). There were no significant differences between sexes, as may have been hypothesized (Colantuoni et al., 2008).

A more recent study (Harris et al., 2009) assessed type I and IV NRG1 expression in the human PFC (Brodmann area 46) of healthy individuals aged 0-49, finding a similar pattern. Specifically, NRG1 expression was highest at birth and decreased significantly until the mid-20's, at which point it became largely constant over the rest of the lifespan. Importantly, Harris et al. (2009) point out that this gene is "minimally expressed" during the late adolescent period in healthy humans. The findings from Colantuoni et al. (2008) and Harris et al. (2009) suggest that, in addition to its role in early brain development, NRG1 expression is also important in the maturation of the brain, particularly the PFC, via a changing expression pattern.

At this time, the distribution of individual NRG1 proteins is largely unknown (Buonanno, 2010); however, type I isoform is expressed at high levels during the early phases of development and is believed to be a factor underlying neuronal plasticity due to its activity-dependent regulation and involvement with NMDAR regulation and expression (Hashimoto et al., 2004). In fact, LTP has been found to increase type I expression in multiple brain regions (Hashimoto et al., 2004). Type II is expressed in the adult human central nervous system (CNS), while type III is mostly related to sensory and motor neuron function, and both have some role in development and plasticity (Hashimoto et al., 2004).

Overall, studies have consistently suggested that NRG1 is expressed throughout the lifespan, with the highest expression levels seen during childhood and early adolescence, and a dramatic reduction in expression near the peak age of onset for schizophrenia. This temporal relationship makes investigating the role that NRG1 variants play in cognition across the lifespan particularly interesting.

1.5.3.2 NRG1 Expression in Schizophrenia Patients. A recent study of post-mortem gene expression in normal control individuals and schizophrenia patients by Torkamani et al. (2010) found that the genetic pattern that distinguished the two participant groups was at the level of gene expression. Specifically, previous studies have shown that genes related to neurodevelopment are naturally down-regulated with age, most significantly between birth and the early-mid twenties (Torkamani, Dean, Schork, & Thomas, 2010). Torkamani et al. (2010) found that the age-related decreases in the expression of multiple genes seen in normal controls were not present in the schizophrenia sample, suggesting that there is not the normal age-related expression decrease of these genes, which may trigger the onset of overt symptoms and functional changes. Although this study did not assess NRG1, studies have found that NRG1

expression is not uniform across the lifespan, such that the expression slope (rate of change) is associated with age, similar to other neurodevelopmental genes.

There are no studies that assess NRG1 expression over the lifespan in patients with schizophrenia, to our knowledge; however, several cross-sectional studies have found differences between patient and control groups. One study (Zhang et al., 2008) found that the total level of NRG1 mRNA expression in peripheral blood lymphocytes was significantly lower in unmedicated patients compared to their unaffected siblings and healthy control individuals, and that this level increased in the patient group with the introduction of antipsychotic medication. Another study that assessed peripheral blood lymphocytes found increased expression of the type III isoform β3 in patients compared to their healthy siblings (Petryshen et al., 2005).

Altered NRG1 expression has also been found in the CNS tissue of patients with schizophrenia when compared to healthy samples. Specifically, three studies found increased expression of NRG1. Type I NRG1 mRNA was found to be upregulated in the DLPFC (Hashimoto et al., 2004) and hippocampus of patients (Law et al., 2006), while levels of NRG1-intracellular domain protein were found to be increased in the PFC of patients (Chong et al., 2008). One study found that an element of NRG1 expression was decreased in patients: expression of NRG1 type Iα, which is only expressed by white matter interstitial neurons and some GABAergic cortical interneurons, was reduced in patients with schizophrenia compared to healthy individuals (Bertram et al., 2007). Another study (Boer, Berk, & Dean, 2009) found no association between NRG1 type Iα protein expression in Brodmann's area 46 within the PFC and schizophrenia when compared to healthy controls.

When isoform ratios were considered, Hashimoto et al. (2004) found decreased type II NRG1 expression relative to types I and III, while Law et al. (2006) found that types II and III

were not altered, but there was a relative increase of type I to types II-IV. Type I-III isoform levels have also been positively correlated with participant age in normal controls, but not in schizophrenia patients (Hashimoto 2004), while type I expression was significantly positively correlated with antipsychotic medication dosage in patients (Tabares-Seisdedos & Rubenstein, 2009).

Taken together, the results of expression studies in schizophrenia patients suggest that there are alterations of NRG1 expression in multiple brain regions and blood components, but that these patterns likely vary significantly by brain region and isoform assessed. Overall, most studies found an increased pattern of expression in patients compared to healthy samples, congruent with the idea that neurodevelopmental gene expression may not appropriately down-regulate in patients with the disorder.

1.5.4 NRG1 & Neurocognitive Functioning

Six previous studies assessing specific NRG1 gene variants and cognition in humans have found mixed results. One NRG1 variant used in studies of cognition has been SNP8NRG221533 (renamed: rs35753505). In healthy participants rs35753505 was found to have no association with working memory performance (Krug et al., 2008), but was associated with semantic verbal fluency (Kircher et al., 2009) and sustained attention (Stefanis et al., 2007). The same single nucleotide polymorphism (SNP) was also tested in patients with schizophrenia, finding significant effects on blood flow in several brain regions, but not task performance (Kircher et al., 2008).

A second NRG1 variant studied in the context of cognition is SNP8NRG243177 (renamed: rs6994992). This SNP was associated with premorbid IQ and fronto-temporal activation in patients (Hall et al., 2006), as well as verbal IQ and brain activation in verbal

fluency tasks in participants at high-risk for developing the disorder (Hall et al., 2006), although non-significant findings between rs6994992 and premorbid IQ in patients have also been reported (Crowley et al., 2008). A third study found that rs6994992 was moderately associated with spatial working memory in a general population sample (Stefanis et al., 2007). Microsatellite 433E1006 has also been tested for association with cognition in a study of Greek male military conscripts, finding that it was modestly associated with sustained attention and verbal working memory (Stefanis et al., 2007).

Overall, there is evidence that NRG1 is associated with cognitive function in healthy, at risk, and schizophrenia samples. The exact nature of this relationship or how it may change across the lifespan, however, is not understood.

1.6 AIMS & HYPOTHESES

Given its potential roles in lifespan development, cognition, and schizophrenia, we hypothesize that NRG1 gene variants may contribute to liability to the disorder through their impact on cognition in an age-dependent manner. This is the first study of its kind, to our knowledge, as previous studies have relied on postmortem non-schizophrenia samples and lacked antemortem cognitive assessment.

The specific questions this study aims to address are:

1) Is cognition genetically correlated with schizophrenia in this multiplex family sample? Significant genetic correlation is a prerequisite for performing the proposed analyses. We hypothesize that there will be significant genetic correlations between schizophrenia and most cognitive domains, indicating joint genetic effects on these traits.

2) Is cognition heritable in this multiplex family sample? Trait heritability is a prerequisite for performing genetic analyses and we hypothesize that there will be significant heritability for most domains.
3) Is cognitive functioning associated with age in this sample, and if so, how? We hypothesize that there will be a significant relationship between age and cognitive function for all domains. Special attention will be paid to cognitive functions that show changes in schizophrenia's peak age of onset range.
4) What is the relationship between NRG1 SNPs and cognition in the present sample? Although it is not necessary to have significant main effects in order to have significant interaction effects, it is important to know whether those main effects exist when interpreting the impact of the interactions, as these associations would suggest that NRG1's mechanism of increasing risk for schizophrenia may be due, in part, to its relationship to cognition. We hypothesize that there will be multiple significant relationships between NRG1 variants and most cognitive domains.
5) Most importantly, are there significant interactions between NRG1 SNPs and age in predicting cognitive function in multiple domains? We hypothesize that there will be significant interactions and that there will be larger effects of NRG1 variants on cognition during the peak age of onset time period (ages 18-30) than for other ages. If there are not significant interactions, however, this would suggest that NRG1's effects are generally consistent across different ages and that differential effects at age of onset are rather minimal.

2.0 METHODS

2.1 PARTICIPANTS

2.1.1 Recruitment and Inclusion Criteria

Probands and their family members were identified by the University of Pittsburgh (PITT) and/or the University of Pennsylvania (PENN) through mental health and consumer organizations in Pennsylvania, New Jersey, Delaware, Ohio, West Virginia, Kentucky, Michigan, and Indiana. Probands were included if they had a diagnosis of schizophrenia, were of European-American origin, 18 years or older, and competent to provide informed consent. In addition, they also had to have one or more first degree relatives with a diagnosis of schizophrenia or schizoaffective disorder-depressed type, and have a large, multigenerational family with ten or more first and second degree relatives. Probands were excluded if they did not provide consent to contact their family members, their IQ was lower than 70, they were not proficient in English, and/or their diagnosis was complicated by the effects of substance use, prescription medications, or medical conditions.

Relatives had to be 15 years or older and willing to provide signed consent. Exclusion criteria for this group included: IQ < 70, not being proficient in English, and/or a CNS disorder that would interfere with the interpretation of cognitive measures.

Control participants were recruited from the same areas as patients and relatives and were included if they met the inclusion/exclusion criteria during a standardized screening. Attempts were made to group match potential control participants to index family members on age, sex, and ethnic background. Recruitment at the PITT site was done through random digit dialing in the area codes where probands and family members were recruited. After the study was described to potential participants, a telephone screen was used to exclude those with psychosis or cognitive disorders. All participants who passed the telephone screen and matching criteria were consented and enrolled in the study.

Recruitment for PENN controls was done through advertisements and word of mouth. A screening interview was used to detect psychotic and cognitive disorders. In addition, a second group of PENN controls was included whose data had been gathered prior to the current study. These controls were administered the same interview as the other PENN control participants to screen for psychopathology and completed the same study procedures. For both sites, control participants were excluded if they had any Axis I disorder with psychotic features or a cluster A personality disorder, if they were taking psychotropic medications, or had a first degree relative with psychosis. They also had to be medically and neurologically healthy.

Written informed consent was obtained after the study procedures had been fully explained in accordance with the Institutional Review Boards of PITT, PENN, and the Texas Biomedical Research Institute. For participants younger than the age of 18, the participant's assent and parents' consents were obtained.

2.2 PROCEDURES

2.2.1 Diagnostic Assessment

Clinical evaluation included the Diagnostic Interview for Genetic Studies, version 2.0 (DIGS) (Nurnberger et al., 1994), the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992), and a review of medical records. Assessment was conducted by trained interviewers with established reliability under the supervision of investigators; however, interviewers were not blind to the status (proband, relative, control) of the individuals participating in the study. To further ensure reliability, investigators who had not evaluated the individual reviewed each case independently and provided DSM-IV multiaxial lifetime diagnoses, with differences being resolved by consensus. In addition, complex cases were discussed between sites. At each site, interrater reliability among investigators and interviewers was tested at regular intervals using videotaped interviews and bimonthly joint interviews. Each team of interviewers reviewed 10 videotaped DIGS evaluations from the other site. Kappa values for exchanged tapes were maintained at or above 0.8. Finally, the two teams met twice a year for further diagnostic and reliability training. In place of the DIGS, 109 control participants from the PENN site were administered a diagnostic checklist to make diagnoses and rule-out schizophrenia-spectrum disorders.

2.2.2 Neurocognitive Measures

Participants were administered a computerized neurocognitive battery previously tested in both healthy and patient samples (R. C. Gur, Ragland, Moberg, Bilker et al., 2001; R. C. Gur, Ragland, Moberg, Turner et al., 2001). The battery took approximately 60 minutes to complete and was administered by research assistants using desktop or laptop computers. The tests included training modules and had automated scoring to ensure reliability of results. Tests were

administered in a fixed order. Raw scores were converted to z-scores using the mean and standard deviation (SD) from the control group used in the present study. Z-scores for domains with more than one test (Emotional Processing) or with two conditions (Attention: letter and number) were calculated by converting the raw scores for both tasks to z-scores using the method described above and then averaging the standard scores. Domain scores for tasks with immediate and delayed conditions (Verbal, Facial, and Spatial Memory) were calculated by averaging the performance on both conditions and then converting the raw average to a z-score.

Three performance indices were calculated: accuracy (number of correct responses), speed (median reaction time for correct responses), and efficiency (ratio of accuracy to the log of speed). Efficiency was analyzed in the current study because it is a single score that incorporates both accuracy and speed to provide an index of correct responses per unit of time that reflects general ideas of good performance (i.e., for a given level of accuracy, quicker responses are better and for a given level of speed, more accurate responses are better). In addition, using the combined efficiency index also reduces the number of statistical comparisons relative to analyzing both accuracy and speed separately.

The battery assessed the following domains (as previously reported in R.E. Gur et al., 2007):

2.2.2.1 Abstraction/Mental Flexibility. The Penn Conditional Exclusion Test (Kurtz, Ragland, Moberg, & Gur, 2004) presents four objects at a time, and the participant selects the object that does not belong with the other three based on one of three sorting principles. Sorting principles change and feedback guides their identification (time: 12 minutes).

2.2.2.2 Attention. The Penn Continuous Performance Test (Kurtz, Ragland, Bilker, Gur, & Gur, 2001) uses a continuous performance test paradigm where the participant responds to

seven-segment displays whenever they form a digit or letter, depending on the condition. Working memory demands are eliminated because the stimulus is present (time: 8 minutes).

2.2.2.3 Spatial Processing. Judgment of Line Orientation (Benton, Varney, & Hamsher, 1975) is a computer adaptation of Benton's test. Participants see two lines at an angle and indicate the corresponding lines on a simultaneously presented array (time: 6 minutes).

2.2.2.4 Emotion Processing. Identification of facial affect was tested with two 40-item tasks. During the Penn Emotion Recognition Task, participants labeled faces as being happy, sad, angry, fearful, or neutral. During the second task, the Emotion Intensity Discrimination Test (R. E. Gur et al., 2006), each stimulus was comprised of two faces of the same individual showing the same emotion (happy or sad) with different intensities. The participant selects the more intense expression. Sets were balanced for gender, age, and ethnicity (5 minutes).

2.2.2.5 Verbal Memory. The Penn Word Memory Test (R. C. Gur et al., 1993) presents 20 target words followed by an immediate recognition trial with targets interspersed with 20 distractors equated for frequency, length, concreteness, and low imageability using Paivio's norms. Delayed recognition is measured at 20 minutes (time: 4 minutes).

2.2.2.6 Facial Memory. The Penn Face Memory Test (R. C. Gur et al., 1993) presents 20 digitized faces subsequently intermixed with 20 foils equated for age, gender, and ethnicity. Participants indicate whether or not they recognize each face immediately and after a 20 minute delay (time: 4 minutes).

2.2.2.7 Spatial Memory. The Visual Object Learning Test (Glahn, Gur, Ragland, Censits, & Gur, 1997) presents 20 Euclidean shapes subsequently interspersed with foils immediately and after a 20 minute delay (time: 4 minutes).

2.2.2.8 Sensorimotor Dexterity. The participant uses a mouse to click on squares appearing at varied locations on the screen (R. C. Gur, Ragland, Moberg, Turner et al., 2001). The stimuli become progressively smaller (time: 2 minutes).

2.2.2.9 Quality Control. To help ensure the validity of the cognitive data, participants with missing data on 10 or more measures (i.e., the accuracy, speed, and efficiency variables of the 8 cognitive tests) in the battery were excluded from the sample prior to this study.

2.2.2.10 Estimation of IQ. All participants were administered the reading subtest of the Wide Range Achievement Test-III (WRAT) as an estimate of intelligence. This measure is commonly used to estimate crystallized verbal intelligence and is relatively robust to the effects of most psychiatric symptoms and brain injury. Raw scores were age-standardized based on published manual norms.

2.2.3 Selection of SNPs and Primer Design

The SNP set for the current study is based on a previously designed primer. The primer incorporated SNPs that were positively associated with schizophrenia by at least one study, near microsatellite haplotype blocks previously associated with schizophrenia (e.g. HapICE, HapIRE, etc.), and those that are exonic. This SNP pool was submitted to Applied Biosystems, Inc. (ABI) SNPlex Genotyping System 48-plex Assay Design and Ordering System (accessed 07/2007) in order to create the primer. The design system checked for a non-competitive reaction, deleterious pooling, and small pooling within the proposed primer set. This design cleared the algorithm as being able to function appropriately within one primer pool. All SNPs have a minor allele frequency of at least 5% in European American populations according to Ensembl (release 43), dbSNP (build 127), and HapMap (release 21a).

Nineteen of the 36 SNPs previously incorporated into this primer have been chosen as the "target" SNPs for the current study. These SNPs were chosen based on their previous positive associations with schizophrenia reported in the literature (N=15), a history of consistent positive associations with cognition within the current participant pool (N=2), or both (N=2). The remaining 17 SNPs are "non-target" SNPs, as they do not meet any of the above criteria. SNP information and a line diagram of the gene with the SNP set are shown in Table 1 and Figure 1.

Table 1. Characteristics of individual SNPs included in the current study

Marker	Position	Type	Category	Major Allele	Minor Allele	Minor Allele Frequency	HWE (p)	SNP Failure (%)
SNP8NRG221132*	31,473,740	Upstream	Target	G	A	0.1112	0.2860	0 (0.00%)
SNP8NRG221533/rs35753505	31,474,141	Upstream	Target	T	C	0.3300	0.5655	3 (0.83%)
rs4298458	31,484,870	Upstream	Target	G	C	0.4230	0.3518	0 (0.00%)
SNP8NRG241930*	31,494,334	Intron	Target	G	T	0.3391	0.8434	4 (1.11%)
rs1081062	31,500,264	Intron	Target	T	C	0.2637	0.7076	1 (0.28%)
rs4566990	31,573,695	Intron	Non-target	G	A	0.3649	0.2224	0 (0.00%)
rs1354335	31,640,979	Intron	Non-target	C	G	0.1695	0.2893	8 (2.22%)
rs1354336	31,644,871	Intron	Non-target	T	C	0.2508	0.3228	5 (1.39%)
rs1354334	31,680,070	Intron	Non-target	G	T	0.3841	0.6355	0 (0.00%)
SNP8NRG444511/rs13268724	31,698,396	Intron	Target	T	A	0.1730	0.1840	0 (0.00%)
rs776401	31,716,962	Intron	Target	T	C	0.3677	0.7222	2 (0.55%)
rs1473438	31,733,759	Intron	Non-target	A	G	0.3675	0.7583	2 (0.55%)
rs1462893	31,831,015	Intron	Non-target	C	G	0.2141	0.4446	1 (0.28%)
rs10954821	31,898,990	Intron	Non-target	G	A	0.3084	0.7686	0 (0.00%)
rs726908	32,058,628	Intron	Non-target	A	G	0.4809	0.4549	3 (0.83%)
rs10954855	32,382,236	Intron	Target	T	A	0.2510	0.5524	0 (0.00%)
rs2439306	32,425,591	Intron	Non-target	G	A	0.2201	0.3435	19 (5.26%)
rs2466062	32,443,090	Intron	Non-target	A	G	0.2809	0.2068	1 (0.28%)
rs3924999	32,453,358	Exon (Arg to Gln change)	Target	C	T	0.3808	0.4807	1 (0.28%)
rs2466060	32,475,691	Intron	Target	G	A	0.4842	0.5194	39 (10.80%)
rs2439272	32,493,092	Intron	Target	C	T	0.4268	0.5262	1 (0.28%)
rs6468121	32,500,809	Intron	Non-target	G	T	0.4598	0.3310	4 (1.11%)
rs2466058	32,507,149	Intron	Target	G	A	0.0893	0.6600	0 (0.00%)
rs2466049	32,514,916	Intron	Target	C	T	0.0799	0.5201	2 (0.55%)
rs723811	32,527,281	Intron	Non-target	T	C	0.0929	0.4824	0 (0.00%)
rs6988339	32,545,916	Intron	Target	A	G	0.4233	0.9869	0 (0.00%)
rs2975498	32,552,189	Intron	Target	A	G	0.1843	0.1476	0 (0.00%)
rs2919382	32,560,765	Intron	Target	T	C	0.1692	0.0329	0 (0.00%)
rs2976525	32,572,983	Intron	Target	A	C	0.0884	0.8648	0 (0.00%)
rs4262285	32,582,701	Intron	Target	C	T	0.0410	0.4502	0 (0.00%)
rs3735776	32,585,434	Intron	Non-target	C	A	0.1568	0.8928	47 (13.02%)
rs4512342	32,607,874	Intron	Non-target	T	G	0.1024	0.8893	1 (0.28%)
rs10503929	32,613,983	Exon (Met to Thr change)	Target	T	C	0.1779	0.4907	0 (0.00%)
rs6992642	32,624,387	Downstream	Non-target	T	C	0.4059	0.6583	12 (3.32%)
rs3735781	32,624,828	Downstream	Non-target	A	G	0.4185	0.4703	2 (0.55%)
rs3735782	32,624,857	Downstream	Non-target	C	A	0.4773	0.4553	1 (0.28%)

Note. *Position estimated as these deCODE SNPs do not have dbSNP ID.

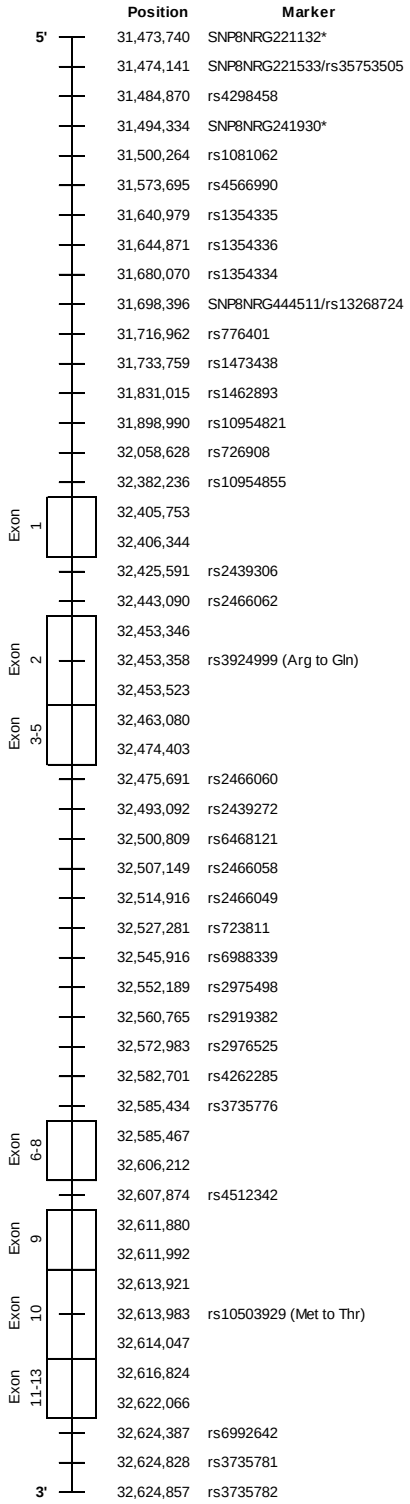


Figure 1. Line diagram of NRG1 with current SNP set marker locations

2.2.4 Genotyping Methods

Blood was collected in ethylenediamine tetraacetic acid (EDTA) tubes and frozen until bulk DNA extraction was performed. At that time, blood was thawed in a 47°C water bath and DNA was extracted according to the phenol-chloroform method. Quantification of DNA was then completed using the Invitrogen Quant-iT PicoGreen method (Chadwick et al., 1996). After extraction and quantification, samples were transferred to 96-well plates to begin ABI's SNPlex Genotyping method (SNPlex 3130xl, data collection v3). This system allowed simultaneous genotyping of up to 48 SNPs per well of DNA.

2.2.4.1 Quality Control Procedures: Individual Analysis of DNA Samples. After the SNPlex procedure, data were uploaded into GeneMapper 4.0 software to assess the quality of results. Each DNA sample was assessed separately for low peaks, failure of the size standard, and failure of the sample as a means of detecting procedural error and poor quality DNA samples. In addition, all participant samples with a peak intensity of less than 100Rfu were excluded on a SNP-by-SNP basis, as this generally suggests that the sample's peak at the given SNP was not high enough to genotype accurately. Any problem samples identified using the above methods were rerun using the procedures outlined above. Samples that failed both genotyping stages, failed on 10 or more individual SNPs, or had any Mendelian Errors, as assessed by PedCheck (O'Connell & Weeks, 1998), were excluded from analysis prior to this study.

2.2.4.2 Quality Control Procedures: SNP-wise Analysis. After the analysis of individual DNA samples was complete, cluster analysis was used to determine genotyping outliers at the level of each individual SNP. These outliers were suggestive of either poor DNA quality or competition between primers at annealing sites during the reaction. Five SNPs (rs3735776,

rs726908, rs3735781, rs2919382, and SNP8NRG241930) could not be clustered by GeneMapper, but were clustered independently by three of the investigators (JLY, KP, and MET) and results were checked for fidelity. Every SNP was in Hardy-Weinberg Equilibrium, except rs2919382 (p=0.0329; as calculated by SOLAR), with between 0% and 13.02% genotyping failure per SNP (mean failure = 1.22%, SD = 2.85). The LD patterns of the final SNP set, as measured by rho in SOLAR, can be seen in Figure 2. As expected, most of the SNPs were in very low LD with each other.

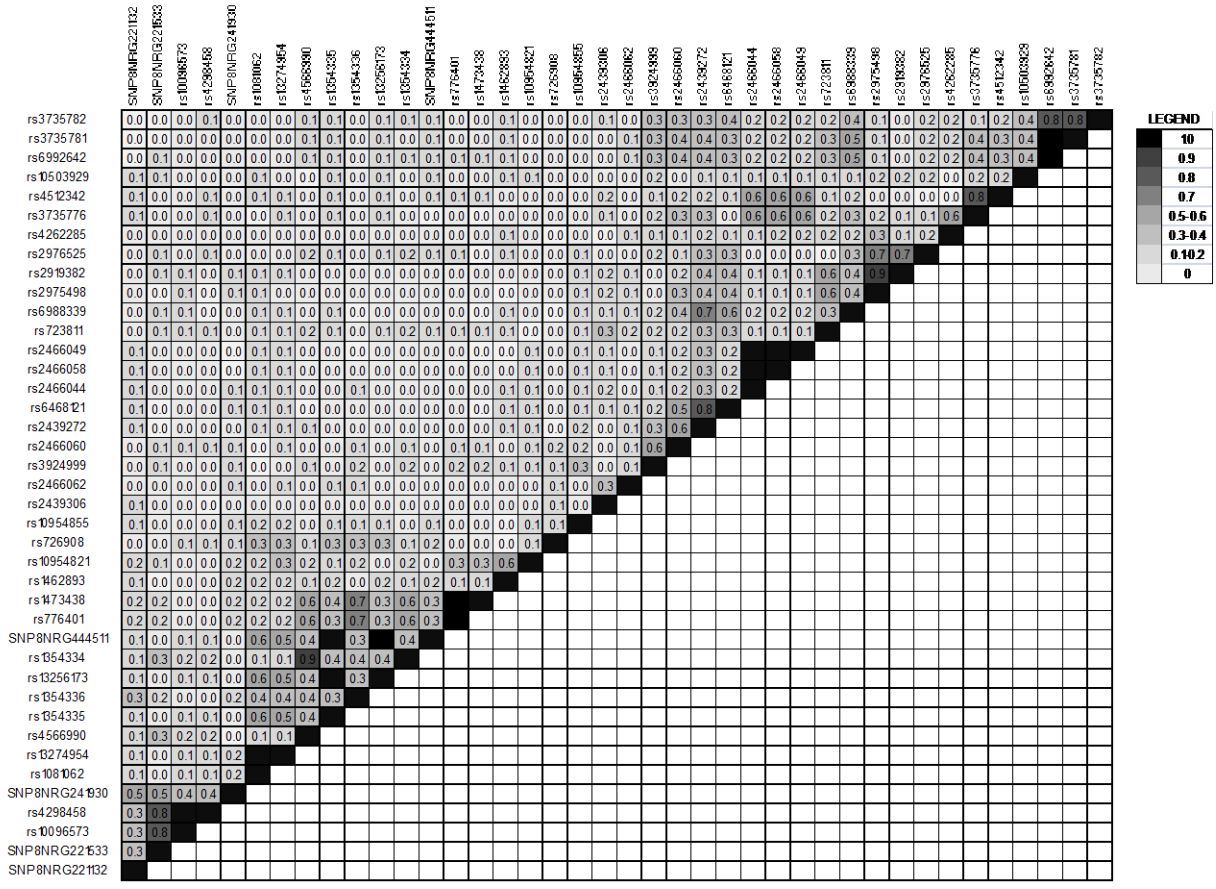


Figure 2. Linkage disequilibrium (rho) values between SNPs

2.2.5 Statistical Analysis

Genetic analyses were performed using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) program. SOLAR is a maximum likelihood variance component analytic program designed for multigenerational pedigrees of variable size and complexity (Almasy & Blangero, 1998). However, SOLAR does not have any graphing capabilities, necessitating the use of SPSS for graphing, as well as for descriptive statistical procedures and some inferential analyses. In addition, the P-values Adjusted for Correlated Tests (pACT) (Conneely & Boehnke, 2007) program, as implemented in the statistical program R, was used for multiple comparison correction. The specific program used for each analysis is noted below.

3.0 RESULTS

3.1 COMPOSITION OF THE SAMPLE

In total, there were 675 pedigree members and 230 normal controls enrolled in the overall study. A total of 603 pedigree members and 218 controls completed the diagnostic portion of the study, and 568 pedigree members and 199 controls also completed the cognitive battery (<10 missing test scores). Five hundred fifty-three of these pedigree members provided DNA. Of these, 419 pedigree members were successfully genotyped for NRG1 and also completed the diagnostic and cognitive portions of the study (meeting quality control indices for genotyping and cognitive assessment), thus forming the final sample for this study. No controls were genotyped, thus the final sample of controls included those who were enrolled and completed the diagnostic and cognitive portions of the study (N=199), without regard NRG1 genotyping.

Within the 419 individuals, 58 were affected and 361 were unaffected participants drawn from 39 multiplex, multigenerational families. For the purposes of this study, "affected" members are those who were diagnosed with schizophrenia or schizoaffective disorder-depressed type, while "unaffected" participants were defined as those diagnosed with any psychopathology other than schizophrenia or schizoaffective disorder-depressed type, including those with no diagnosis. As shown in Table 2, family size (counting only individuals included in the current study) ranged from one to 37 members (average members per family = 9.26, SD=1.39).

Table 2. Frequency distribution of family size (included members) in the sample

Included Members in	Count of Families
1	5
2	3
3	4
4	2
5	2
6	2
7	5
8	1
9	3
10	1
11	1
12	1
13	1
17	1
19	1
20	1
23	2
24	1
29	1
37	1
Mean	9.256
Standard Deviation	1.393

As shown in Table 3, there were 58 first-degree relatives of the index proband, 86 second-degree relatives, 95 third-degree relatives, 90 biological relatives extended past the third-degree, and 32 non-biological relatives (e.g., spouses) within the final sample.

Table 3. Demographics and genetic relationships in the study sample

	N	% Male	Age	Age range	Participant Education	Participant Education range	WRAT	% Right handed	% Pitt Site
Affected Individuals	**58**	**67.24**	**43.94 (9.37)**	**22-60**	**12.45 (2.73)**	**7-20**	**89.60 (17.07)**	**91.38**	**41.38**
Unaffected Relatives	**361**	**47.65**	**44.92 (17.42)**	**15-85**	**13.33 (2.89)**	**6-20**	**100.83 (13.25)**	**85.04**	**43.77**
First-degree relatives	*58*	*41.38*	*48.76 (16.80)*	*15-83*	*12.88 (3.07)*	*7-20*	*100.02 (12.04)*	*82.76*	*48.28*
Parents of proband	16	37.50	65.00 (10.86)	49-83	11.56 (2.94)	7-18	98.25 (12.66)	75.00	62.50
Sibling of proband	33	42.42	48.42 (9.77)	18-68	13.58 (3.01)	9-20	99.24 (12.47)	84.85	36.36
Child of proband	9	44.44	21.11 (4.37)	15-27	12.67 (3.00)	9-17	105.22 (8.97)	88.89	66.67
Second-degree relatives	*86*	*55.81*	*47.55 (21.05)*	*15-85*	*12.84 (2.94)*	*6-20*	*101.32 (14.25)*	*80.23*	*43.02*
Grandparent of proband	3	66.67	71.00 (1.00)	70-72	12.67 (3.51)	9-16	108.67 (9.07)	100.00	100.00
Aunt/Uncle of proband	42	57.14	64.69 (11.03)	42-85	12.50 (2.79)	8-18	101.79 (11.13)	78.57	40.48
Half-sibling of proband	2	100.00	32.00 (1.41)	31-33	11.00 (1.41)	10-12	91.50 (7.78)	100.00	50.00
Niece/Nephew of proband	39	51.28	28.08 (9.47)	15-56	13.31 (3.11)	6-20	100.84 (17.04)	79.49	41.03
Third-degree relatives	*95*	*48.42*	*44.97 (12.55)*	*18-74*	*13.88 (2.91)*	*9-20*	*101.71 (11.48)*	*84.21*	*56.84*
1st Cousin of proband	95	48.42	44.97 (12.55)	18-74	13.88 (2.91)	9-20	101.71 (11.48)	84.21	56.84
Extended relatives	*90*	*44.44*	*35.90 (16.33)*	*16-81*	*13.24 (2.61)*	*8-19*	*99.82 (14.44)*	*90.00*	*42.22*
Non-biological relatives	*32*	*43.75*	*56.13 (11.56)*	*26-78*	*14.06 (2.81)*	*10-20*	*101.33 (14.63)*	*90.63*	*3.13*
Controls	**199**	**42.71**	**47.24 (19.06)**	**18-84**	**15.03 (2.76)**	**8-20**	**108.34 (8.40)**	**87.94**	**44.20**

Note. Means and standard deviations are presented, unless otherwise labelled. Affected: schizophrenia or schizoaffective-depressed type, Unaffected: any diagnosis other than affected diagnoses (including those with no diagnosis), WRAT: Wide Range Achievement Test (age-standardized value), Pitt: University of Pittsburgh site

As seen in Figure 3, the unaffected relative sample had a wide age range, with participants as young as 15 and as old as 85.

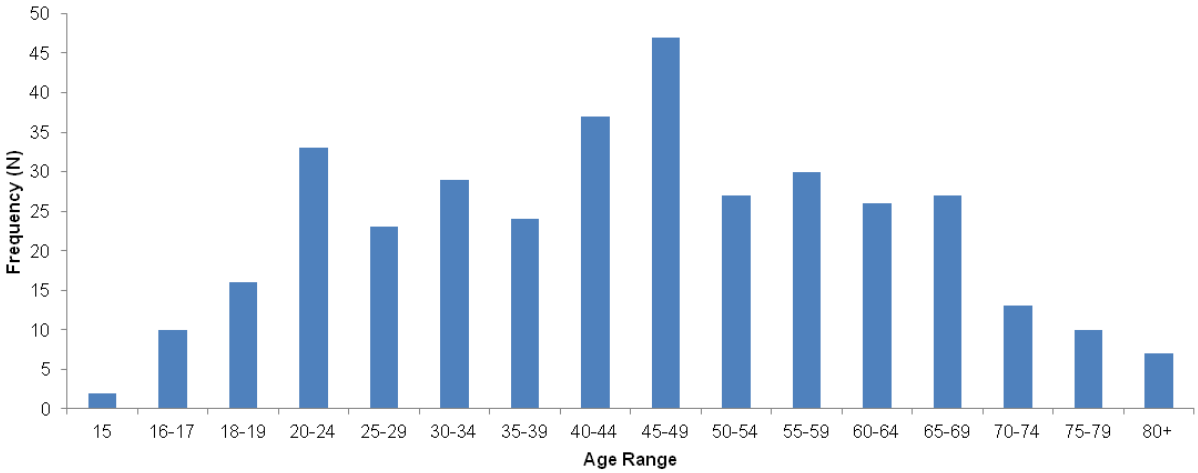


Figure 3. Frequency distribution of age in the unaffected relative sample.

The clinical composition of the sample is shown in Table 4. Affected individuals were diagnosed with schizophrenia (94.8%) or schizoaffective disorder-depressed type (5.2%). In addition, 32.8% of the affected sample had a comorbid diagnosis of a substance-related disorder, and 10.3% had a comorbid non-psychotic mood disorder that was not major depressive disorder (including mood disorders due to substance use or general medical conditions, and mood disorder NOS).

Table 4. Clinical composition of the study sample by genetic relationship

	Schizophrenia	Schizoaffective disorder, Depressed	Schizoaffective disorder, Bipolar	Bipolar I & II	Other Psychosis	Cluster A Personality Disorder	MDD with Psychotic Features	MDD	Other Mood Disorder	Substance-related Disorder
Affected Individuals	**55**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**6**	**19**
Unaffected Relatives	**0**	**0**	**2**	**5**	**9**	**18**	**2**	**70**	**37**	**82**
First-degree relatives	*0*	*0*	*0*	*0*	*4*	*2*	*0*	*15*	*5*	*14*
Parents of proband	0	0	0	0	1	1	0	5	1	3
Sibling of proband	0	0	0	0	3	1	0	10	3	8
Child of proband	0	0	0	0	0	0	0	0	1	3
Second-degree relatives	*0*	*0*	*1*	*2*	*1*	*7*	*1*	*15*	*4*	*20*
Grandparent of proband	0	0	0	0	0	1	0	0	0	1
Aunt/Uncle of proband	0	0	1	1	1	4	0	4	1	10
Half-sibling of proband	0	0	0	0	0	0	0	1	0	1
Niece/Nephew of proband	0	0	0	1	0	2	1	10	3	8
Third-degree relatives	*0*	*0*	*1*	*2*	*2*	*2*	*0*	*14*	*12*	*21*
1st Cousin of proband	0	0	1	2	2	2	0	14	12	21
Extended relatives	*0*	*0*	*0*	*0*	*1*	*6*	*0*	*20*	*15*	*20*
Non-biological relatives	*0*	*0*	*0*	*1*	*1*	*1*	*1*	*6*	*1*	*7*
Controls	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**24**	**4**	**16**

Note. The following categories are mutually exclusive: schizophrenia, schizoaffective disorder-depressed, schizoaffective disorder-bipolar, Bipolar I & II, and MDD (major depressive disorder) with and without psychotic features. Non-mutually exclusive diagnoses (e.g., other psychosis, cluster A personality disorder, other mood disorder, and substance-related disorder) may be comorbid with each other and/or mutually exclusive conditions.

As shown in Table 5, unaffected relatives were further classified into three hierarchical mutually exclusive groups: spectrum, other psychopathology, and no diagnosis. The "spectrum" group consisted of 36 individuals diagnosed with disorders believed to fall in the "schizophrenia spectrum," including schizoaffective disorder-bipolar type (5.6%), bipolar disorder I and II (13.9%), major depressive disorder (MDD) with psychotic features (5.6%), other organic or nonorganic psychosis (25.0%), and cluster A personality disorder (50.0%). Some individuals in this group also met criteria for comorbid non-psychotic MDD or other mood disorder (22.2%) and substance disorders (44.4%).

Table 5. Clinical composition of the unaffected relative sample by diagnostic category

	Schizoaffective disorder, Bipolar	Bipolar I & II	Other Psychosis	Cluster A Personality Disorder	MDD with Psychotic Features	MDD	Other Mood Disorder	Substance-related Disorder
Spectrum	2	5	9	18	2	3	5	16
Other Psychopathology	0	0	0	0	0	67	32	66
No diagnosis	0	0	0	0	0	0	0	0

Note. The following categories are mutually exclusive: schizoaffective disorder-bipolar, Bipolar I & II, and MDD (major depressive disorder) with and without psychotic features. Non-mutually exclusive diagnoses (e.g., other psychosis, cluster A personality disorder, other mood disorder, and substance-related disorder) may be comorbid with each other and/or mutually exclusive conditions. Spectrum: schizoaffective disorder-bipolar type, bipolar disorder I & II, MDD with psychotic features, other organic or nonorganic psychosis, and cluster A personality disorder; Other Psychopathology: individuals with psychopathology falling into any non-spectrum diagnosis; No Diagnosis: individuals with no diagnosable psychopathology on any clinical measure.

Individuals who did not meet criteria for schizophrenia-spectrum diagnoses were grouped into either the other psychopathology group or the no diagnosis group. The "other psychopathology" group consisted of 147 individuals diagnosed with MDD and other mood disorders (67.3%) and substance disorders (44.9%). Those individuals with no diagnosis on the clinical evaluations were grouped into the "no diagnosis" group (N=178). Demographic information for all diagnostic groups is provided in Table 6.

Table 6. Demographic and pedigree information for the unaffected relative sample by diagnostic category

	N	% Male	Age	Age range	Education	Education range	WRAT	% Right handed	% Pitt Site	First-degree relatives	Second-degree relatives	Third-degree relatives	Extended biological relatives	Non-biological relatives
Spectrum	36	69.44	47.75 (16.25)	20-83	13.28 (2.91)	8-20	99.57 (16.02)	91.67	13.89	6	12	7	7	4
Other Psychopathology	147	51.02	42.87 (15.82)	16-82	13.21 (2.89)	6-20	98.62 (12.67)	85.03	48.30	25	25	40	46	11
No Diagnosis	178	40.44	46.04 (18.67)	15-85	13.44 (2.89)	8-20	103.05 (12.80)	83.71	46.07	27	49	48	37	17

Note. Means and standard deviations are presented, unless otherwise labelled. Spectrum: schizoaffective disorder-bipolar type, bipolar disorder I & II, MDD with psychotic features, other organic or nonorganic psychosis, and cluster A personality disorder; Other Psychopathology: individuals with psychopathology falling into any non-spectrum diagnosis; No Diagnosis: individuals with no diagnosis on any clinical measure; WRAT: Wide Range Achievement Test (age-standardized value); Pitt: University of Pittsburgh site

Among the 199 controls, 19.6% were diagnosed with some type of psychopathology on the clinical measures (non-psychotic MDD or another mood disorder: 14.1%; substance-related disorder: 8.0%), while 70.4% had no diagnosis. No participant in any group met criteria for a cognitive disorder.

3.2 DEMOGRAPHIC COMPARISONS

Pairwise demographic comparisons between the affected, unaffected, and normal control samples showed multiple significant differences, as seen in Table 7. Comparisons between the affected and control samples revealed that there were significantly more females, higher levels of education, and higher WRAT scores in the control group, with no group difference in participant age. The same pattern of findings was found when comparing affected and unaffected relatives

on these variables. Comparisons between unaffected relatives and controls found that controls had significantly higher education and WRAT scores, but that there were no differences between these groups on age or sex.

Table 7. Demographic comparisons between groups in the study sample

	Affecteds vs. Controls		**Affecteds vs. Unaffected Relatives**		**Unaffected Relatives vs. Controls**	
	Statistic (df)	*p-value*	*Statistic (df)*	*p-value*	*Statistic (df)*	*p-value*
Sex	10.82 (1)	**0.001**	7.68 (1)	**0.006**	1.26 (1)	0.262
Age	-1.80 (195.43)	0.074	-0.42 (417)	0.678	-1.42 (378.26)	0.157
Education	-5.91 (255)	**0.000000**	-2.18 (417)	**0.030**	-6.16 (558)	**0.000000**
WRAT	-7.84 (69.62)	**0.000000**	-4.74 (67.73)	**0.00001**	-7.09 (315.73)	**0.000000**

Note. Chi-square statistics are reported for sex comparisons, while t-tests are reported for age, education, and WRAT. WRAT: Wide Range Achievement Test (age-standardized value), df: degrees of freedom. Significant ($p<0.05$) values are bolded. Variable coding: group (1: affected, 2: unaffected, 3: controls), sex (1: male, 2: female). See text for a description of the direction of effects.

When the unaffected relative sample was broken down into spectrum, other psychopathology, and no diagnosis groups, few significant demographic differences were found, as seen in Table 8. Comparisons between spectrum and no diagnosis groups found no significant differences in age, education, or WRAT score, but the spectrum group had significantly more males than the no diagnosis group. This same pattern was found when comparing spectrum individuals with the other psychopathology group. Comparisons between the other psychopathology and no diagnosis groups found no differences in sex, age, or education; however, the no diagnosis group had significantly higher WRAT scores than the other psychopathology group.

Table 8. Demographic comparisons between diagnostic groups in the unaffected relative sample

	Spectrum vs. No Diagnosis		Spectrum vs. Other Psychopathology		Other Psychopathology vs. No Diagnosis	
	Statistic (df)	*p-value*	*Statistic (df)*	*p-value*	*Statistic (df)*	*p-value*
Sex	10.16 (1)	**0.001**	3.96 (1)	**0.047**	3.63 (1)	0.057
Age	-0.51 (212)	0.611	-1.65 (181)	0.101	1.65 (322.85)	0.100
Education	0.30 (212)	0.762	-0.12 (181)	0.901	0.71 (323)	0.481
WRAT	1.39 (196)	0.166	-0.38 (176)	0.706	3.04 (304)	**0.003**

Note. Chi-square statistics are reported for sex comparisons, while t-tests are reported for age, education, and WRAT. WRAT: Wide Range Achievement Test (age-standardized value), df: degrees of freedom. Significant ($p<0.05$) values are bolded. Variable coding: group (0: no diagnosis, 1: other psychopathology, 2: spectrum), sex (1: male, 2: female). See text for a description of the direction of effects.

3.3 NEUROCOGNITIVE PERFORMANCE

3.3.1 Missing Data Rates and Outlier Analysis

Averaging over all of the tests in the computerized neurocognitive battery, the rate of missing efficiency data per test in the whole sample was 3.24%, with affected, unaffected, and control groups having 3.88%, 2.08%, and 5.15% rates of missing data, respectively. Average rates of missing efficiency domain scores (out of eight) per person were 0.310, 0.166, and 0.412 for affected, unaffected, and control groups, respectively. Missing data could have been the result of computer malfunction, participant's unwillingness to complete the test, and/or data that was deemed invalid due to either participant's behavior during testing or non-standard testing conditions. PENN controls recruited prior to the current study had higher rates of missing data due to tests that were added to the battery at a later time.

Data from each cognitive domain were checked for outliers by box plot analysis collapsed over the affected, unaffected, and control groups. There were no extreme outliers, defined as a data point six or more standard deviations from the next most extreme score, for any domain. Table 9 presents skewness and kurtosis for each participant group and cognitive domain.

For affected participants, six domains showed negative skew (only Abstraction/Mental Flexibility and Spatial Memory did not) in the range of -1.43 to 0.85. Kurtosis for this group was in the range of -1.06 to 2.41. The pattern in unaffected participants was somewhat different as each domain was negatively skewed (range: -4.14 to -0.08) and kurtosis had a much larger range (range: -0.47 to 24.56). The control group's cognitive performance was also all negatively skewed (range: -2.50 to -0.10) with a smaller kurtosis range than unaffected individuals (range: -0.48 to 9.09). Attention and Sensorimotor Dexterity showed the highest levels of skewness and kurtosis for all participant groups, thus there is little evidence of heteroscedasticity.

Table 9. Cognitive performance by group and domain: Skewness & Kurtosis

		Abstraction & Mental Flexibility	Attention	Spatial Processing	Emotional Processing	Verbal Memory	Facial Memory	Spatial Memory	Sensorimotor Dexterity
Affected	*Skewness (SE)*	0.846 (0.322)	-1.426 (0.322)	-0.351 (0.330)	-0.144 (0.316)	-0.364 (0.314)	-0.049 (0.314)	0.069 (0.316)	-1.234 (0.325)
	Kurtosis (SE)	-0.884 (0.634)	2.407 (0.634)	-1.055 (0.650)	0.023 (0.623)	-0.106 (0.618)	-0.777 (0.618)	-0.943 (0.623)	1.635 (0.639)
Unaffected	*Skewness (SE)*	-0.515 (0.131)	-2.040 (0.131)	-0.880 (0.130)	-0.743 (0.129)	-0.808 (0.129)	-0.612 (0.129)	-0.078 (0.129)	-4.136 (0.131)
	Kurtosis (SE)	-1.058 (0.261)	5.069 (0.262)	0.878 (0.259)	0.491 (0.257)	0.681 (0.256)	0.362 (0.256)	-0.471 (0.257)	24.559 (0.262)
Controls	*Skewness (SE)*	-0.828 (0.172)	-2.329 (0.192)	-0.544 (0.174)	-0.879 (0.175)	-0.430 (0.179)	-0.373 (0.177)	-0.096 (0.172)	-2.503 (0.176)
	Kurtosis (SE)	-0.384 (0.343)	8.749 (0.383)	0.271 (0.346)	1.954 (0.347)	0.302 (0.355)	0.004 (0.353)	-0.477 (0.343)	9.086 (0.350)

Note. SE: standard error

3.3.2 Cognitive Performance by Group

Table 10 presents the standardized means and standard deviations for each group using the control group's performance data as the reference group.

Table 10. Cognitive performance by group and domain: Means & Standard Deviations

		Abstraction & Mental Flexibility	Attention	Spatial Processing	Emotional Processing	Verbal Memory	Facial Memory	Spatial Memory	Sensorimotor Dexterity
Affected	*N*	55	55	52	57	58	58	57	54
	Mean (SD)	-1.227 (1.17)	-1.805 (2.07)	-1.123 (1.45)	-1.550 (1.28)	-1.206 (1.37)	-0.921 (0.93)	-0.786 (1.00)	-1.397 (1.75)
Unaffected	*N*	348	344	353	359	360	360	359	345
	Mean (SD)	-0.169 (1.11)	-0.344 (1.43)	-0.211 (1.10)	-0.289 (1.15)	-0.33 (1.09)	-0.256 (1.00)	-0.174 (0.95)	-0.235 (1.27)
Controls	*N*	199	159	195	194	185	188	199	191
	Mean (SD)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)

Note. SD: standard deviation

As expected, there were significant performance differences between groups on every domain, as seen in Table 11, with omnibus F statistics ranging from 14.51 to 42.42 (p=0.000). Pairwise contrasts showed significant differences between affected and control individuals, as well as affected compared to unaffected individuals on each domain, in which the affected group consistently performed worse than other groups. Contrasts between the unaffected relatives and control group found significant differences for all domains, except Abstraction/Mental Flexibility. The performance of the unaffected group was consistently worse than the control group.

Table 11. Cognitive performance comparisons between groups

	Omnibus (all groups)		Affecteds vs. Controls		Affecteds vs. Unaffected Relatives		Unaffected Relatives vs. Controls	
	Statistic (df)	*p-value*	*Statistic (df)*	*p-value*	*Statistic (df)*	*p-value*	*Statistic (df)*	*p-value*
Abstraction & Mental Flexibility	28.47 (2, 599)	**0.000**	55.76 (1, 599)	**0.000**	45.67 (1, 599)	**0.000**	3.12 (1, 599)	0.078
Attention	34.41 (2, 555)	**0.000**	67.86 (1, 555)	**0.000**	51.59 (1, 555)	**0.000**	6.56 (1, 555)	**0.011**
Verbal Memory	26.90 (2, 600)	**0.000**	53.59 (1, 600)	**0.000**	31.97 (1, 600)	**0.000**	11.12 (1, 600)	**0.001**
Facial Memory	19.17 (2 ,603)	**0.000**	38.13 (1, 603)	**0.000**	22.42 (1, 603)	**0.000**	8.19 (1, 603)	**0.004**
Spatial Memory	14.51 (2 ,612)	**0.000**	29.08 (1, 612)	**0.000**	19.57 (1, 612)	**0.000**	4.12 (1, 612)	**0.043**
Sensorimotor Dexterity	21.29 (2, 597)	**0.000**	52.96 (1 ,587)	**0.000**	40.68 (1, 587)	**0.000**	4.35 (1, 587)	**0.037**
Spatial Processing	26.73 (2, 587)	**0.000**	42.50 (1, 597)	**0.000**	30.93 (1, 597)	**0.000**	4.60 (1, 597)	**0.032**
Emotional Processing	42.42 (2, 607)	**0.000**	84.39 (1, 607)	**0.000**	62.30 (1, 607)	**0.000**	8.42 (1, 607)	**0.004**

Note. All analyses used the F statistic. Significant (p<0.05) values are bolded. Variable coding: group (1: affected, 2: unaffected, 3: controls). See text for a description of the direction of effects.

Some differences in cognitive performance by diagnostic group among unaffected relatives were also found (means for each group shown in Table 12).

Table 12. Cognitive performance by diagnostic category in the unaffected sample

		Abstraction & Mental Flexibility	Attention	Spatial Processing	Emotional Processing	Verbal Memory	Facial Memory	Spatial Memory	Sensorimotor Dexterity
Spectrum	*N*	34	33	33	35	36	36	36	34
	Mean (SD)	-0.491 (1.27)	-0.343 (1.58)	-0.088 (1.15)	-0.900 (1.39)	-0.843 (1.40)	-0.915 (1.16)	-0.382 (1.09)	-0.796 (2.60)
Other Psychopathology	*N*	142	147	145	147	146	147	147	144
	Mean (SD)	-0.170 (1.09)	-0.490 (1.63)	-0.155 (1.08)	-0.254 (1.03)	-0.362 (1.07)	-0.134 (0.94)	-0.109 (0.91)	-0.152 (1.06)
No Diagnosis	*N*	172	164	175	177	178	177	176	167
	Mean (SD)	-0.105 (1.08)	-0.214 (1.18)	-0.281 (1.11)	-0.198 (1.17)	-0.201 (1.02)	-0.223 (0.97)	-0.186 (0.95)	-0.191 (0.99)

Note. SD: standard deviation

Specifically, omnibus effects were detected for the domains of Verbal and Facial Memory, Sensorimotor Dexterity, and Emotional Processing, as seen in Table 13. Pairwise contrasts for these domains found significant differences between spectrum and no diagnosis groups, as well as spectrum compared to other psychopathology groups. The same pattern was found for a comparison between the spectrum and combined no diagnosis/other psychopathology groups. In all contrasts, the spectrum group's performance was always poorer than the comparison group. No contrasts between the other psychopathology and no diagnosis groups were significant. There were also no significant differences on any domain between control individuals with a diagnosis and those without ($0.200 \leq p \leq 0.924$; data not tabled), indicating that non-schizophrenia-related psychopathology does not significantly impair cognitive performance in this sample.

Table 13. Cognitive performance comparisons between diagnostic categories in the unaffected sample

	Omnibus (all groups)		Spectrum vs. No Diagnosis		Spectrum vs. Other Psychopathology		Other Psychopathology vs. No Diagnosis		Spectrum vs. (No Diagnosis & Other Psychopathology)	
	Statistic (df)	*p-value*	*Statistic (df)*	*p-value*	*Statistic (df)*	*p-value*	*Statistic (df)*	*p-value*	*Statistic (df)*	*p-value*
Abstraction & Mental Flexibility	1.73 (2, 345)	0.180	3.45 (1, 345)	0.064	2.32 (1, 345)	0.129	0.26 (1, 345)	0.610	3.13 (1, 345)	0.078
Attention	1.44 (2, 341)	0.238	0.22 (1, 341)	0.638	0.29 (1, 341)	0.594	2.88 (1, 341)	0.090	0.00 (1, 341)	0.972
Spatial Processing	0.74 (2, 350)	0.479	0.84 (1, 350)	0.359	0.10 (1, 350)	0.753	1.03 (1, 350)	0.312	0.41 (1, 350)	0.521
Emotional Processing	5.68 (2, 356)	**0.004**	11.11 (1, 356)	**0.001**	9.12 (1, 356)	**0.003**	0.19 (1, 356)	0.663	11.07 (1, 356)	**0.001**
Verbal Memory	5.40 (2, 357)	**0.005**	10.59 (1, 357)	**0.001**	5.73 (1, 357)	**0.017**	1.78 (1, 357)	0.183	8.75 (1, 357)	**0.003**
Facial Memory	9.44 (2, 357)	**0.000**	15.01 (1, 357)	**0.000**	18.49 (1, 357)	**0.000**	0.67 (1, 357)	0.414	18.41 (1, 357)	**0.000**
Spatial Memory	1.22 (2, 356)	0.297	1.27 (1, 356)	0.260	2.39 (1, 356)	0.123	0.52 (1, 356)	0.470	1.97 (1, 356)	0.161
Sensorimotor Dexterity	3.76 (2, 342)	**0.024**	6.46 (1, 342)	**0.011**	7.14 (1, 342)	**0.008**	0.07 (1, 342)	0.785	7.47 (1, 342)	**0.007**

Note. Comparisons based on significant omnibus F tests (p<0.05) discussed in text. All analyses used the F statistic. Significant (p<0.05) values are bolded. Variable coding: group (0: no diagnosis, 1: other psychopathology, 2: spectrum). See text for a description of the direction of effects.

3.3.3 Relationship between Cognitive Performance and Demographic Characteristics

As seen in Table 14, Pearson correlations between demographic characteristics and individual cognitive domains in the unaffected sample found multiple significant relationships: 1) education and WRAT were both significantly positively associated with all cognitive tasks; 2) handedness was negatively associated only with Spatial Processing performance (right handed individuals performing better); and 3) site was negatively associated with Abstraction/Mental Flexibility performance (PENN individuals performing better).

Table 14. Pearson correlations between demographic characteristics and cognitive performance in the unaffected sample

	Abstraction & Mental Flexibility		Attention		Spatial Processing		Emotional Processing		Verbal Memory		Facial Memory		Spatial Memory		Sensorimotor Dexterity	
	Statistic	*p-value*	*Statistic*	*p-value*	*Statistic*	*p-value*	*Statistic*	*p-value*	*Statistic*	*p-value*	*Statistic*	*p-value*	*Statistic*	*p-value*	*Statistic*	*p-value*
Education	0.27	**0.000**	0.30	**0.000**	0.39	**0.000**	0.27	**0.000**	0.25	**0.000**	0.21	**0.000**	0.12	**0.021**	0.28	**0.000**
WRAT	0.32	**0.000**	0.34	**0.000**	0.45	**0.000**	0.36	**0.000**	0.38	**0.000**	0.23	**0.000**	0.12	**0.027**	0.23	**0.000**
Handedness	-0.04	0.488	0.03	0.646	-0.17	**0.002**	0.01	0.879	-0.04	0.457	-0.01	0.792	-0.04	0.503	-0.01	0.843
Site	-0.11	**0.050**	-0.06	0.270	-0.07	0.197	-0.04	0.490	-0.01	0.843	0.09	0.078	0.00	0.946	-0.02	0.661

Note. WRAT: Wide Range Achievement Test (age-standardized values). Coding: handedness (right = 1, left = 2); site (PENN = 70, PITT = 71). Significant ($p<0.05$) values are bolded.

3.3.4 Genetic Correlations between Affected Status and Cognitive Performance

Genetic correlations (R_g) were estimated between affected status and each cognitive domain using the combined affected and unaffected groups. Age and sex were screened as covariates for each analysis and retained if $p<0.1$. No covariates were used in modeling R_g for Verbal Memory due to recurrent convergence failure when covariates were included.

As seen in Table 15, R_g ranged from -0.143 to -0.604 and was negative in direction for all analyses indicating that the closer the genetic relationship with an affected individual, the poorer the cognitive performance. R_g was significantly different from zero ($p<0.05$) for four domains: Abstraction/Mental Flexibility, Attention, Spatial Processing, and Verbal Memory. Emotional processing tended towards significance ($p=0.054$). These findings indicate that genetic effects on affected status and cognitive domains are shared by some degree (i.e., pleiotropy). However, R_g was significantly different from 1.0 for all eight domains indicating that the genetic effects on affected status and cognition are not identical.

Table 15. Genetic correlations between affected status and cognitive performance

	N	R_g	p-value (different from 0)	p-value (different from +/-1)	Significant Covariates (Cognitive Domain)^	Significant Covariates (Affected Status)^
Abstraction & Mental Flexibility	419	-0.604	**0.0047**	**0.0105**	age, sex	sex
Attention	419	-0.552	**0.0147**	**0.0045**	age	sex
Spatial Processing	419	-0.459	**0.0072**	**0.0000005**	age, sex	sex
Emotional Processing	419	-0.358	0.0542	**0.000009**	age, sex	sex
Verbal Memory	419	-0.466	**0.0120**	**0.00001**	none#	none#
Facial Memory	419	-0.318	0.1374	**0.00005**	age, sex	sex
Spatial Memory	419	-0.237	0.2147	**0.000001**	age	sex
Sensorimotor Dexterity	419	-0.143	0.5587	**0.0011**	age	sex

Note. R_g: genetic correlation. ^Age and sex were screened as covariates for each analysis. # No covariates could be included in the estimation of R_g for this domain due to recurrent convergence failure. Significant ($p<0.05$) values are bolded.

3.3.5 Heritability of Cognitive Domains

Heritabilities for each cognitive domain are shown in Table 16. Heritability was estimated in the unaffected sample including age and sex as potential covariates that were retained in the model if $p<0.1$. Heritability ranged from 0.169 to 0.583 and was significant for all domains except Abstraction/Mental Flexibility. These analyses indicate that a significant proportion of variation in cognitive performance is due to genetic variation for nearly all of the cognitive domains.

Table 16. Heritability of cognitive domains

	N	h^2	p-value	Significant Covariate(s)
Abstraction & Mental Flexibility	348	0.172	0.059	age, sex
Attention	344	0.169^	**0.023**	age
Spatial Processing	353	0.583^	**1.00×10^{-7}**	age, sex
Emotional Processing	359	0.394^	**3.00×10^{-5}**	age, sex
Verbal Memory	360	0.535	**9.00×10^{-9}**	age, sex
Facial Memory	360	0.323	**0.0002**	age
Spatial Memory	359	0.453	**9.00×10^{-6}**	age
Sensorimotor Dexterity	345	0.187^	**0.0278**	age

Note. h^2: heritability. ^The tdist command was used to normalize the distribution when kurtosis (as measured by SOLAR) was high. Potential covariates screened for inclusion (and included if $p<0.10$) included age and sex for all cognitive domains. Significant ($p<0.05$) values are bolded.

3.3.6 Designation of Target Domains

Five "target" domains for the staged statistical analysis were designated based on the genetic correlation and heritability findings. The target domains were defined as those with R_g

significantly ($p<0.1$) different from zero and significant ($p<0.1$) heritability. This liberal significance threshold ($p<0.1$) was used in order to capture those traits that showed high R_g values, as R_g provides information about the genetic variance underlying the relationship between cognition and schizophrenia, which heritability estimates do not. The target domains included: Abstraction/Mental Flexibility, Attention, Spatial Processing, Emotional Processing, and Verbal Memory. The R_g for these domains ranged from -0.358 to -0.604, while heritability estimates ranged from 0.172 to 0.583. Facial memory, Spatial Memory, and Sensorimotor Dexterity were designated as non-target domains.

3.4 STAGED ANALYSES

3.4.1 Stage 1 Analysis: Covarying Cognitive Domains with Sex

All cognitive domains were independently covaried with sex to determine the proportion of variance explained by this variable using SOLAR. As seen in Table 17, four domains (Abstraction/Mental Flexibility, Spatial Processing, Emotional Processing, and Verbal Memory) showed nominally significant ($p<0.05$) covariance with sex. In these domains, sex explained between 1.02% and 9.23% of the variance in cognitive performance. The sex effects indicated that females performed better on Abstraction/Mental Flexibility and Verbal Memory, while males performed better on the domains of Spatial and Emotional Processing performance.

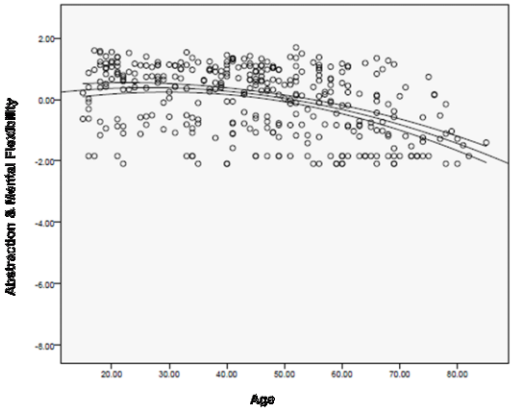
Table 17. Stage 1 analysis: Covarying cognitive domains with sex

	p-value	Proportion of variance explained by sex
Abstraction & Mental Flexibility	**0.0334**	0.01021
Attention	0.2327	0.00229
Spatial Processing	**4.00×10^{-7}**	0.09225
Emotional Processing	**6.00×10^{-5}**	0.03911
Verbal Memory	**0.0033**	0.03739
Facial Memory	0.0881	0.00628
Spatial Memory	0.3138	0.00393
Sensorimotor Dexterity	0.2079	0.01383

Note. Nominally significant ($p<0.05$) values are bolded. See text for a description of the direction of effects.

3.4.2 Stage 2 Analysis: Estimation of the Main Effects of Age on Cognition

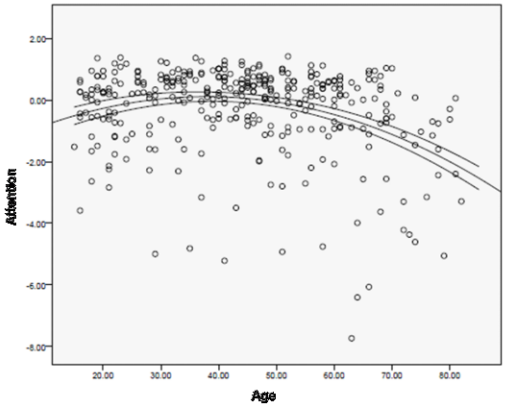
3.4.2.1 Linear & Curvilinear Regression in SPSS. SPSS was used for linear and curvilinear regression and graphing as a means of investigating the relationship between cognitive performance and age using the residuals from the previous stage. The model data are provided in Figures 4 (target domains) and 5 (non-target domains). Tolerance levels were sufficient (>0.18) for every model. First derivatives (F'(x)) of the regression equations enabled the localization of the slope change when the average age of the sample was added (this was done to aid interpretability, as the regression was done on a mean age-centered variable to reduce multicollinearity).



$F(x) = 0.175 - 0.023age - 0.0009age^2$
$F'(x) = -18.1657 + 44.9197 = 25.7530$

$R^2_{\Delta linear} = 0.149$ (p=0.000)
$R^2_{\Delta quadratic} = 0.032$ (p=0.000)*
$R^2_{\Delta cubic} = 0.000$ (p=0.865)

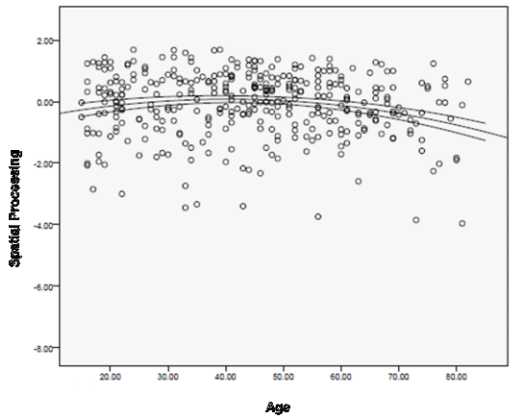
$R^2_{Total} = 0.181$



$F(x) = 0.052 - 0.017age - 0.001age^2$
$F'(x) = -8.5 + 44.9197 = 36.4197$

$R^2_{\Delta linear} = 0.044$ (p=0.000)
$R^2_{\Delta quadratic} = 0.064$ (p=0.000)*
$R^2_{\Delta cubic} = 0.000$ (p=0.870)

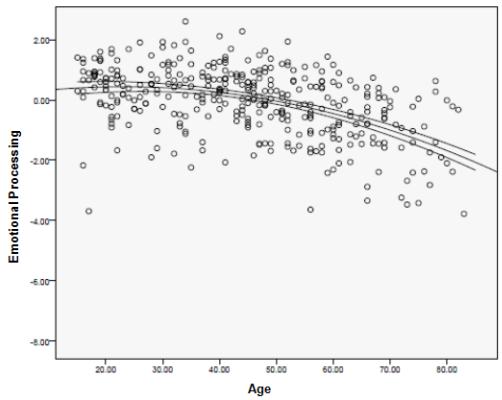
$R^2_{Total} = 0.109$



$F(x) = 0.071 - 0.005age - 0.00054age^2$
$F'(x) = -4.6296 + 44.9197 = 40.2901$

$R^2_{\Delta linear} = 0.008$ (p=0.104)
$R^2_{\Delta quadratic} = 0.025$ (p=0.003)*
$R^2_{\Delta cubic} = 0.001$ (p=0.585)

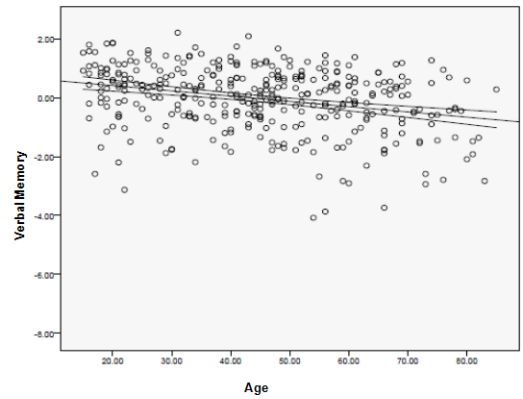
$R^2_{Total} = 0.032$



$F(x) = 0.129 - 0.029age - 0.00065age^2$
$F'(x) = -22.3077 + 44.9197 = 22.6120$

$R^2_{\Delta linear} = 0.205$ (p=0.000)
$R^2_{\Delta quadratic} = 0.032$ (p=0.000)*
$R^2_{\Delta cubic} = 0.001$ (p=0.529)

$R^2_{Total} = 0.238$



$F(x) = -0.034 - 0.018age$

$R^2_{\Delta linear} = 0.083$ (p=0.000)*
$R^2_{\Delta quadratic} = 0.004$ (p=0.238)
$R^2_{\Delta cubic} = 0.001$ (p=0.476)

$R^2_{Total} = 0.083$

Figure 4. Graphs of the relationship between age and performance on target cognitive domains

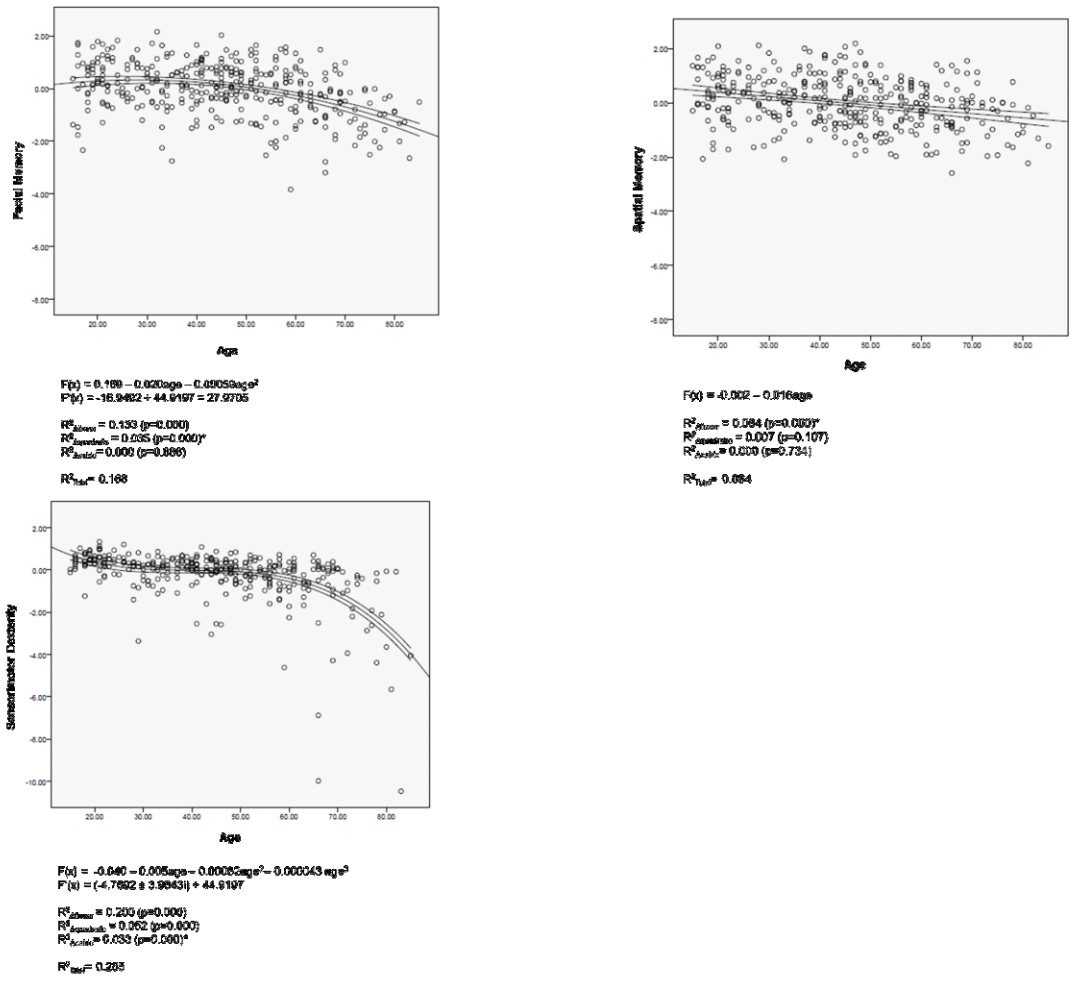


Figure 5. Graphs of the relationship between age and performance on non-target cognitive domains

3.4.2.1.1 Target Domains. Of the five target domains, one showed a linear relationship with age and four showed a significant quadratic function. Verbal Memory showed a linear function with a negative slope. The quadratic and cubic terms in this equation did not significantly increase the prediction of task performance and were dropped from the model. In this domain, cognitive performance declined with age.

The remaining four target domains showed a significant quadratic relationship between age and cognitive performance. The cubic term did not significantly increase the prediction of task performance and was dropped from each of the models. In these domains, task performance

increased at a decreasing rate until reaching a peak and then decreased with continued age. The ages at which cognitive performance shifted from improving to declining for Attention (36.4 years) and Spatial Processing (40.3 years) are generally past the time of highest onset risk for schizophrenia, although Emotional Processing (22.6 years) and Abstraction/Mental Flexibility (25.8 years) are well within the peak risk range for the disorder, particularly for women.

3.4.2.1.2 Non-Target Domains. Of the three non-target domains, one showed a linear relationship with age, one a quadratic relationship, and one a cubic function. Like Verbal Memory, Spatial Memory showed a linear function with a negative slope. In this domain, each additional year of age is associated with task performance decline. Facial Memory did not follow the pattern seen in Verbal and Spatial Memory and instead showed a quadratic function. In this domain, task performance increased at a decreasing rate until reaching a peak at age 28.0 years and then decreased with continued aging. This peak is somewhat beyond the greatest risk period for men, although it does fall within the women's risk period.

Sensorimotor dexterity showed a cubic function with age: a negative slope until approximately 20 years of age, a generally flat slope between 20 and 55 years, and a decline in performance thereafter. Specific estimates of slope change could not be established for this domain due to its unsolvable derivative (see Figure 5 for the equation which includes imaginary numbers). This pattern of function and age is suggestive of a change near the first risk period for both sexes and the peri-menopausal risk period for women.

3.4.2.2 Estimation of the Main Effects of Age Using SOLAR. SOLAR was used to estimate the main effects of age on cognitive performance and confirm the SPSS findings because of its unique ability to take into account the pedigree structure of the data. This

estimation was done separately for each cognitive domain using the residuals from the stage 1 analyses. The linear, quadratic, and cubic effects of age were estimated for every domain.

The linear component of age (age^1) was a nominally significant ($p<0.05$) covariate for every domain. Table 18 presents the percentage of variance in performance explained by age, which ranged from 2.67% to 22.83% overall. Increased age was associated with poorer performance on all cognitive tasks. The estimates of the quadratic main effects of age (age^2) on cognition (after accounting for the linear effects) are also presented in Table 18.

Table 18. Stage 2 analysis: Estimation of the linear, quadratic, and cubic main effects of age on cognition

	Linear		**Quadratic**		**Cubic**	
	p-value	*Proportion of variance explained by age*	*p-value*	*Proportion of variance explained by age²*	*p-value*	*Proportion of variance explained by age³*
Abstraction & Mental Flexibility	**2.02×10^{-13}**	0.14931	**0.00009**	0.03747	0.7524	0.00499
Attention	**0.00004**	0.02671	**1.93×10^{-8}**	0.03727	0.1976	*
Spatial Processing	**0.0245**	0.02987	**0.00009**	0.03554	1.0000	0.00815
Emotional Processing	**4.38×10^{-21}**	0.22834	**4.38×10^{-6}**	0.04366	1.0000	0.06391
Verbal Memory	**6.16×10^{-10}**	0.10141	0.0607	0.00347	1.0000	0.05556
Facial Memory	**7.54×10^{-13}**	0.13325	**2.22×10^{-6}**	0.04538	1.0000	0.01118
Spatial Memory	**3.34×10^{-8}**	0.07808	0.1062	0.00815	1.0000	0.02046
Sensorimotor Dexterity	**4.20×10^{-18}**	0.18401	**0.0116**	*	0.5100	*

Note. Nominally significant ($p<0.05$) values are bolded. *Proportion of variance cannot be estimated due to its relatively small effect and repeated instability in the model.

As expected, age^2 was a nominally significant covariate for every domain that showed a quadratic function in the regression analyses (i.e., Abstraction/Mental Flexibility, Attention, Spatial and Emotional Processing, and Facial Memory), as well as the domain that showed a cubic function (i.e., Sensorimotor Dexterity), but was not significant for Verbal and Spatial Memory. The amount of variation explained by age^2 ranged between 3.6% and 4.5% for individual domains with significant quadratic effects, excluding Sensorimotor Dexterity, which was not able to be estimated due to repeated instability in the model. The estimate of the cubic main effects of age (age^3) after accounting for both linear and quadratic elements was not significant for any domain, including Sensorimotor Dexterity ($p=0.51$), also presented in Table 18.

Given the general agreement between the SOLAR and SPSS findings, the main effects of age retained in succeeding analyses will include the following: age^1 (Verbal and Spatial Memory); age^1 and age^2 (Abstraction/Mental Flexibility, Attention, Spatial and Emotional Processing, and Facial Memory); and age^1, age^2, and age^3 (Sensorimotor Dexterity).

3.4.3 Stage 3 Analysis: Estimation of the Main Effects of SNPs on Cognition

The main effects of individual SNPs on cognitive performance were estimated separately for each cognitive domain in SOLAR using the residuals from the stage 2 analyses. Given the different elements that were estimated in stage 2, the residuals used are those from the highest power of age that was significant in the SPSS regression analysis. The unadjusted significance levels and proportion of variance explained by individual SNPs for each domain are shown in Table 19. In general, there were few nominally significant main effects on cognition.

Table 19. Stage 3 analysis: Estimation of the main effects of individual SNPs on cognitive domains

Marker	Abstraction & Mental Flexibility	Attention	Spatial Processing	Emotional Processing	Verbal Memory	Facial Memory	Spatial Memory	Sensorimotor Dexterity
SNP8NRG221132	0.2520	0.8693	0.3030	0.3404	0.1162	0.3395	0.8177	0.9770
SNP8NRG221533/rs35753505	0.5350	0.3424	0.4214	0.1361	0.3858	0.7403	0.5595	0.6610
rs4298458	0.9784	0.4516	0.9209	0.5283	0.3939	0.6376	0.6775	0.3142
SNP8NRG241930	0.9402	0.5343	0.8788	0.4890	**0.0425 (0.022)**	0.9365	0.8912	0.8350
rs1081062	0.9403	0.1397	0.5759	0.6432	0.8431	0.9507	0.1292	0.9652
rs4566990	0.6432	0.9590	0.6804	0.2174	0.8944	0.6732	0.3318	0.6332
rs1354335	0.3591	0.2992	0.3672	0.8881	0.2335	0.5497	0.3577	0.6957
rs1354336	0.2946	0.3458	0.0823	0.3252	0.3519	0.4742	0.8719	0.9630
rs1354334	0.8508	0.5324	0.8472	0.2613	0.8507	0.5884	0.4321	0.7127
SNP8NRG444511/rs13268724	0.5544	0.3419	0.3202	0.9311	0.1703	0.4919	0.2713	0.7814
rs776401	0.8514	0.9383	0.2119	0.6189	0.5062	0.9355	0.4022	0.5203
rs1473438	0.7507	0.9728	0.2265	0.4699	0.4140	0.8055	0.3456	0.7655
rs1462893	0.3604	0.2714	0.9185	0.3237	0.1200	**0.0131 (0.023)**	0.1126	0.0721
rs10954821	0.7787	0.3068	0.1331	0.4330	0.3042	0.9510	0.8138	0.4661
rs726908	0.0907	0.4044	0.2821	**0.0420 (0.018)**	0.7670	0.6588	0.4061	0.3483
rs10954855	0.2500	**0.0166 (0.029)**	0.3035	**0.0188 (0.021)**	0.3264	0.7271	0.3240	0.6958
rs2439306	0.8060	0.8288	0.4571	0.8125	0.1491	0.4653	0.4546	0.3082
rs2466062	0.2264	0.7845	0.4280	0.6526	0.1311	0.7911	0.5724	0.1323
rs3924999	0.8181	0.3768	0.5874	**0.0385 (0.010)**	0.5847	0.8977	0.3372	0.3186
rs2466060	0.2160	0.9826	0.4599	0.5684	0.2674	0.8833	**0.0385 (0.017)**	0.7273
rs2439272	0.4028	0.8263	0.5728	0.7959	0.2594	0.7421	0.6480	0.3968
rs6468121	0.8639	0.2945	0.4629	0.4187	0.2657	0.6247	0.5506	0.1788
rs2466058	0.4109	0.6298	0.8797	0.2303	0.9124	0.4946	0.9233	0.3463
rs2466049	0.4504	0.5703	0.9095	0.2021	0.9608	0.5717	0.8729	0.3280
rs723811	0.8013	0.4230	0.8675	0.6819	0.1888	0.8495	0.7670	0.3860
rs6988339	0.3896	0.2970	0.2100	0.1821	0.0582	0.4671	0.7459	0.8483
rs2975498	0.5835	0.1918	0.4504	0.3883	0.1172	0.5959	0.6326	0.3343
rs2919382	0.3927	0.1027	0.7496	0.3305	0.1864	0.4616	0.6166	0.4842
rs2976525	0.3644	0.6613	0.2356	0.6710	0.3588	0.7750	0.6231	0.9819
rs4262285	0.6980	0.7109	0.2343	0.9053	0.7277	0.8213	0.3799	0.2147
rs3735776	**0.0418 (0.014)**	0.9080	0.7620	0.1525	0.4795	0.3793	0.5046	0.1032
rs4512342	**0.0340 (0.015)**	0.8609	0.5976	0.2434	0.6651	0.4437	0.9133	0.5008
rs10503929	0.1991	0.1316	0.8424	0.8202	0.9940	0.4382	0.7762	0.2453
rs6992642	0.9862	0.7022	0.2130	0.5331	0.6914	0.0778	0.4459	0.8685
rs3735781	0.6797	0.7176	0.0962	0.3141	0.3559	0.0824	0.4846	0.8705
rs3735782	0.5613	0.9162	0.1245	0.6337	0.5994	0.1364	0.4706	0.7968

Note. Unadjusted p-values are reported. Nominally significant (p<0.05) values are bolded and proportion of variance is provided in parentheses when the effect is significant (p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations.

3.4.3.1 Target Domains. In total, there were six SNPs that had nominally significant effects on cognitive performance across the target cognitive domains and explained a small proportion of the variance in cognition, ranging from 1.0% to 2.9%. These effects were evenly divided between target SNPs and non-target SNPs.

3.4.3.1.1 Target SNPs. There were four nominally significant target domain, target SNP effects across three SNPs. Emotional processing had the most unadjusted significant SNP effects (rs10954855 and rs3924999), while Attention and Verbal Memory each had one (rs10954855

and SNP8NRG241930, respectively), and Abstraction/Mental Flexibility and Spatial Processing had no significant target SNP effects. Across these four effects, the individual SNPs explained only a small proportion of the variance, in the range of 1.0-2.9%.

3.4.3.1.2 Non-target SNPs. There were three nominally significant target domain, non-target SNP effects across three SNPs. Abstraction/Mental Flexibility had the most unadjusted significant SNP effects (rs3735776 and rs4512342), while Emotional Processing had one (rs726908), and Attention, Spatial Processing, and Verbal Memory had none. Across these three effects, individual SNPs explained a small proportion of the variance, in the range of 1.4-1.8%.

3.4.3.2 Non-Target Domains. In total, there were two SNPs that had nominally significant effects on cognitive performance across the three non-target cognitive domains. Across these effects, the individual SNPs explained a small proportion of the variance of cognition, ranging from 1.7% to 2.3%.

3.4.3.2.1 Target SNPs. There was one nominally significant non-target domain, target SNP effect, which was seen in Spatial Memory (rs2466060), while Facial Memory and Sensorimotor Dexterity had no significant findings. Marker rs2466060 explained 1.7% of the variance in Spatial Memory performance.

3.4.3.2.2 Non-target SNPs. There was one nominally significant non-target domain, non-target SNP effect, which was seen in Facial Memory (rs1462893), while Spatial Memory and Sensorimotor Dexterity had no significant findings. Marker rs1462893 explained 2.3% of the variance in Facial Memory performance.

3.4.4 Stage 4 Analysis: Estimation of Age x SNP Interactions on Cognition

Using the residuals from the stage 3 analyses, the interactions between age and individual SNPs on cognitive performance were estimated separately for each cognitive domain. Interactions

estimated in this step included age^1 x SNP, age^2 x SNP, and age^3 x SNP, based on the findings of the previously reported main effects of age. For example, interactions for Verbal Memory were limited to age^1 x SNP, while interactions for Abstraction/Mental Flexibility included age^1 x SNP and age^2 x SNP, and Sensorimotor Dexterity included age^1 x SNP, age^2 x SNP, and age^3 x SNP. In the case of domains with quadratic or cubic patterns, all interactions for an individual SNP were estimated simultaneously, providing individual p-values for each component and the total proportion of variance explained.

Because the joint effects of the different levels of interactions (e.g., linear plus quadratic) might be significant even if an individual level was not, the log likelihood (LL) difference [$-2(LL_{Model1} - LL_{Model2})$] was calculated for the model with sex, age (linear, quadratic, and cubic, as appropriate), and SNP main effects ($Model_1$) compared to the model with those components plus the age x SNP interactions (linear, quadratic, and cubic, as appropriate) ($Model_2$). The LL difference score is distributed as a chi-square function and has two degrees of freedom for domains with a quadratic function and three for those with a cubic shape. The log likelihoods, their differences, interaction significance levels, and the total proportion of variance explained can be found in Tables 20-27 and are discussed individually below. Graphs for significant model changes and interactions are presented in Figures 6-11 and discussed individually below. Importantly, the number of participants in each genotype group differs for individual SNPs due to the frequency of the minor allele (see Table 1 for minor allele frequencies for each SNP), and thus the number of participants who have the minor allele homozygote (genotype 2) is sometimes very small, and in these cases, should not be interpreted graphically. This is noted for each domain below. Overall there were several nominally significant total age interaction effects (LL model differences) and specific component interactions (linear, quadratic, and/or cubic)

across multiple SNPs and domains. All nominally significant ($p<0.05$) effects are discussed below. These are considered nominal because they have not undergone correction for multiple comparisons, which will be discussed in the coming sections.

3.4.4.1 Target Domains. Across all target domains, there were nine nominally significant total age interaction effects and 19 nominally significant specific interaction components, for a total of 20 nominally significant independent SNP-domain findings.

3.4.4.1.1 Abstraction/Mental Flexibility. As seen in Table 20, there were five nominally significant findings in this domain. Among target SNPs, there was one significant total age interaction effect and a significant linear interaction component (but not a quadratic one) for rs35753505. Target markers rs3924999, rs2466058, and rs2466049 also all had significant linear interaction components without quadratic components or a significant total age interaction effect. There was only one non-target SNP, rs726908, with a significant finding in this domain, which was a significant linear component.

Table 20. Abstraction & Mental Flexibility (Target): Model comparisons and estimation of specific components of the SNP x age interaction in cognitive performance prediction

	Total *NRG1* x Age Interaction Effect			Interaction Components		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132	-169.6226	-169.2579	0.7293	0.4843	0.6605	
SNP8NRG221533/rs35753505	-169.3440	-165.9100	**6.8679 (p=0.032)**	**0.0089**	0.4939	0.019
rs4298458	-170.2784	-169.3661	1.8246	0.1793	0.9496	
SNP8NRG241930	-169.7219	-168.2671	2.9095	0.1024	0.6902	
rs1081062	-169.5706	-169.4597	0.2218	0.7094	0.8056	
rs4566990	-170.1715	-170.0550	0.2331	0.7620	0.6876	
rs1354335	-165.7326	-165.1457	1.1738	0.3371	0.6035	
rs1354336	-169.0830	-168.7487	0.6686	0.8810	0.4180	
rs1354334	-170.2611	-169.6595	1.2032	0.3332	0.5406	
SNP8NRG444511/rs13268724	-170.1040	-169.8385	0.5312	0.5020	0.7731	
rs776401	-168.8111	-168.3538	0.9146	0.5026	0.4231	
rs1473438	-169.4962	-169.0970	0.7982	0.6124	0.4150	
rs1462893	-169.5417	-167.9233	3.2368	0.0720	0.8000	
rs10954821	-170.2393	-169.6992	1.0802	0.3006	0.7995	
rs726908	-168.1517	-166.1425	4.0184	**0.0469**	0.9888	0.010
rs10954855	-169.6172	-169.1525	0.9295	0.3369	0.9884	
rs2439306	-161.6780	-161.2424	0.8712	0.3507	0.9161	
rs2466062	-166.3367	-166.1724	0.3288	0.6151	0.7780	
rs3924999	-169.8575	-167.8946	3.9259	**0.0495**	0.9838	0.009
rs2466060	-152.9567	-151.8587	2.1960	0.1398	0.9268	
rs2439272	-169.8808	-169.8496	0.0624	0.8715	0.8587	
rs6468121	-168.2628	-167.9787	0.5681	0.4510	0.9625	
rs2466058	-169.9406	-167.5779	4.7254	**0.0371**	0.5254	0.015
rs2466049	-169.2609	-166.7365	5.0489	**0.0304**	0.5370	0.016
rs723811	-170.2471	-169.9058	0.6827	0.9579	0.4244	
rs6988339	-169.9087	-169.7267	0.3641	0.7913	0.5899	
rs2975498	-170.1284	-168.8231	2.6106	0.1062	0.8543	
rs2919382	-169.9135	-168.8754	2.0762	0.1498	0.8438	
rs2976525	-169.8674	-168.2792	3.1764	0.0967	0.5097	
rs4262285	-170.2035	-170.0869	0.2331	0.6691	0.6318	
rs3735776	-140.9455	-139.4364	3.0183	0.0827	0.6701	
rs4512342	-167.7131	-166.0192	3.3879	0.0683	0.5282	
rs10503929	-169.4543	-168.2288	2.4511	0.1322	0.9203	
rs6992642	-165.5366	-164.5481	1.9770	0.1628	0.8166	
rs3735781	-169.8271	-169.1467	1.3607	0.2587	0.7169	
rs3735782	-167.2990	-167.2932	0.0117	0.9942	0.9142	

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, and each individual SNP. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded.

The individual patterns of cognitive function by age for each SNP (minor allele dosage) for this domain are graphed in Figure 6. Quadratic curve-fitting was applied due to the quadratic nature of this domain with age. Compared to the "wild type" genotype (major allele homozygote; genotype 0), the effect of the minor allele is generally advantageous to Abstraction/Mental Flexibility performance, especially in later life. Although there is some divergence in

performance by genotype early in life, especially for rs35753505, the age at which the performance-allele patterns begin to diverge is in midlife, between age 30 and 50. In the case of rs35753505, having two copies of the minor allele results in a pattern in which performance is low earlier in life, better at midlife, and again low later in life. Given that rs2466058 and rs2466049 each only have three minor allele homozygotes, the pattern of this genotype should not be interpreted.

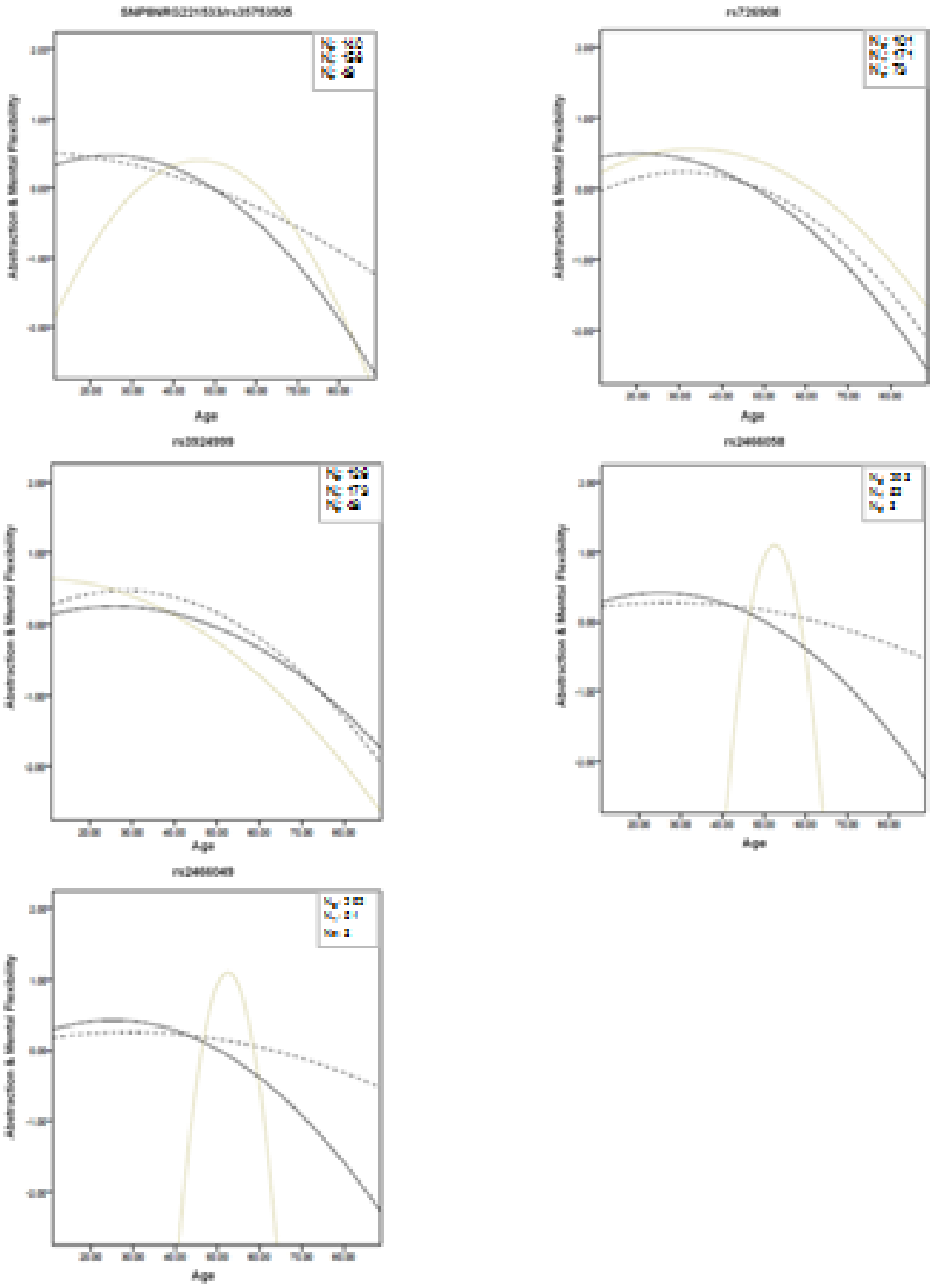


Figure 6. Abstraction & Mental Flexibility (Target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

3.4.4.1.2 Attention. As seen in Table 21, there were five nominally significant findings in this domain. Target markers rs2439272 and rs6988339 had significant linear interaction components without quadratic components or a significant total age interaction effect. Among non-target SNPs, there was one significant total age interaction effect: rs6468121. This SNP also showed a significant linear interaction component, but not a quadratic one. One additional non-target SNP, rs3735782, had a significant finding in this domain with a significant linear component.

Table 21. Attention (Target): Model comparisons and estimation of specific components of the SNP x age interaction in cognitive performance prediction

	Total *NRG1* x Age Interaction Effect			**Interaction Components**		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132	-357.4238	-356.6516	1.5444	0.5081	0.2292	
SNP8NRG221533/rs35753505	-354.6446	-353.4421	2.4051	0.8122	0.1220	
rs4298458	-357.1540	-355.7184	2.8712	0.8417	0.0916	
SNP8NRG241930	-352.9311	-352.4656	0.9310	0.3575	0.7929	
rs1081062	-355.5673	-355.3827	0.3693	0.8077	0.5641	
rs4566990	-357.4360	-354.9695	4.9331	0.2551	**0.0337**	0.035
rs1354335	-349.0740	-348.5732	1.0016	0.3173	0.9878	
rs1354336	-348.7144	-348.5910	0.2469	0.8625	0.6466	
rs1354334	-357.2424	-355.6170	3.2509	0.2895	0.0913	
SNP8NRG444511/rs13268724	-356.9857	-355.8392	2.2930	0.1510	0.5981	
rs776401	-352.3736	-352.3417	0.0637	0.8030	0.9501	
rs1473438	-355.8434	-355.6956	0.2955	0.6262	0.8427	
rs1462893	-356.2099	-355.6803	1.0593	0.3122	0.7507	
rs10954821	-356.9151	-356.2272	1.3759	0.2887	0.5952	
rs726908	-354.7936	-353.6934	2.2004	0.2986	0.2810	
rs10954855	-354.5674	-352.7588	3.6173	0.3997	0.1195	
rs2439306	-342.6799	-341.5797	2.2004	0.1570	0.7168	
rs2466062	-356.2178	-354.2491	3.9374	0.0913	0.3736	
rs3924999	-351.9861	-351.1125	1.7473	0.2670	0.5775	
rs2466060	-332.2580	-330.9639	2.5883	0.1742	0.4560	
rs2439272	-357.1377	-354.8512	4.5729	**0.0397**	0.6445	0.013
rs6468121	-346.8393	-343.5527	**6.5732 (p=0.037)**	**0.0108**	0.8677	0.032
rs2466058	-357.3211	-356.1929	2.2564	0.9767	0.1338	
rs2466049	-355.6136	-354.4161	2.3949	0.9331	0.1248	
rs723811	-357.1163	-355.2227	3.7871	0.4561	0.1078	
rs6988339	-356.8935	-354.5159	4.7552	**0.0431**	0.4175	0.006
rs2975498	-356.5854	-355.7432	1.6845	0.9133	0.1978	
rs2919382	-356.1061	-355.2213	1.7696	0.6277	0.2350	
rs2976525	-357.3414	-357.1336	0.4156	0.5334	0.8045	
rs4262285	-357.3686	-356.0036	2.7300	0.1155	0.2430	
rs3735776	-306.9429	-306.1164	1.6531	0.5266	0.2030	
rs4512342	-356.7993	-354.5329	4.5328	0.2071	0.1552	
rs10503929	-356.3004	-355.9509	0.6990	0.5051	0.4826	
rs6992642	-350.0546	-349.4034	1.3022	0.2598	0.9735	
rs3735781	-355.2641	-354.4073	1.7137	0.2077	0.9753	
rs3735782	-353.5881	-351.1248	4.9265	**0.0535**	0.4659	0.041

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, and each individual SNP. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded.

The individual patterns of cognitive function by age for each SNP (minor allele dosage) for this domain are graphed in Figure 7. Quadratic curve-fitting was applied due to the quadratic nature of this domain with age. Compared to the wildtype, the effect of the minor allele is mixed with regard to Attention performance. For rs4566990, the effect of the minor allele is generally advantageous, with minor allele homozygotes performing best, followed by heterozygotes, and then major allele homozygotes. There is notable divergence in the pattern of the minor allele homozygote from the other two genotypes, where individuals with this genotype perform better at early and late life, and approximately the same as the other groups at midlife. Markers rs2439272 and rs6988339 also show a minor allele advantage with less dramatic differences between groups. The point at which the genotype patterns diverge for these SNPs is at approximately 30 years of age. For SNPs rs6468121 and rs3735782, the minor allele confers a slight disadvantage, especially later in life. The age at which the genotypes diverge is at approximately age 20 and 60 for rs6468121, and age 30 for rs3735782.

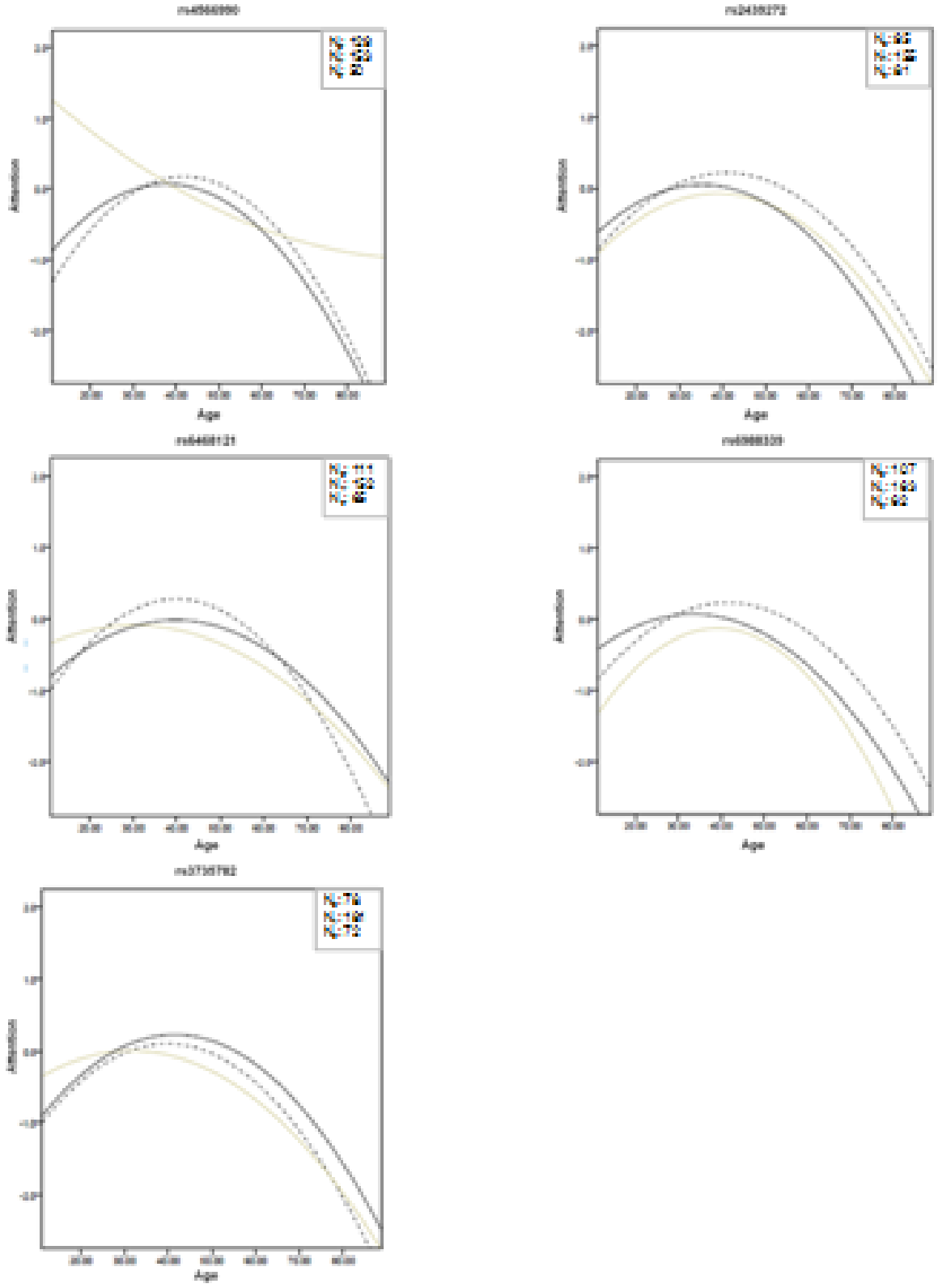


Figure 7. Attention (Target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

3.4.4.1.3 Spatial Processing. As seen in Table 22, there was one nominally significant finding in this domain. No target markers had significant total age interaction effects or significant specific interactions. Among non-target SNPs, there were no significant total age interaction effects, but rs1473438 showed a significant quadratic interaction component. No other non-target SNPs showed significant interaction components for this domain.

Table 22. Spatial Processing (Target): Model comparisons and estimation of specific components of the SNP x age interaction in cognitive performance prediction

	Total *NRG1* x Age Interaction Effect			**Interaction Components**		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132	-292.9784	-292.7258	0.5051	0.7736	0.5015	
SNP8NRG221533/rs35753505	-292.0196	-291.8825	0.2741	0.6397	0.9280	
rs4298458	-293.5039	-293.4815	0.0448	0.8330	0.9636	
SNP8NRG241930	-281.1672	-281.1198	0.0947	0.7733	0.9057	
rs1081062	-290.2224	-290.1878	0.0693	0.8200	0.8875	
rs4566990	-293.4240	-292.7884	1.2712	0.4814	0.3348	
rs1354335	-287.3766	-287.3003	0.1526	0.8435	0.7305	
rs1354336	-288.6889	-288.3353	0.7072	0.6020	0.4845	
rs1354334	-293.4903	-292.8605	1.2595	0.4551	0.3512	
SNP8NRG444511/rs13268724	-293.0147	-292.9227	0.1840	0.7918	0.7301	
rs776401	-292.0766	-290.7771	2.5990	0.8198	0.1075	
rs1473438	-287.8408	-286.6230	2.4357	0.8892	**0.0453**	0.013
rs1462893	-292.9988	-292.8675	0.2626	0.6237	0.8500	
rs10954821	-292.3808	-292.1795	0.4025	0.8980	0.5259	
rs726908	-286.1235	-285.7226	0.8017	0.3960	0.7305	
rs10954855	-292.9794	-292.5096	0.9396	0.5000	0.4482	
rs2439306	-279.1184	-278.8578	0.5211	0.5129	0.7182	
rs2466062	-288.0496	-286.9711	2.1569	0.8156	0.1573	
rs3924999	-292.7086	-291.3963	2.6247	0.8235	0.1175	
rs2466060	-266.7944	-266.3585	0.8717	0.5680	0.5367	
rs2439272	-292.3204	-291.9451	0.7507	0.4999	0.7064	
rs6468121	-290.5676	-290.1262	0.8829	0.3640	0.9069	
rs2466058	-293.4974	-293.2266	0.5416	0.5483	0.7071	
rs2466049	-288.3463	-288.0539	0.5847	0.5317	0.7001	
rs723811	-293.4949	-293.1548	0.6802	0.6921	0.5040	
rs6988339	-292.7232	-290.7077	4.0311	0.0619	0.4248	
rs2975498	-293.2240	-292.3485	1.7509	0.1964	0.5776	
rs2919382	-293.4579	-292.9276	1.0607	0.3515	0.7157	
rs2976525	-292.8056	-291.8695	1.8720	0.1718	0.9393	
rs4262285	-292.8015	-292.4215	0.7600	0.6078	0.8703	
rs3735776	-257.3983	-256.7800	1.2365	0.3258	0.4051	
rs4512342	-292.8648	-292.5431	0.6433	0.4235	0.9014	
rs10503929	-293.4891	-292.9120	1.1540	0.3560	0.8962	
rs6992642	-274.0872	-273.7098	0.7550	0.0988	0.6789	
rs3735781	-291.3142	-290.7864	1.0557	0.0593	0.6650	
rs3735782	-289.9949	-289.8918	0.2061	0.6638	0.9152	

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, and each individual SNP. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded.

The pattern of cognitive function by age for rs1473438 is graphed in Figure 8. Quadratic curve-fitting was applied due to the quadratic nature of this domain with age. Compared to the wildtype, the effect of the minor allele changed with age. Although the patterns are somewhat divergent earlier in life, the genotype effects are most significant after approximately age 30, when each copy of the minor allele confers additional benefit to performance. However, the minor allele homozygote's performance decreases dramatically at approximately age 60 and after, while the heterozygote and major allele homozygote's performance remains largely stable.

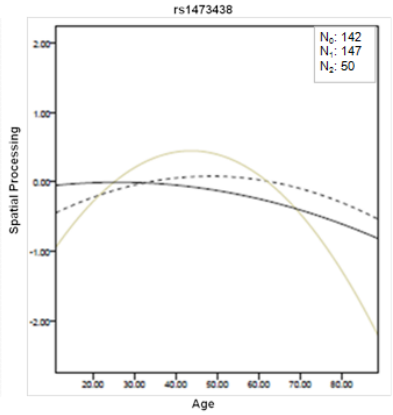


Figure 8. Spatial Processing (Target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

3.4.4.1.4 Emotional Processing. As seen in Table 23, there were nine nominally significant findings in this domain. Among target SNPs, there were three significant total age interaction effects for markers rs35753505, rs4298458, and rs776401. Both rs35753505 and rs4298458 showed a significant linear component, while rs776401 had a significant quadratic component. Target marker rs4262285 had a significant linear interaction component without a quadratic component or a significant total age interaction effect.

Among non-target SNPs, there were four significant total age interaction effects for markers rs4566990, rs1354334, rs1473438, and rs10954821. Markers rs4566990, rs1354334, and rs1473438 showed only significant quadratic components, while rs10954821 had no

significant specific interaction components. Non-target marker rs723811 had a significant quadratic interaction component without a linear component or a significant total age interaction effect.

Table 23. Emotional Processing (Target): Model comparisons and estimation of specific components of the SNP x age interaction in cognitive performance prediction

	Total *NRG1* x Age Interaction Effect			Interaction Components		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132	-163.4921	-161.5331	3.9180	0.2198	0.1205	
SNP8NRG221533/rs35753505	-162.9386	-157.7109	**10.4554 (p=0.005)**	**0.0061**	0.2702	0.032
rs4298458	-163.7478	-160.0451	**7.4055 (p=0.025)**	**0.0133**	0.2936	0.023
SNP8NRG241930	-161.9187	-160.5955	2.6464	0.3437	0.1794	
rs1081062	-163.7745	-161.8262	3.8966	0.1268	0.2633	
rs4566990	-163.1861	-159.7209	**6.9302 (p=0.031)**	0.8872	**0.0085**	0.019
rs1354335	-160.0081	-158.7853	2.4454	0.3153	0.2140	
rs1354336	-160.5400	-159.5251	2.0298	0.7263	0.1569	
rs1354334	-163.3157	-159.1082	**8.4150 (p=0.015)**	0.6761	**0.0049**	0.023
SNP8NRG444511/rs13268724	-163.9429	-163.1453	1.5952	0.4718	0.2867	
rs776401	-158.0433	-153.2594	**9.5679 (p=0.008)**	0.5094	**0.0020**	0.026
rs1473438	-163.0141	-158.4875	**9.0531 (p=0.011)**	0.3408	**0.0028**	0.025
rs1462893	-163.5226	-161.2465	4.5522	0.0875	0.1476	
rs10954821	-163.6393	-160.5289	**6.2208 (p=0.045)**	0.1934	0.0590	
rs726908	-161.7459	-161.7296	0.0326	0.9269	0.8806	
rs10954855	-161.1848	-160.6171	1.1353	0.5298	0.3532	
rs2439306	-158.6094	-158.4656	0.2877	0.5965	0.8871	
rs2466062	-161.3080	-160.8756	0.8648	0.9411	0.3530	
rs3924999	-158.7767	-158.0150	1.5235	0.4938	0.3541	
rs2466060	-145.1698	-144.9138	0.5120	0.4916	0.9469	
rs2439272	-164.0009	-163.0586	1.8846	0.1764	0.9225	
rs6468121	-160.7770	-160.4460	0.6620	0.4486	0.7487	
rs2466058	-163.2273	-162.8096	0.8354	0.5996	0.4614	
rs2466049	-162.7925	-162.3530	0.8790	0.5474	0.4817	
rs723811	-163.8627	-161.6383	4.4487	0.3171	**0.0420**	0.011
rs6988339	-163.0563	-162.4097	1.2933	0.8001	0.2741	
rs2975498	-163.5746	-162.9669	1.2153	0.4641	0.4771	
rs2919382	-163.4732	-163.2286	0.4892	0.9093	0.4867	
rs2976525	-163.8565	-163.5694	0.5742	0.5152	0.6781	
rs4262285	-163.9396	-160.9494	5.9804	**0.0168**	0.1014	0.012
rs3735776	-249.5935	-249.5626	0.0617	0.8822	0.8811	
rs4512342	-163.3292	-162.9954	0.6675	0.4271	0.9826	
rs10503929	-163.9209	-163.6166	0.6085	0.9631	0.4723	
rs6992642	-162.3351	-161.7737	1.1226	0.4373	0.4499	
rs3735781	-162.3219	-161.8263	0.9913	0.5809	0.3821	
rs3735782	-162.8334	-162.5371	0.5927	0.8152	0.4562	

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, and each individual SNP. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded.

The individual patterns of cognitive function by age for each SNP (minor allele dosage) for this domain are graphed in Figure 9. Quadratic curve-fitting was applied due to the quadratic nature of this domain with age. Compared to the wildtype, the minor allele had mixed effects on Emotional Processing performance over the lifespan. The minor alleles of SNPs rs4566990, rs1354334, rs10954821, and rs723811 showed a benefit to performance both in early (at or before age 30) and later life (at or after age 60), with less dramatic effects at midlife. Markers rs776401and rs1473438, however, showed the opposite pattern whereby the minor allele conferred a detriment to performance early and late in life. The minor alleles for SNPs rs35753505 and rs4298458 conferred a detriment to performance early in life, but an advantage later in life, with the change in direction occurring at approximately age 50. Finally, rs4262285 showed the opposite pattern, with minor alleles conferring an advantage early and a detriment at age 40 and later. Given that rs723811 and rs4262285 each have fewer than three minor allele homozygotes, the pattern of this genotype should not be interpreted.

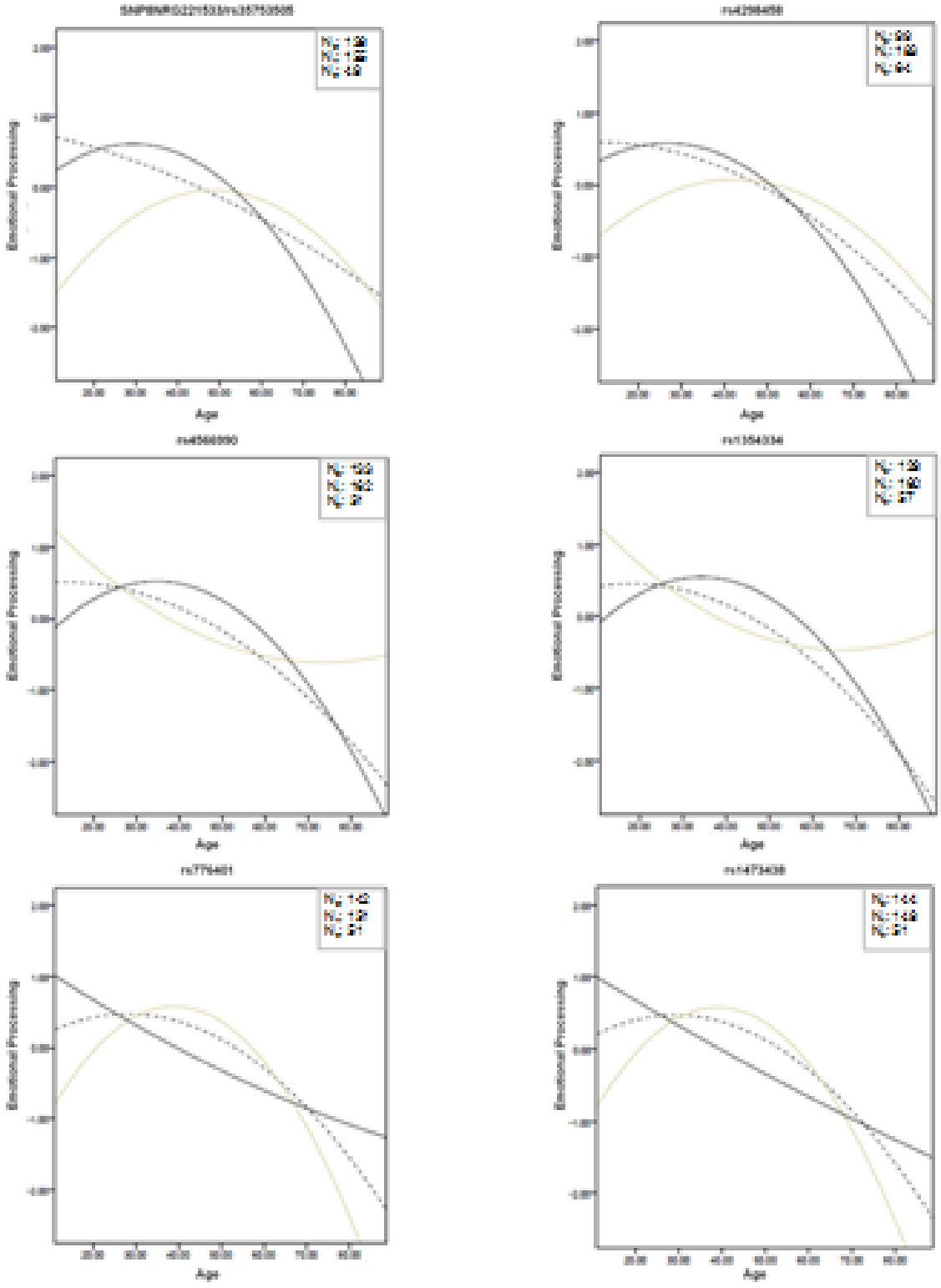


Figure 9. Emotional Processing (Target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

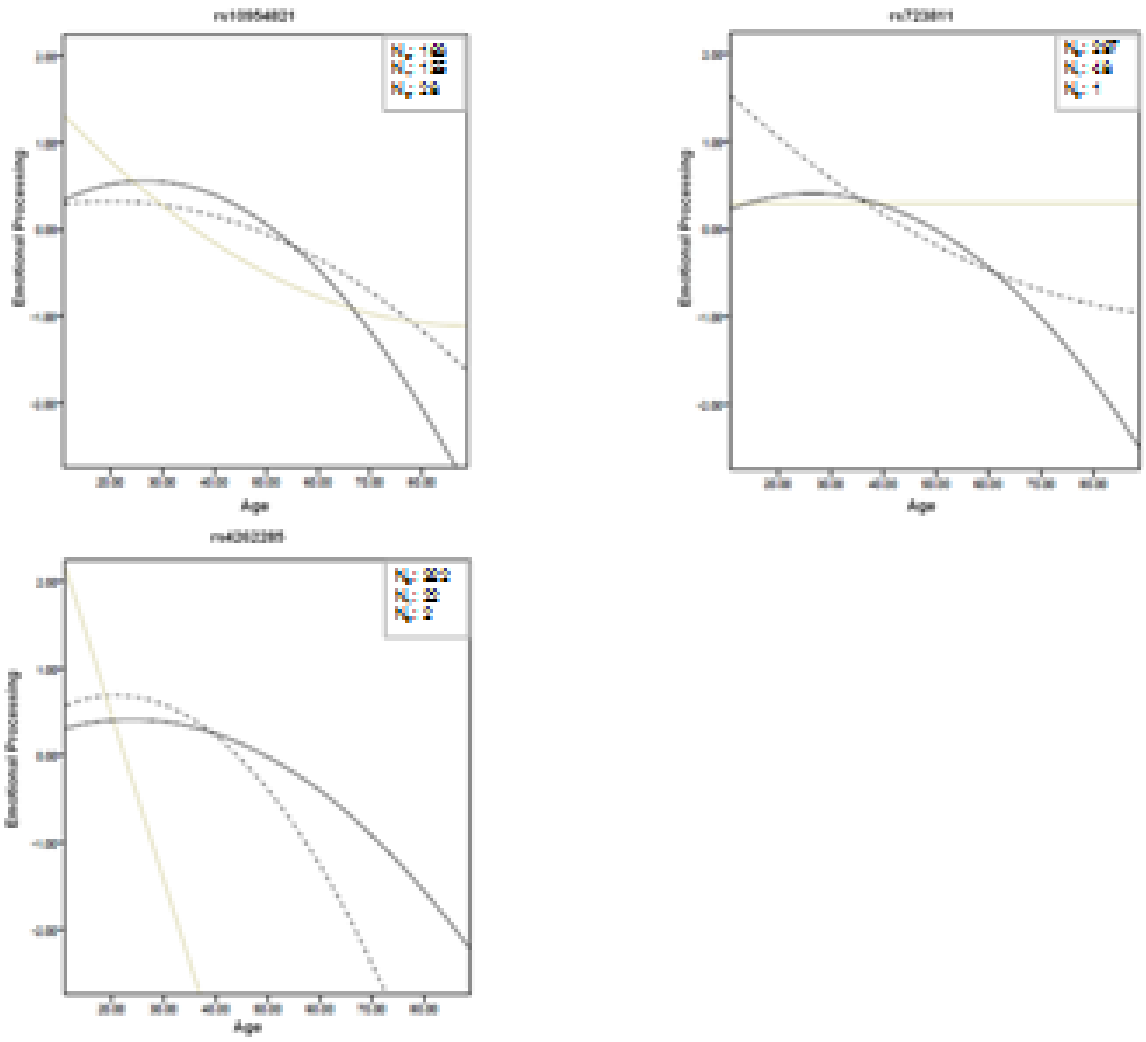


Figure 9 (continued). Emotional Processing (Target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

3.4.4.1.5 Verbal Memory. As seen in Table 24, there were no significant linear interaction components for target or non-target SNPs.

Table 24. Verbal memory (Target): Model comparisons and estimation of specific components of the SNP x age interaction in cognitive performance prediction

	Verbal Memory	
	Linear Component	
Marker	*p-value*	*Proportion of Variance*
SNP8NRG221132	0.9928	
SNP8NRG221533/rs35753505	0.8596	
rs4298458	0.9433	
SNP8NRG241930	0.7826	
rs1081062	0.7966	
rs4566990	0.9255	
rs1354335	0.8591	
rs1354336	0.7387	
rs1354334	0.5145	
SNP8NRG444511/rs13268724	0.9658	
rs776401	0.4429	
rs1473438	0.5844	
rs1462893	0.4600	
rs10954821	0.4771	
rs726908	0.2185	
rs10954855	0.9144	
rs2439306	0.8344	
rs2466062	0.5271	
rs3924999	0.8884	
rs2466060	0.2243	
rs2439272	0.0814	
rs6468121	0.0675	
rs2466058	0.8256	
rs2466049	0.9406	
rs723811	0.2750	
rs6988339	0.6490	
rs2975498	0.2376	
rs2919382	0.4963	
rs2976525	0.3643	
rs4262285	0.4174	
rs3735776	0.7835	
rs4512342	0.7455	
rs10503929	0.7098	
rs6992642	0.3471	
rs3735781	0.5392	
rs3735782	0.5588	

Note. P-values are reported. Proportion of variance is provided for significant ($p<0.05$) effects. Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant ($p<0.05$) values are bolded.

3.4.4.2 Non-Target Domains. Across all non-target domains, there were 11 nominally significant total age interaction effects and 12 nominally significant specific interactions, for a total of 14 nominally significant findings.

3.4.4.2.1 Facial Memory. As seen in Table 25, there were no significant findings in this domain at the level of total age interaction effects or individual interaction components.

Table 25. Facial Memory (Non-target): Model comparisons and estimation of specific components of the SNP x age interaction in cognitive performance prediction

	Total *NRG1* x Age Interaction Effect			Interaction Components		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132	-134.2221	-133.6261	1.1920	0.3165	0.6917	
SNP8NRG221533/rs35753505	-134.9580	-134.9158	0.0845	0.9989	0.7765	
rs4298458	-134.5674	-134.5308	0.0731	0.8385	0.8540	
SNP8NRG241930	-130.9093	-129.6435	2.5316	0.1223	0.6652	
rs1081062	-134.7651	-134.4270	0.6764	0.7695	0.4285	
rs4566990	-134.5894	-133.2575	2.6638	0.1201	0.5185	
rs1354335	-134.0868	-133.5772	1.0193	0.3222	0.8781	
rs1354336	-134.7986	-134.6521	0.2929	0.5884	0.9322	
rs1354334	-134.5319	-133.8978	1.2683	0.3072	0.5601	
SNP8NRG444511/rs13268724	-134.4422	-133.6122	1.6599	0.1984	0.9003	
rs776401	-129.5437	-129.5301	0.0271	0.9739	0.8695	
rs1473438	-134.8768	-134.8463	0.0609	0.8528	0.9093	
rs1462893	-131.7359	-130.9798	1.5123	0.2754	0.6508	
rs10954821	-134.6764	-133.9660	1.4208	0.6143	0.2501	
rs726908	-134.3478	-134.1883	0.3191	0.5802	0.9389	
rs10954855	-134.6174	-134.4258	0.3833	0.9936	0.5384	
rs2439306	-132.8196	-132.1287	1.3817	0.5217	0.2949	
rs2466062	-134.2010	-133.2364	1.9293	0.6732	0.1816	
rs3924999	-134.8057	-134.5792	0.4529	0.9162	0.5223	
rs2466060	-118.8033	-118.3805	0.8456	0.3602	0.9375	
rs2439272	-134.6315	-134.1058	1.0514	0.6721	0.3316	
rs6468121	-134.5519	-134.1539	0.7960	0.6248	0.4639	
rs2466058	-134.4451	-133.1268	2.6367	0.1136	0.7144	
rs2466049	-134.3041	-133.1398	2.3286	0.1394	0.7082	
rs723811	-134.6603	-134.2938	0.7331	0.3975	0.9597	
rs6988339	-134.4139	-133.5752	1.6773	0.9044	0.1956	
rs2975498	-134.5377	-134.5248	0.0258	0.8801	0.9737	
rs2919382	-134.4074	-134.1756	0.4635	0.4964	0.9456	
rs2976525	-134.6375	-134.5366	0.2017	0.7283	0.7651	
rs4262285	-134.6528	-133.7665	1.7727	0.2440	0.5691	
rs3735776	-120.7999	-120.6977	0.2045	0.7263	0.8587	
rs4512342	-134.5231	-134.2192	0.6080	0.9699	0.4532	
rs10503929	-134.3778	-133.6123	1.5310	0.2795	0.8405	
rs6992642	-131.4009	-129.6965	3.4089	0.3586	0.1071	
rs3735781	-132.2077	-130.6589	3.0976	0.3647	0.1235	
rs3735782	-133.7047	-131.9772	3.4551	0.5991	0.0747	

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, and each individual SNP. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Nominally significant (p<0.05) difference values are bolded.

3.4.4.2.2 Spatial Memory. As seen in Table 26, there was one nominally significant linear component: rs4566990. The pattern of the interaction between this SNP and age in predicting performance in this domain is shown in Figure 10.

Table 26. Spatial memory (Non-target): Model comparisons and estimation of specific components of the SNP x age interaction in cognitive performance prediction

	Spatial Memory	
Marker	*Linear Component p-values*	*Proportion of Variance*
SNP8NRG221132	0.4957	
SNP8NRG221533/rs35753505	0.1251	
rs4298458	0.1221	
SNP8NRG241930	0.3615	
rs1081062	0.8944	
rs4566990	**0.0381**	0.007
rs1354335	0.2997	
rs1354336	0.1883	
rs1354334	0.1191	
SNP8NRG444511/rs13268724	0.2055	
rs776401	0.1315	
rs1473438	0.0969	
rs1462893	0.3605	
rs10954821	0.5599	
rs726908	0.1348	
rs10954855	0.8411	
rs2439306	0.4186	
rs2466062	0.7366	
rs3924999	0.6787	
rs2466060	0.1037	
rs2439272	0.7940	
rs6468121	0.6693	
rs2466058	0.3620	
rs2466049	0.2467	
rs723811	0.7559	
rs6988339	0.8954	
rs2975498	0.4546	
rs2919382	0.8370	
rs2976525	0.4285	
rs4262285	0.1664	
rs3735776	0.9560	
rs4512342	0.5004	
rs10503929	0.5756	
rs6992642	0.5045	
rs3735781	0.3520	
rs3735782	0.6542	

Note. P-values are reported. Proportion of variance is provided for significant ($p<0.05$) effects. Nominally significant ($p<0.05$) values are bolded.

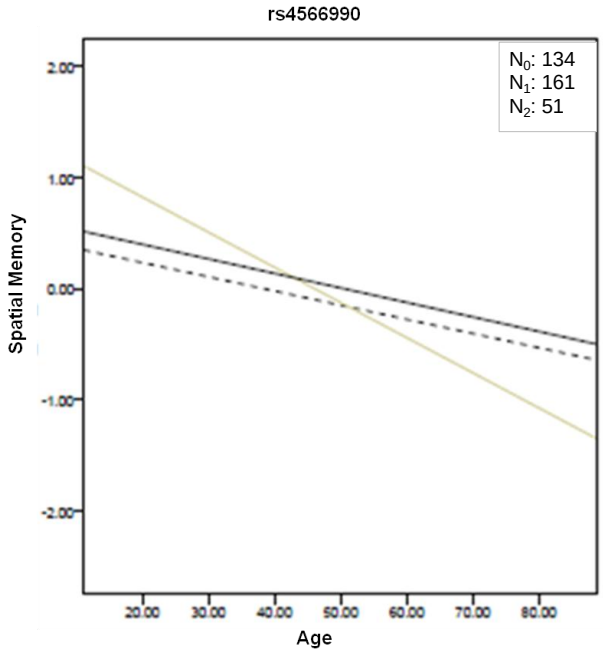


Figure 10. Spatial Memory (Non-target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

Linear curve-fitting was applied due to the linear relationship between this domain and age. Compared to the wildtype, the minor allele had inconsistent effects depending on the dosage. The major allele homozygote and heterozygote show a consistent pattern throughout life, with the major allele homozygote consistently performing slightly better than the heterozygote. The minor allele homozygote shows a different pattern: it shows better performance early in life, but worse later in life, intersecting with the major allele homozygote and heterozygote between age 40 and 50.

3.4.4.2.3 Sensorimotor Dexterity. As seen in Table 27, there were 13 nominally significant findings in this domain, the most of any domain studied. Among target SNPs, there were seven significant total age interaction effects across markers SNP8NRG221132, SNP8NRG241930, rs776401, rs2439272, rs2466058, rs2466049, and rs2976525. SNP8NRG221132 and rs776401 had significant linear and cubic components without significant quadratic contributions. SNP8NRG241930, rs2439272, and rs2466058 had significant cubic

components without linear or quadratic contributions. Marker rs2466049 and rs2976525 had significant total age interaction effects without specific linear, quadratic, or cubic components.

Table 27. Sensorimotor Dexterity (Non-target): Model comparisons and estimation of specific components of the SNP x age interaction in cognitive performance prediction

	Total *NRG1* x Age Interaction Effect			Interaction Components			
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Cubic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132	-233.3970	-223.5782	**19.6376 (p=0.0002)**	**0.0041**	0.1293	**0.0001**	0.0311
SNP8NRG221533/rs35753505	-233.8804	-230.8417	6.0773	0.1682	0.5729	0.1282	
rs4298458	-232.8909	-232.1265	1.5289	0.7569	0.3893	1.0000	
SNP8NRG241930	-229.2886	-221.1448	**16.2877 (p=0.001)**	0.1082	0.1569	**0.0052**	0.0784
rs1081062	-233.5803	-232.1197	2.9211	0.8407	0.3210	0.3207	
rs4566990	-233.2836	-231.9239	2.7193	0.5624	0.3306	0.3015	
rs1354335	-228.6087	-228.7543	-0.2913	1.0000	0.5486	1.0000	
rs1354336	-232.4276	-228.9710	6.9131	0.1253	0.4524	**0.0216**	*
rs1354334	-233.3296	-231.2885	4.0822	0.6978	0.3016	0.2545	
SNP8NRG444511/rs13268724	-233.3589	-233.0771	0.5636	1.0000	0.4078	1.0000	
rs776401	-213.0308	-196.3154	**33.4307 (p=0.000000)**	**0.0321**	0.1883	**0.00006**	0.0332
rs1473438	-231.6103	-219.6078	**24.0050 (p=0.00003)**	**8.437×10^{-7}**	0.1729	**0.0002**	0.0246
rs1462893	-231.8464	-226.6286	**10.4356 (p=0.015)**	0.7602	0.0831	0.1965	
rs10954821	-233.1319	-229.4734	7.3169	0.2850	1.0000	0.0998	
rs726908	-232.1440	-224.1396	**16.0087 (p=0.001)**	0.0574	0.2973	1.0000	
rs10954855	-233.3210	-231.7313	3.1793	0.2496	0.4636	0.1099	
rs2439306	-216.2951	-214.0053	4.5797	0.0972	0.2915	0.0954	
rs2466062	-231.7141	-230.6562	2.1159	0.4905	1.0000	0.2031	
rs3924999	-233.4284	-232.2748	2.3073	**0.0431**	0.9337	1.0000	0.0255
rs2466060	-202.1204	-200.1234	3.9940	0.0595	0.3170	0.3478	
rs2439272	-233.5364	-228.0079	**11.0572 (p=0.011)**	0.1373	0.2049	**0.0189**	0.0320
rs6468121	-219.4760	-214.7174	**9.5172 (p=0.023)**	0.6223	0.2083	0.0648	
rs2466058	-232.9539	-228.8462	**8.2153 (p=0.042)**	0.1711	0.0902	**0.0500**	0.0725
rs2466049	-231.5896	-227.3691	**8.4409 (p=0.038)**	0.2011	0.0902	0.0549	
rs723811	-233.0217	-229.8754	6.2925	1.0000	0.7377	0.1565	
rs6988339	-233.3791	-232.5267	1.7048	0.2439	0.7194	0.3356	
rs2975498	-232.9313	-232.9638	-0.0650	0.2601	0.7340	1.0000	
rs2919382	-233.1528	-232.8808	0.5440	0.3797	1.0000	1.0000	
rs2976525	-233.3972	-226.4623	**13.8697 (p=0.003)**	1.0000	0.1337	0.1113	
rs4262285	-232.6278	-231.9624	1.3309	0.7630	0.5465	1.0000	
rs3735776	-206.4748	-206.0988	0.7519	1.0000	0.2378	1.0000	
rs4512342	-233.2366	-231.8204	2.8324	1.0000	0.1638	0.4377	
rs10503929	-232.7226	-232.7172	0.0108	0.5518	0.6485	1.0000	
rs6992642	-224.9860	-224.0740	1.8240	1.0000	0.2812	1.0000	
rs3735781	-233.7141	-233.0421	1.3439	1.0000	0.2677	1.0000	
rs3735782	-230.4901	-228.9297	3.1208	0.2204	0.2851	0.7335	

Note. Total *NRG1* x age interaction effect includes linear, quadratic, and cubic components for this domain. Model 1 includes the main effects of sex, age, age^2, and age^3, and each individual SNP. Model 2 provides the same elements as Model 1 plus SNP x age, SNP x age^2, and SNP x age^3. -2LL Difference is distributed as a chi-square function (critical values: 7.82, df=3, p<0.05). *Proportion of variance cannot be estimated due to its relatively small effect and repeated instability in the model. Nominally significant (p<0.05) difference values are bolded.

Among non-target SNPs, there were four significant total age interaction effects for markers rs1473438, rs1462893, rs726908, and rs6468121. SNP rs1473438 had significant linear and cubic components. Markers rs1462893, rs726908, and rs6468121 had significant total age interaction effects with no significant contributions from the linear, quadratic, or cubic elements.

Non-target marker rs1354336 had a significant cubic interaction component without linear or quadratic components or a significant total age interaction effect.

The individual patterns of cognitive function by age for each SNP (minor allele dosage) for this domain are graphed in Figure 11. Cubic curve-fitting was applied due to the cubic nature of this domain with age. Given that SNP8NRG221132, rs2466058, rs2466049, and rs2976526 each have less than five minor allele homozygotes, the pattern for these genotypes should not be interpreted. SNPs rs1354336 and rs1462893 have between 12 and 16 minor allele homozygotes, thus these specific genotypes should be interpreted with caution. As can be seen across all graphs, the interaction patterns in this domain vary widely by marker. In some cases, the minor allele confers an advantage (rs1462893, rs726908, and rs2439272), while it confers a detriment in others (SNP8NRG221132, SNP8NRG241930, rs1354336, rs776401, rs1473438, rs6468121, rs2466058, rs2466049, and rs2976525), and little impact for rs3924999. The age pattern varied widely. Some SNP-performance patterns showed only one point of divergence, while others had multiple ages of divergence across the lifespan. Among SNPs where the minor allele conferred an advantage, the age of divergence ranged from 25 to 50 years; while among SNPs where the minor allele was disadvantageous, the age of divergence ranged from 30 to 70 years.

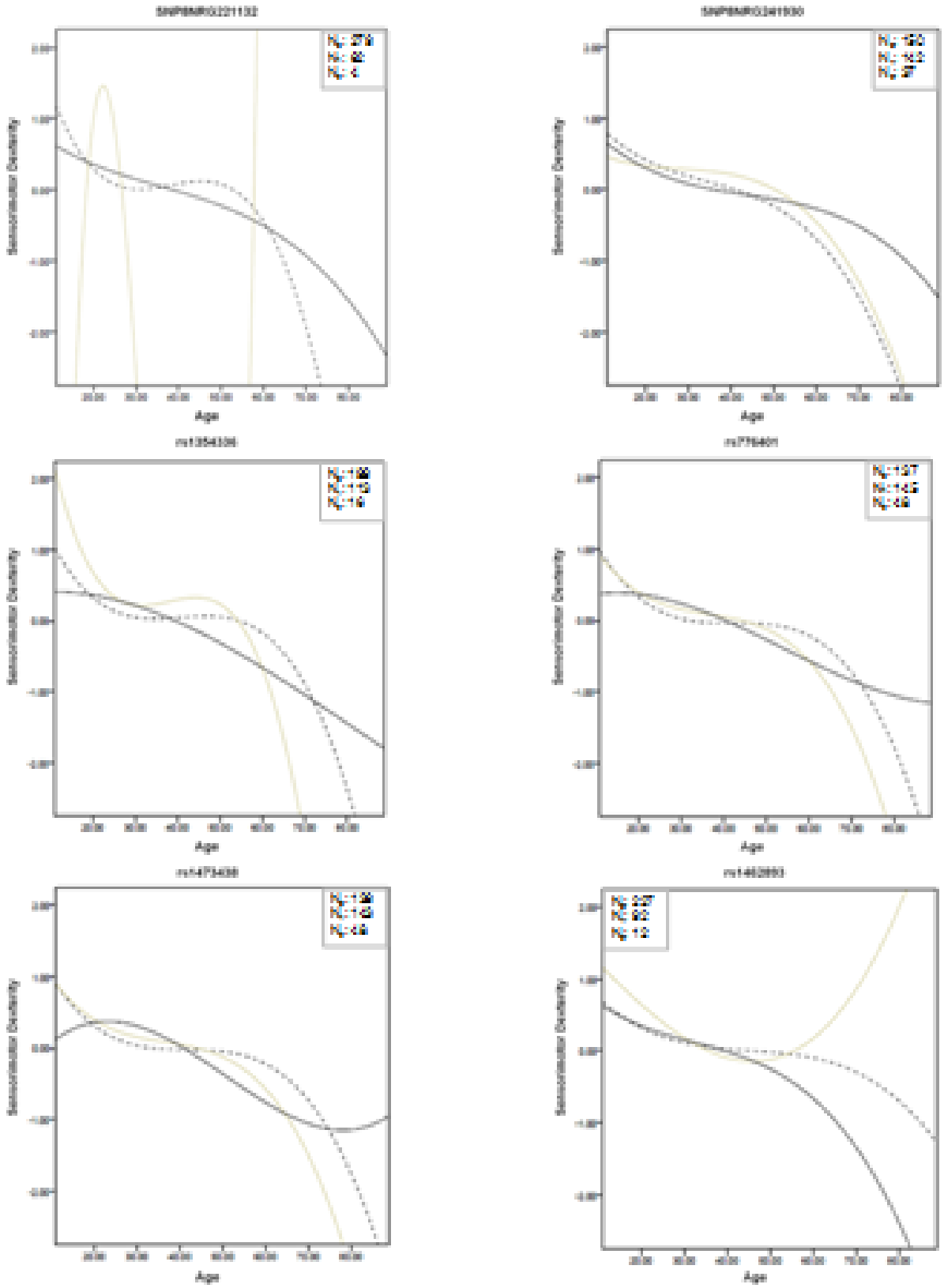


Figure 11. Sensorimotor Dexterity (Non-target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

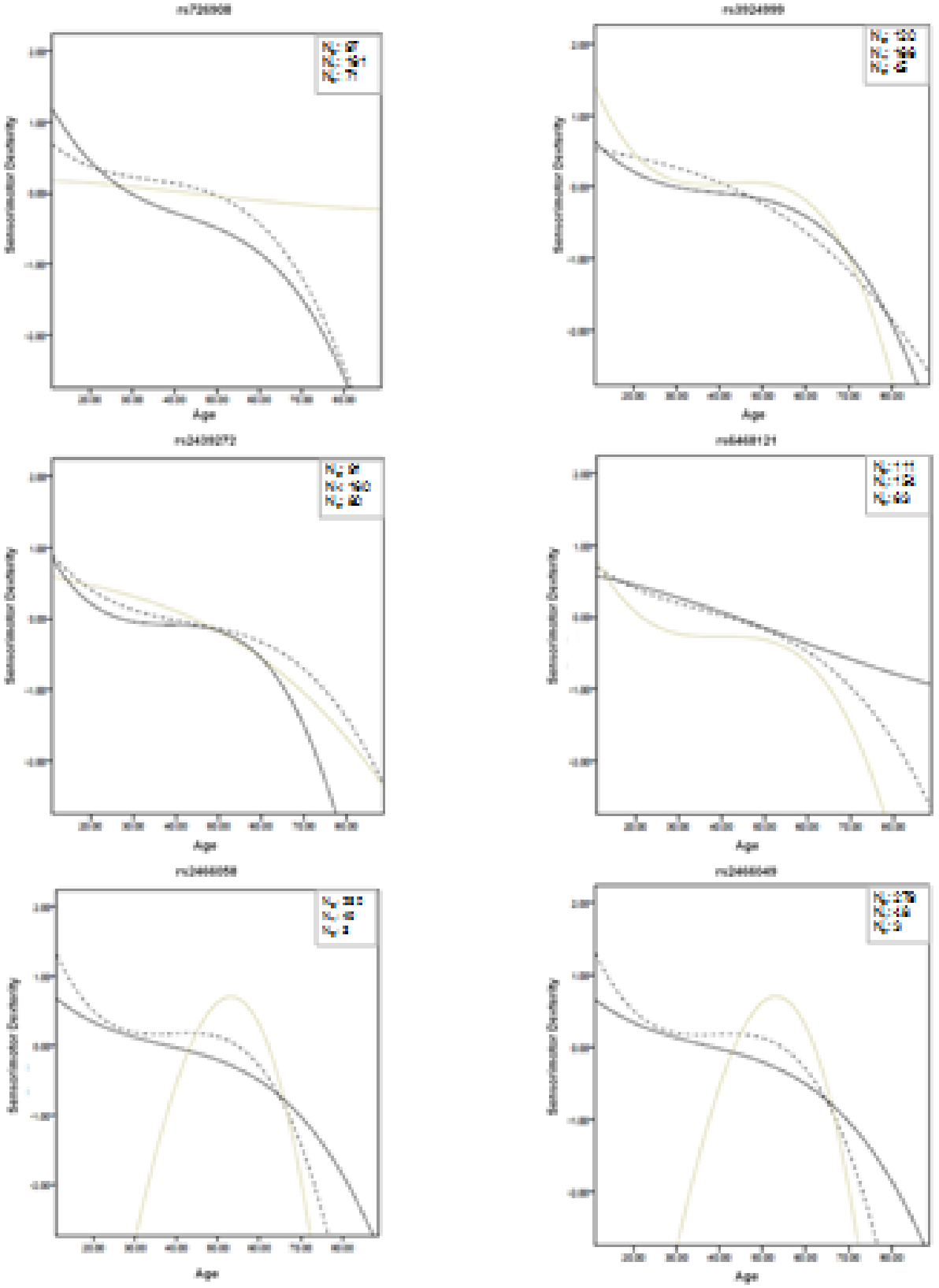


Figure 11 (continued). Sensorimotor Dexterity (Non-target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

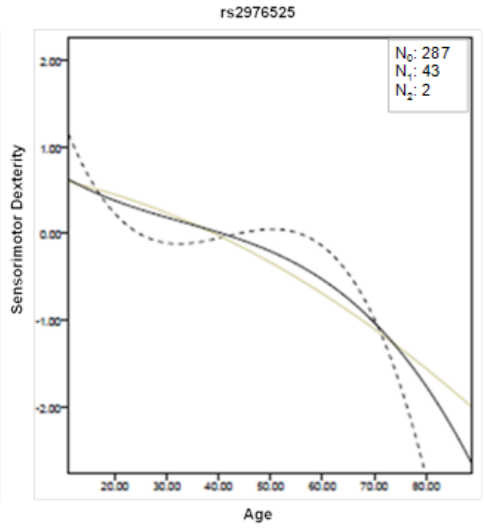


Figure 11 (continued). Sensorimotor Dexterity (Non-target): Graphs of the interactions between SNP minor allele dose and age in predicting cognitive performance.

3.4.4.3 Correction for Multiple Comparisons. The use of Bonferroni correction, and other similar methods, to control experiment-wise alpha error is overly conservative when tests are performed on correlated variables. In the present study, the SNPs are correlated with one another (through linkage disequilibrium), as are the cognitive domains. In order to more accurately control experiment-wise alpha error, the P-values Adjusted for Correlated Tests (pACT) program (Conneely & Boehnke, 2007), which takes into account the intercorrelations among both the cognitive domains and the SNP set, was used. This program was not designed to be used with interaction data, thus the trait values were residualized on sex, age, and the mean SNP effect for each participant and domain. Given the SNP effects are small, this probably introduced little or no bias. In addition, only the linear component of the SNP x age interaction could be tested, without the quadratic and/or cubic components. To our knowledge, no other program exists that can account for the intercorrelations between both independent and dependent variables when interactional data is used.

Two pACT analyses were performed: one for the target SNPs and target domains, and the other on all SNPs and all domains. Based on the observed correlational structure among both the target SNPs and target cognitive domains, pACT estimated that the 95 (19 SNPs * 5 domains) correlated tests performed was equivalent to 49 independent tests. Thus, the significance level needed to yield an experiment-wise alpha error of .05 was .001 (.05/49) for the target-target analyses. At this level of significance, none of the nominally significant interactions remained significant at the level of the linear component.

For the analysis including all SNPs and all domains, pACT estimated that the 288 (36 SNPs * 8 domains) correlated tests was equivalent to 156 independent tests. The significance level needed to yield an experiment-wise alpha error of .05 was .0004 (.05/156) for the overall analyses. At this level of significance, none of the nominally significant interactions remain significant for the linear component. However, there are multiple highly significant -2LL model differences for the sensorimotor domain that exceed this threshold. Specifically, the interactions for SNP8NRG221132, rs776401, and rs1473438 remain significant after correction. In all three of these cases, the significance of the model change difference is driven by nominally significant linear and cubic components. Although the pACT was not able to be performed on the -2LL model data, the pACT results from the linear interaction provide a guideline for estimating the significance threshold.

3.4.5 Sensitivity Analyses: Re-estimation of Models with Diagnosis and IQ as Covariates

To better understand the previous results and assess the importance of characteristics that might be causally related to cognitive function in this sample, the models were re-estimated with diagnosis and intelligence as covariates. Because schizophrenia-spectrum diagnoses have been related to mild-to-moderate impairments in cognition, and because intelligence level is closely

related to cognitive function, spectrum diagnosis (i.e., schizoaffective disorder-bipolar type, bipolar disorder I and II, MDD with psychotic features, other organic or nonorganic psychosis, and cluster A personality disorder) versus other psychopathology plus no diagnosis group status and age-standardized WRAT scores were included as covariates in sensitivity analyses of the previously nominally significant interaction findings. This step provided the ability to gauge whether the previous significant findings might be the result of diagnostic and intelligence-related variance that is causally related to cognitive function, allowing a better understanding of the relationships between these characteristics and test the robustness of our previous results.

These analyses were conducted using the stage 3 residuals, thus stages 1-3 of the previous models did not change. Spectrum diagnosis and WRAT were entered as covariates for only those SNP and domain combinations in which one or more components (linear, quadratic, and/or cubic) or the overall -2LL model difference was nominally significant. As can be seen in Tables 28 (target domains) and 29 (non-target domains), the spectrum variable was only a significant covariate for eight of nine SNPs in the Emotional Processing task and not significant for any other domain. This is consistent with the previous analyses (Tables 12 and 13) that showed that the performance of the spectrum group was not always significantly different when compared to the no diagnosis and/or other psychopathology groups. The effects of WRAT as a covariate, however, were highly significant for nearly every SNP-domain analysis, which is consistent with the significant Pearson correlations (Table 14) between WRAT and domain performance within this sample.

Table 28. Target Domains: Significance of spectrum diagnoses and IQ as covariates for previously significant interaction models

	Abstraction & Mental Flexibility			Attention			Spatial Processing			Emotional Processing		
Marker	*Spectrum*	*WRAT*	*Total Proportion of Variance Explained*	*Spectrum*	*WRAT*	*Total Proportion of Variance Explained*	*Spectrum*	*WRAT*	*Total Proportion of Variance Explained*	*Spectrum*	*WRAT*	*Total Proportion of Variance Explained*
SNP8NRG221132												
SNP8NRG221533/rs35753505	0.1842	**1.587×10^{-10}**	0.1370							**0.0143**	**4.235×10^{-12}**	0.1667
rs4298458										**0.0161**	**3.869×10^{-12}**	0.1643
SNP8NRG241930												
rs1081062												
rs4566990				0.4035	**1.507×10^{-8}**	0.0513				**0.0087**	**2.054×10^{-12}**	0.1776
rs1354335												
rs1354336												
rs1354334										**0.0092**	**1.825×10^{-12}**	0.1766
SNP8NRG444511/rs13268724												
rs776401										0.0736	**1.757×10^{-12}**	0.1584
rs1473438							0.4540	**3.928×10^{-18}**	0.2541	**0.0061**	**3.316×10^{-12}**	0.1745
rs1462893												
rs10954821										**0.0144**	**3.497×10^{-12}**	0.1694
rs726908	0.0921	**3.818×10^{-10}**	0.1316									
rs10954855												
rs2439306												
rs2466062												
rs3924999	0.2324	**1.267×10^{-10}**	0.1355									
rs2466060												
rs2439272				0.4313	**2.258×10^{-8}**	0.0470						
rs6468121				0.6778	**3.573×10^{-7}**	0.0308						
rs2466058	0.1953	**1.543×10^{-10}**	0.1337									
rs2466049	0.1951	**1.668×10^{-10}**	0.1335									
rs723811										**0.0148**	**4.395×10^{-12}**	0.1612
rs6988339				0.4765	**6.893×10^{-8}**	0.0399						
rs2975498												
rs2919382												
rs2976525												
rs4262285										**0.0130**	**3.612×10^{-12}**	0.1667
rs3735776												
rs4512342												
rs10503929												
rs6992642												
rs3735781												
rs3735782				0.3693	**2.484×10^{-8}**	0.0279						

Note. Unadjusted p-values are reported and nominally significant ($p<0.05$) values are bolded. Covariate estimation w as only performed for SNP-domain combinations that had significant interactions previously. Shaded cells indicate target SNPs-target cognitive domain estimations. Proportion of variance explained incorporates both the spectrum and WRAT contributions. Spectrum: schizoaffective disorder-bipolar type, bipolar disorder I & II, MDD w ith psychotic features, other organic or nonorganic psychosis, and cluster A personality disorder; WRAT: Wide Range Achievement Test, reading subtest.

Table 29. Non-Target Domains: Significance of spectrum diagnoses and IQ as covariates for previously significant interaction r

	Spatial Memory			Sensorimotor Dexterity		
Marker	*Spectrum*	*WRAT*	*Total Proportion of Variance Explained*	*Spectrum*	*WRAT*	*Total Proportion of Variance Explained*
SNP8NRG221132				0.7704	**3.890×10^{-6}**	0.0924
SNP8NRG221533/rs35753505						
rs4298458						
SNP8NRG241930				0.8046	**3.550×10^{-6}**	0.0976
rs1081062						
rs4566990	0.6068	**0.0350**	0.0194			
rs1354335						
rs1354336				0.5716	**7.966×10^{-6}**	0.0712
rs1354334						
SNP8NRG444511/rs13268724						
rs776401				0.7600	**1.215×10^{-6}**	0.0897
rs1473438				0.5704	**4.039×10^{-6}**	0.0950
rs1462893				0.7413	**0.00002**	0.0765
rs10954821						
rs726908				0.6148	**4.687×10^{-6}**	0.0932
rs10954855						
rs2439306						
rs2466062						
rs3924999				0.5223	**3.485×10^{-6}**	0.0920
rs2466060						
rs2439272				0.7341	**1.846×10^{-6}**	0.0979
rs6468121				0.4828	**8.533×10^{-7}**	0.1131
rs2466058				0.8022	**3.941×10^{-6}**	0.0969
rs2466049				0.7845	**3.399×10^{-6}**	0.0978
rs723811						
rs6988339						
rs2975498						
rs2919382						
rs2976525				0.7718	**3.902×10^{-6}**	0.0925
rs4262285						
rs3735776						
rs4512342						
rs10503929						
rs6992642						
rs3735781						
rs3735782						

Note. Unadjusted p-values are reported and nominally significant (p<0.05) values are bolded. Covariate estimation was only performed for SNP-domain combinations that had significant interactions previously. Proportion of variance explained incorporates both the spectrum and WRAT contributions. Spectrum: schizoaffective disorder-bipolar type, bipolar disorder I & II, MDD with psychotic features, other organic or nonorganic psychosis, and cluster A personality disorder; WRAT: Wide Range Achievement Test, reading subtest.

3.4.5.1 SNP x Age Interactions in Target Domains with Spectrum and WRAT Covaried.

Overall, there were numerous changes in the significance levels of interactions that had been previously nominally significant at the level of the linear, quadratic, or cubic components and/or

-2LL model difference in the target cognitive domains. These results can be compared to the previous findings in Tables 20-23.

At the level of the -2LL model difference, as seen in Tables 30-33, there were 11 changes in significance in the target domains, eight of which were interactions that were previously nominally significant but became non-significant when spectrum and WRAT were added into the model. Three previously non-significant interactions became nominally significant with these covariates, one in the domain of Abstraction/Mental Flexibility and two in the Attention domain. At the level of the linear component, nine changes in significance were found, eight of which became non-significant, and one, in the Emotional Processing domain, became nominally significant. At the level of the quadratic component, seven changes were detected, all involving previously nominally significant interactions that became non-significant. In general, the proportion of variance explained by the interaction components when these covariates were included in the model was reduced compared to the previous analyses.

Table 30. Abstraction and Mental Flexibility (Target): Model comparisons and estimation of specific components of the SNP x age interaction for previously significant findings when spectrum diagnosis and IQ are included as covariates

	Total *NRG1* x Age Interaction Effect			Interaction Components		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132						
SNP8NRG221533/rs35753505	-141.9276	-138.9148	**6.0256 (0.049) [0.032]**	**0.0146 [0.0089]**	0.6875	0.018
rs4298458						
SNP8NRG241930						
rs1081062						
rs4566990						
rs1354335						
rs1354336						
rs1354334						
SNP8NRG444511/rs13268724						
rs776401						
rs1473438						
rs1462893						
rs10954821						
rs726908	-141.7220	-138.4714	**6.5011 (0.038) [ns]**	**0.0112 [0.0469]**	0.6353	0.020
rs10954855						
rs2439306						
rs2466062						
rs3924999	-142.4214	-141.0236	2.7957	0.1173	0.4905	0.009
rs2466060						
rs2439272						
rs6468121						
rs2466058	-142.5702	-140.7777	3.5850	0.0623	0.8217	0.011
rs2466049	-142.5981	-140.7472	3.7018	0.0587	0.8127	0.011
rs723811						
rs6988339						
rs2975498						
rs2919382						
rs2976525						
rs4262285						
rs3735776						
rs4512342						
rs10503929						
rs6992642						
rs3735781						
rs3735782						

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, each individual SNP, and the main effects of spectrum diagnostic status and IQ. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded. P-values for the previous model are provided in square brackets ([ ]) for currently significant effects. ns: non-significant.

Table 31. Attention (Target): Model comparisons and estimation of specific components of the SNP x age interaction for previously significant findings when spectrum diagnosis and IQ are included as covariates

	Total *NRG1* x Age Interaction Effect			**Interaction Components**		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132						
SNP8NRG221533/rs35753505						
rs4298458						
SNP8NRG241930						
rs1081062						
rs4566990	-318.3273	-317.1517	2.3512	0.1323	0.6667	0.031
rs1354335						
rs1354336						
rs1354334						
SNP8NRG444511/rs13268724						
rs776401						
rs1473438						
rs1462893						
rs10954821						
rs726908						
rs10954855						
rs2439306						
rs2466062						
rs3924999						
rs2466060						
rs2439272	-318.4292	-316.8517	3.1550	0.1344	0.2900	0.011
rs6468121	-311.5479	-309.8980	3.2998	0.0694	0.9428	0.015
rs2466058						
rs2466049						
rs723811						
rs6988339	-319.5867	-315.5571	**8.0592 (0.018) [ns]**	**0.0136 [0.0431]**	0.1038	0.031
rs2975498						
rs2919382						
rs2976525						
rs4262285						
rs3735776						
rs4512342						
rs10503929						
rs6992642						
rs3735781						
rs3735782	-315.0592	-310.3720	**9.3744 (0.009) [ns]**	**0.0050 [0.0535]**	0.0954	0.043

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, each individual SNP, and the main effects of spectrum diagnostic status and IQ. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded. P-values for the previous model are provided in square brackets ([ ]) for currently significant effects. ns: non-significant.

Table 32. Spatial Processing (Target): Model comparisons and estimation of specific components of the SNP x age interaction for previously significant findings when spectrum diagnosis and IQ are included as covariates

	Total *NRG1* x Age Interaction Effect			Interaction Components		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132						
SNP8NRG221533/rs35753505						
rs4298458						
SNP8NRG241930						
rs1081062						
rs4566990						
rs1354335						
rs1354336						
rs1354334						
SNP8NRG444511/rs13268724						
rs776401						
rs1473438	-112.4051	-111.9380	0.9342	0.3955	0.5989	0.004
rs1462893						
rs10954821						
rs726908						
rs10954855						
rs2439306						
rs2466062						
rs3924999						
rs2466060						
rs2439272						
rs6468121						
rs2466058						
rs2466049						
rs723811						
rs6988339						
rs2975498						
rs2919382						
rs2976525						
rs4262285						
rs3735776						
rs4512342						
rs10503929						
rs6992642						
rs3735781						
rs3735782						

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, each individual SNP, and the main effects of spectrum diagnostic status and IQ. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded. P-values for the previous model are provided in square brackets ([ ]) for currently significant effects.

Table 33. Emotional Processing (Target): Model comparisons and estimation of specific components of the SNP x age interaction for previously significant findings when spectrum diagnosis and IQ are included as covariates

	Total *NRG1* x Age Interaction Effect			Interaction Components		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132						
SNP8NRG221533/rs35753505	-235.0025	-233.0888	3.8274	0.0611	0.8665	0.010
rs4298458	-237.2113	-236.1266	2.1694	0.1394	0.9384	0.007
SNP8NRG241930						
rs1081062						
rs4566990	-235.2268	-234.7914	0.8708	0.6933	0.3959	*
rs1354335						
rs1354336						
rs1354334	-235.1049	-234.8154	0.5790	0.8419	0.4645	*
SNP8NRG444511/rs13268724						
rs776401	-231.1943	-229.8751	2.6384	0.1021	0.1909	0.012
rs1473438	-235.1721	-233.2794	3.7854	**0.0439 [ns]**	0.2606	0.015
rs1462893						
rs10954821	-236.8999	-236.1654	1.4689	0.4247	0.3864	0.004
rs726908						
rs10954855						
rs2439306						
rs2466062						
rs3924999						
rs2466060						
rs2439272						
rs6468121						
rs2466058						
rs2466049						
rs723811	-237.9115	-237.4147	0.9936	0.3479	0.6341	0.001
rs6988339						
rs2975498						
rs2919382						
rs2976525						
rs4262285	-237.5613	-235.6574	3.8078	0.0880	0.4212	0.011
rs3735776						
rs4512342						
rs10503929						
rs6992642						
rs3735781						
rs3735782						

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2, each individual SNP, and the main effects of spectrum diagnostic status and IQ. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded. P-values for the previous model are provided in square brackets ([ ]) for currently significant effects. ns: non-significant.

3.4.5.2 SNP x Age Interactions in Non-target Domains with Spectrum and WRAT Covaried. There were also numerous changes in the significance levels of interactions that had been previously nominally significant in the non-target cognitive domains. These results can be directly compared to the previous findings in Tables 26 and 27.

At the level of the -2LL model difference, as seen in Tables 34-35, there were eight changes in significance, all of which became non-significant with the addition of spectrum and WRAT as covariates. At the level of the linear component, five changes in significance were found, all of which became non-significant. There were zero changes at the level of the quadratic component. At the level of the cubic component (Sensorimotor Dexterity), five changes were detected, all involving previously nominally significant interactions that became non-significant. Overall, the proportion of variance explained by the interaction components when these covariates were included in the model was mixed; sometimes including these covariates increased the variance explained and sometimes it decreased it.

Table 34. Spatial Memory (Non-Target): Model comparisons and estimation of specific components of the SNP x age interaction for previously significant findings when spectrum diagnosis and IQ are included as covariates

	Spatial Memory	
Marker	*Linear Component p-values*	*Proportion of Variance*
SNP8NRG221132		
SNP8NRG221533/rs35753505		
rs4298458		
SNP8NRG241930		
rs1081062		
rs4566990	0.0861	0.004
rs1354335		
rs1354336		
rs1354334		
SNP8NRG444511/rs13268724		
rs776401		
rs1473438		
rs1462893		
rs10954821		
rs726908		
rs10954855		
rs2439306		
rs2466062		
rs3924999		
rs2466060		
rs2439272		
rs6468121		
rs2466058		
rs2466049		
rs723811		
rs6988339		
rs2975498		
rs2919382		
rs2976525		
rs4262285		
rs3735776		
rs4512342		
rs10503929		
rs6992642		
rs3735781		
rs3735782		

Note. Nominally significant ($p<0.05$) difference values are bolded. P-values for the previous model are provided in square brackets ([ ]) for currently significant effects.

Table 35. Sensorimotor Dexterity (Non-Target): Model comparisons and estimation of specific components of the SNP x age interaction for previously significant findings when spectrum diagnosis and IQ are included as covariates

	Total *NRG1* x Age Interaction Effect			Interaction Components			
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Cubic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132	-914.9657	-914.2265	1.4784	0.9283	0.2950	0.7905	0.0027
SNP8NRG221533/rs35753505							
rs4298458							
SNP8NRG241930	-906.8233	-906.5339	0.5788	0.6810	0.5475	0.6455	*
rs1081062							
rs4566990							
rs1354335							
rs1354336	-903.0893	-901.5359	3.1068	0.1864	0.7483	0.0909	0.026
rs1354334							
SNP8NRG444511/rs13268724							
rs776401	-885.0334	-880.4461	**9.1746 (0.027) [0.000000]**	0.1008	0.9367	**0.01126 [0.00006]**	0.049
rs1473438	-912.0624	-906.9998	**10.1252 (0.018) [0.00003]**	0.0805	0.6730	**0.00843 [0.0002]**	0.070
rs1462893	-912.5865	-909.6540	5.8650	0.7864	0.3353	0.4761	0.048
rs10954821							
rs726908	-911.7173	-905.3300	**12.7746 (0.005) [0.001]**	0.9185	0.7048	0.1659	0.029
rs10954855							
rs2439306							
rs2466062							
rs3924999	-912.8120	-909.4497	6.7246	0.4313	0.4029	0.8453	0.075
rs2466060							
rs2439272	-912.4454	-911.4444	2.0020	0.4633	0.3376	0.3481	*
rs6468121	-907.0537	-906.0711	1.9652	0.8639	0.2348	0.6323	*
rs2466058	-914.5545	-912.2974	4.5142	0.1830	0.2191	0.0759	0.005
rs2466049	-912.1083	-910.0887	4.0392	0.2416	0.2440	0.0972	0.003
rs723811							
rs6988339							
rs2975498							
rs2919382							
rs2976525	-914.9656	-913.3722	3.1868	0.6514	0.2820	0.1834	0.010
rs4262285							
rs3735776							
rs4512342							
rs10503929							
rs6992642							
rs3735781							
rs3735782							

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age, age^2, and age^3, each individual SNP, and the main effects of spectrum diagnostic status and IQ. Model 2 provides the same elements as Model 1 plus SNP x age, SNP x age^2, and SNP x age^3. -2LL Difference is distributed as a chi-square function (critical values: 7.82, df=3, p<0.05). Nominally significant (p<0.05) difference values are bolded. P-values for the previous model are provided in square brackets ([ ]) for currently significant effects.

3.4.5.3 Summary. Overall, there were a number of previously nominally significant interactions that became non-significant when spectrum diagnosis and WRAT were included in the models, as well as a few that became nominally significant. This suggests that some of the variance in WRAT scores accounts for the previous nominally significant findings across multiple domains, while the variance in spectrum diagnosis only accounts for some findings in Emotional Processing. When this variation is removed through covarying, the interactions are no longer nominally significant.

3.4.6 Exploratory Analyses: Estimation of Sex, Age, SNP, and Interaction Effects on the WRAT

Given the highly significant effect of the WRAT as a covariate in the previous analyses and the fact that it generally reduced the significance of previously nominally significant SNP x age interactions on cognitive performance, the raw (non-age-standardized) WRAT scores were examined as a trait of interest. Before proceeding with the multi-stage model, the genetic correlation and heritability of WRAT performance were estimated. As done previously with the cognitive domains, R_g was estimated in the combined affected and unaffected sample with age and sex screened as covariates. R_g was estimated to be -0.29 with sex as a covariate (age was not significant and omitted from the estimation) and was significantly different from both zero ($p=0.043$) and one ($p=7.5 \times 10^{-16}$). WRAT scores were also highly heritable in the unaffected sample: $h^2 = 0.69$ ($p=1.6 \times 10^{-13}$).

In stage 1 of the multi-stage model, the main effect of sex on the WRAT raw score was estimated, finding a trend towards significance ($p=0.092$) where females perform slightly better than males. As done with the cognitive domains, linear and curvilinear regression was performed in SPSS to investigate the relationship between WRAT performance and age using the residuals from the sex effects analysis. This analysis revealed a general lack of age effects on WRAT scores, with no significant R-square change values for linear, quadratic, and/or cubic models. This WRAT performance across age is presented in Figure 12. Using SOLAR, the main effect of the linear age component was also estimated and found to be non-significant ($p=0.208$). Importantly, the lack of age effects on WRAT scores also suggests that there are not potentially problematic cohort effects on WRAT performance, and perhaps other cognitive domains, in this sample.

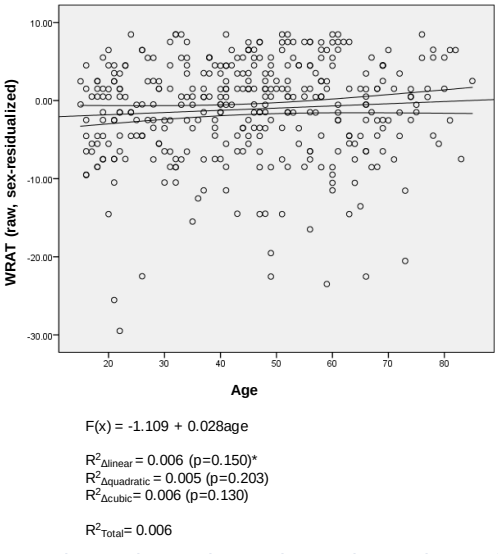


Figure 12. Graph of the relationship between age and WRAT performance

The main effects of individual SNPs on WRAT score were estimated in SOLAR using the residuals from stage 2. Table 36 presents the unadjusted significance levels and proportion of variance explained by individual SNPs. There were no significant main effects of SNPs on WRAT performance.

The linear interactions between age and WRAT performance were estimated using the residuals from the stage 3 analysis. Table 36 also presents the unadjusted significance levels and proportion of variance explained by individual SNPs. Overall, there were very few significant interactions. Only rs2992642 and rs3735781 showed nominally significant interactions with age in predicting WRAT scores, and neither was significant after correction for multiple comparisons using a pACT-modified Bonferroni correction ($p<.0024$).

Table 36. Stages 3 and 4: Estimation of the main effects of individual SNPs and SNP x age interactions in the prediction of WRAT scores

	Stage 3: SNP Effects		Stage 4: SNP x Age Interactions	
Marker	*p-value*	*Proportion of Variance*	*Linear Component p-value*	*Proportion of Variance*
SNP8NRG221132	0.5190		0.8407	
SNP8NRG221533/rs35753505	0.6009		0.5928	
rs4298458	0.6707		0.5217	
SNP8NRG241930	0.2153		0.1348	
rs1081062	0.9286		0.4392	
rs4566990	0.3675		0.8325	
rs1354335	0.1208		0.2037	
rs1354336	0.6187		0.3872	
rs1354334	0.2702		0.8123	
SNP8NRG444511/rs13268724	0.1592		0.2219	
rs776401	0.6947		0.9090	
rs1473438	0.7487		0.7732	
rs1462893	0.2063		0.3988	
rs10954821	0.8147		0.3545	
rs726908	0.3198		0.7520	
rs10954855	0.2821		0.4276	
rs2439306	0.7855		0.6280	
rs2466062	0.2295		0.2335	
rs3924999	0.3150		0.8060	
rs2466060	0.4366		0.9289	
rs2439272	0.4623		0.7004	
rs6468121	0.3113		0.6431	
rs2466058	0.8159		0.8057	
rs2466049	0.7587		0.7531	
rs723811	0.5179		0.7631	
rs6988339	0.2687		0.7486	
rs2975498	0.4466		0.8304	
rs2919382	0.3426		0.7511	
rs2976525	0.3228		0.8272	
rs4262285	0.3104		0.8631	
rs3735776	0.7002		0.2196	
rs4512342	0.4839		0.4420	
rs10503929	0.8146		0.5172	
rs6992642	0.7920		**0.0140**	0.0340
rs3735781	0.7208		**0.0374**	0.0269
rs3735782	0.9595		0.1000	

Note. Unadjusted p-values are reported. Proportion of variance is provided when the effect is significant (p<0.05). Nominally significant (p<0.05) values are bolded.

The models for these two SNPs were re-estimated including spectrum as a covariate. There were no significant main effects of spectrum for either of these SNPs on WRAT performance, and both SNP x age interactions remained significant: rs6992642 (p=0.013,

explaining 3.4% of the variation in WRAT score) and rs3735781 (p=0.037, explaining 2.7% of variation).

3.4.7 Exploratory Analyses: Estimation of Sex, Age, SNP, and Interaction Effects on CNB Factor Scores

Given that the eight cognitive domains included in the computerized neurocognitive battery (CNB) used in this study are theoretically intercorrelated, this common variation was examined. Pearson correlations were calculated and exploratory factor analysis was used to determine whether the variation in these eight observed variables reflected fewer unobserved variables. Both the correlations and the factor analysis were performed on the sex- and age-residualized cognitive scores. As can be seen in Table 37, the domains are moderately to highly correlated with each other, as hypothesized. Every domain-domain correlation was significant, with Pearson coefficients ranging from 0.205 (Attention and Spatial Memory) to 0.513 (Emotional Processing and Spatial Processing).

Table 37. Pearson correlations and p-values among the cognitive domains

	Abstraction & Mental Flexibility	Attention	Spatial Processing	Emotional Processing	Verbal Memory	Facial Memory	Spatial Memory	Sensorimotor Dexterity
Abstraction & Mental Flexibility		0.357	0.452	0.370	0.245	0.313	0.240	0.240
Attention	0.000		0.332	0.453	0.284	0.336	0.205	0.371
Spatial Processing	0.000	0.000		0.513	0.365	0.420	0.306	0.279
Emotional Processing	0.000	0.000	0.000		0.338	0.483	0.210	0.386
Verbal Memory	0.000	0.000	0.000	0.000		0.402	0.303	0.236
Facial Memory	0.000	0.000	0.000	0.000	0.000		0.358	0.303
Spatial Memory	0.000	0.000	0.000	0.000	0.000	0.000		0.219
Sensorimotor Dexterity	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Note. Pearson correlation values are presented above the diagonal with p-values presented below the shaded diagonal.

The significant correlations between all domains suggested reasonable factorability of the data. In addition, the Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.855 (above the suggested threshold of 0.60) and Bartlett's Test of Sphericity was significant ($\chi^2(28) = 585.746$,

p = 0.000). The diagonal values of the anti-image correlation matrix, which are the Measures of Sampling Adequacy, were all above 0.826, supporting the inclusion of each domain in the analysis. Finally, the communalities were all above 0.30, except for Spatial Memory (0.268), as shown in Table 38, further confirming that each domain shared common variance with other domains. Given these overall indicators, an exploratory factor analysis was conducted with all eight cognitive domains.

Principal components analysis extracted one factor. Initial eigenvalues showed that this factor explained 42.06% of the total variance among the eight domains. Given that only one factor was extracted, no rotation was possible. The factor loading matrix for this solution is presented in Table 38. Overall, this analysis indicated that there was one distinct factor underlying the eight cognitive domains. The strength of factor loadings were (in order from highest to lowest): Emotional Processing, Facial Memory, Attention, Abstraction/Mental Flexibility, Verbal Memory, Sensorimotor Dexterity, Spatial Memory, and Spatial Processing.

Table 38. Factor loadings and commonalities based on a principal components analysis with no rotation

	CNB Factor	Communality
Abstraction & Mental Flexibility	0.622	0.386
Attention	0.648	0.420
Spatial Processing	0.272	0.528
Emotional Processing	0.750	0.562
Verbal Memory	0.606	0.367
Facial Memory	0.711	0.506
Spatial Memory	0.518	0.268
Sensorimotor Dexterity	0.572	0.327

Note. CNB: computerized neurocognitive battery.

Factor scores for analyses were calculated from this exploratory factor analysis using the regression method. Although the domain scores used in the factor analysis were based on the sex- and age-residualized variables, the factor score was re-residualized for both sex and age to

eliminate any potential sex or age effects that might have remained in the composite variable. R_g was estimated in the combined affected and unaffected sample; however, age and sex could not be used as covariates due to repeated convergence failure. R_g was estimated to be -0.16 and was not significantly different from zero (p=0.553), but was significantly different from one (p=0.00001). The heritability of the factor score was highly significant: $h^2 = 0.52$ ($p=4.0 \times 10^{-7}$). This factor was also moderately and significantly correlated with the age-standardized WRAT score (r=0.49, p=0.000).

In stage 1 of the multi-stage model, the main effect of sex on the factor score was estimated and found to be non-significant (p=0.773). As in previous analyses, linear and curvilinear regression was performed in SPSS to investigate the relationship between factor score and age using the residuals from the sex effects analysis. This analysis revealed a significant quadratic relationship between age and factor score where the age of slope change was 47.42 years, as seen in Figure 13. Quadratic effects were therefore included in all subsequent analyses. The main effects of age were also estimated in SOLAR in stage 2, and as expected, the linear component was not significant (p=0.198), while the quadratic component was ($p=5.099 \times 10^{-9}$). Together, the linear and quadratic components explained 9.39% of the variation in factor score.

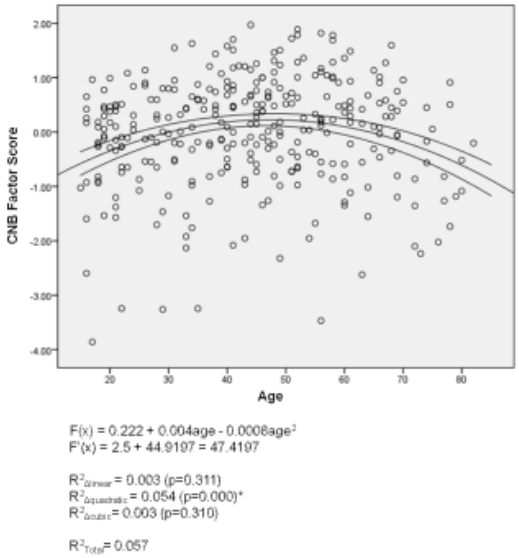


Figure 13. Graph of the relationship between age and factor score

The main effects of individual SNPs on factor score were estimated in SOLAR using the residuals from stage 2. Table 39 presents the unadjusted significance levels and proportion of variance explained by individual SNPs. There were no significant main effects of SNPs on factor score.

Table 39. Estimation of the main effects of individual SNPs on factor score

Marker	*p-value*	*Proportion of Variance*
SNP8NRG221132	0.1011	
SNP8NRG221533/rs35753505	0.5838	
rs4298458	0.3566	
SNP8NRG241930	0.3655	
rs1081062	0.7033	
rs4566990	0.2163	
rs1354335	0.8175	
rs1354336	0.2203	
rs1354334	0.5139	
SNP8NRG444511/rs13268724	0.8714	
rs776401	0.6486	
rs1473438	0.6341	
rs1462893	0.1149	
rs10954821	0.5108	
rs726908	0.3123	
rs10954855	0.1920	
rs2439306	0.3552	
rs2466062	0.1626	
rs3924999	0.3171	
rs2466060	0.6407	
rs2439272	0.6262	
rs6468121	0.4108	
rs2466058	0.9162	
rs2466049	0.9023	
rs723811	0.8796	
rs6988339	0.5265	
rs2975498	0.5483	
rs2919382	0.6015	
rs2976525	0.6665	
rs4262285	0.4442	
rs3735776	0.1759	
rs4512342	0.5879	
rs10503929	0.2469	
rs6992642	0.8196	
rs3735781	0.7654	
rs3735782	0.8422	

Note. Unadjusted p-values are reported. Proportion of variance is provided when the effect is significant (p<0.05). Nominally significant (p<0.05) values are bolded.

The linear and quadratic interactions between age and factor score, as well as the -2LL model differences, were estimated using the residuals from the stage 3 analysis, as seen in Table 40.

Table 40. Factor Score: Model comparisons and estimation of specific components of the SNP x age interaction in predicting factor score

	Total *NRG1* x Age Interaction Effect			Interaction Components		
Marker	*Model1*	*Model2*	*-2LL Difference*	*Linear Component p-values*	*Quadratic Component p-values*	*Total Proportion of Variance Explained*
SNP8NRG221132	-229.0379	-228.4698	1.1361	0.8464	0.2865	
SNP8NRG221533/rs35753505	-229.3833	-228.4739	1.8188	0.3467	0.5292	
rs4298458	-229.9569	-229.5163	0.8812	0.7762	0.4132	
SNP8NRG241930	-226.0461	-224.8021	2.4880	0.2390	0.3391	
rs1081062	-229.5804	-229.2126	0.7356	0.5094	0.6052	
rs4566990	-229.6176	-228.2384	2.7585	0.1555	0.2854	
rs1354335	-225.2893	-225.2757	0.0273	0.9318	0.8837	
rs1354336	-227.3073	-227.2345	0.1454	0.7041	0.9736	
rs1354334	-230.1687	-229.2724	1.7927	0.4764	0.2158	
SNP8NRG444511/rs13268724	-230.3687	-230.2422	0.2531	0.7314	0.7023	
rs776401	-228.9099	-228.0193	1.7813	0.6239	0.2423	
rs1473438	-228.7369	-228.0522	1.3693	0.7588	0.2763	
rs1462893	-229.0634	-227.3030	3.5207	0.1153	0.2279	
rs10954821	-230.1657	-229.6667	0.9979	0.6205	0.4627	
rs726908	-228.1448	-225.8964	4.4967	**0.0376**	0.6368	0.044
rs10954855	-229.5306	-229.1052	0.8507	0.9899	0.3571	
rs2439306	-224.7313	-224.6199	0.2228	0.6636	0.8329	
rs2466062	-223.4538	-223.3081	0.2914	0.8830	0.6195	
rs3924999	-228.5134	-228.2898	0.4470	0.6990	0.6518	
rs2466060	-209.1356	-208.7716	0.7281	0.4194	0.6232	
rs2439272	-230.0226	-229.3255	1.3942	0.2826	0.7777	
rs6468121	-226.3669	-225.6886	1.3566	0.2499	0.9663	
rs2466058	-230.3763	-229.4880	1.7767	0.5597	0.2231	
rs2466049	-228.9428	-228.0668	1.7521	0.5747	0.2230	
rs723811	-230.3704	-230.2575	0.2258	0.7613	0.7697	
rs6988339	-230.1813	-228.4310	3.5006	0.0620	0.8224	
rs2975498	-230.2017	-228.9092	2.5849	0.1091	0.7586	
rs2919382	-230.2454	-229.9245	0.6418	0.5033	0.6894	
rs2976525	-230.2890	-228.4704	3.6370	0.0924	0.3436	
rs4262285	-230.0892	-229.4481	1.2822	0.5749	0.9815	
rs3735776	-201.0062	-200.8983	0.2159	0.6592	0.7753	
rs4512342	-230.1590	-229.1085	2.1010	0.3695	0.3608	
rs10503929	-229.7115	-229.5995	0.2239	0.9576	0.6586	
rs6992642	-224.6785	-224.3944	0.5681	0.4537	0.9066	
rs3735781	-230.1183	-229.8272	0.5821	0.4465	0.9433	
rs3735782	-227.5358	-226.2800	2.5116	0.1316	0.5779	

Note. Total *NGR1* x age interaction effect includes linear and quadratic effects for this domain. Model 1 includes the main effects of sex, age and age^2. Model 2 provides the same elements as Model 1 plus SNP x age and SNP x age^2. -2LL Difference is distributed as a chi-square function (critical value: 5.99, df=2, p<0.05). Shaded cells indicate target SNPs-target cognitive domain estimations. Nominally significant (p<0.05) difference values are bolded.

Overall, there were no significant -2LL model differences, but there was one nominally significant interaction component: the linear component of the interaction with rs726908 explained 4.4% of the variance in factor score. This was no longer significant after correction for multiple comparisons using a pACT-modified Bonferroni correction ($p<.0032$). The model for this SNP was re-estimated including spectrum diagnosis and WRAT performance as covariates. Significant main effects of both spectrum ($p=0.017$) and WRAT score (1.63×10^{-17}) on CNB factor score were found, but the SNP x age interaction on CNB factor score was not significant at the linear ($p=0.194$) or quadratic level ($p=0.550$).

4.0 DISCUSSION

4.1 SUMMARY OF FINDINGS

The current study utilized a multi-stage analytic strategy to better understand NRG1 SNP x age interactions in the prediction of cognitive performance. A summary of each analysis is provided below.

4.1.1 Selection of Target and Non-Target Cognitive Domains

Given the *a priori* hypothesis that cognitive domains with significant genetic correlation with affected status and heritability would be more likely to show significant SNP x age interaction effects than domains without these two characteristics, cognitive domains were designated as being either "target" or "non-target." This designation allowed for a two-step correction for multiple comparisons; first, with the target domains alone and then with all domains. This procedure allowed for a multiple comparison correction that was more appropriate for the target domains, thus allowing a correction that would reduce alpha error risk while maintaining appropriate power to detect significant effects.

The target/non-target designation was based on the genetic correlation with affected status and the heritability of each domain. Genetic correlations provide an estimate of the amount of shared genetic variance that underlies the relationship between cognitive performance and schizophrenia, enabling the identification of variables that are likely important to schizophrenia's

etiology. Thus, the genetic correlation value was weighted more heavily when assessing each domain for the target/non-target designation. Heritability levels estimate the amount of variance in cognitive performance that is due to genetic variation, but are not specific to schizophrenia, thus this value was ranked second when making designations.

Based on the procedure described above, the following five domains were designated as target domains: Abstraction/Mental Flexibility, Attention, Spatial Processing, Emotional Processing, and Verbal Memory. These domains were significantly genetically correlated with schizophrenia in this sample, indicating the presence of shared genetic effects between these domains and affected status (see Table 15), and were significantly heritable, suggesting that a significant amount of variation in performance in these cognitive areas is due to genetic variation (see Table 16). In contrast, Facial Memory, Spatial Memory, and Sensorimotor Dexterity were designated as non-target domains because they were not significantly genetically correlated with schizophrenia, despite having significant heritabilities.

Taken together, these findings suggest that although a large amount of the variation in cognitive function on the present computerized neurocognitive battery is due to genetic variation, it is not all related to schizophrenia, and the amount related to affected status varies among cognitive domains. The discussion of results is organized based on this target/non-target distinction.

4.1.2 Summary of Major Findings: Target Cognitive Domains

As hypothesized, the main effects of age were significant for every target domain, with most showing a curvilinear pattern whereby cognition improved until early- to mid-adulthood and then began to decline, although Verbal Memory showed a linear decline with age (see Figure 4).

There were seven nominally significant SNP main effects across the target cognitive domains that encompassed six individual SNPs (see Table 19), three of which were target markers (SNP8NRG241930 – Verbal Memory; rs10954855 – Attention and Emotional Processing; rs3924999 – Emotional Processing) and three that were non-target (rs726908 – Emotional Processing; rs3735776 – Abstraction/Mental Flexibility; rs4512342 – Abstraction/Mental Flexibility). Spatial processing was the only target domain that had no nominally significant SNP main effects.

Overall, there were numerous nominally significant total SNP x age interaction effects and/or significant specific components of interactions across multiple domains and markers (see Tables 20-24). The target domain with the most nominally significant interactions was Emotional Processing (fifteen), followed by Abstraction/Mental Flexibility and Attention (six each). In contrast, Spatial Processing (one) and Verbal Memory (zero) had few significant interactions. Interestingly, the interactions tended to be clustered toward the 5' end of the gene for Emotional Processing, while they were spread across the gene for Abstraction/Mental Flexibility and Attention. Importantly, however, none of these interactions remained significant after correction by a modified Bonferroni procedure for the target SNPs-target domains ($p<.001$).

Overall, these findings suggest that there are nominally significant NRG1 x age interactions in the prediction of some, but not all, of the target cognitive areas. More specifically, Emotional Processing, Abstraction/Mental Flexibility, and Attention each had potentially promising findings, although none exceeded the correction for multiple comparisons. Given that these domains are significantly genetically correlated with schizophrenia and heritable, this suggests that these interactions may be informative when considering the pathogenesis of schizophrenia.

4.1.3 Summary of Major Findings: Non-target Cognitive Domains

The main effects of age were also significant for every non-target domain. Facial memory showed a quadratic relationship with age whereby cognition improved until mid-life and then began to decline, while Spatial Memory showed a linear decline with age, and Sensorimotor Dexterity had a cubic relationship with age such that performance declined until early adulthood and then remained stable before declining again in mid-late life (see Figure 5). There were only two nominally significant SNP main effects across the non-target cognitive domains (see Table 19), one with target SNP rs2466060 (Spatial Memory) and one with non-target SNP rs1462893 (Facial Memory). Sensorimotor dexterity did not have any nominally significant SNP main effects.

Investigation of the interactions between age and SNPs in the non-target domains (see Tables 25-27) found relatively few effects across individual markers in Facial (zero) and Spatial Memory (one). However, 22 total nominally significant interaction effects were detected for Sensorimotor Dexterity. Three of these interactions (SNP8NRG221132, rs776401, and rs1473438) remained significant after correction by a modified Bonferroni procedure that included all SNPs and all domains ($p<.0004$).

Overall, these findings suggest that there are significant SNP x age interactions in the prediction of Sensorimotor Dexterity, including several that exceeded correction for multiple comparisons. However, although moderately heritable ($h^2 = 0.187$, $p=0.028$), Sensorimotor Dexterity was not significantly genetically correlated with schizophrenia ($R_g = -0.143$, $p=0.559$). Thus these results are likely unrelated to the pathogenesis of the disorder and may instead indicate that these SNPs play an important age-moderated role in Sensorimotor Dexterity in the general population.

4.1.4 Summary of Major Findings: Covariate and Exploratory Analyses

To investigate the robustness of these findings, analyses were conducted to explore the NRG1 x age and cognition relationships while covarying schizophrenia-spectrum diagnostic status and intelligence. These specific covariates were chosen due to a large literature that links them with cognitive function. In the case of spectrum diagnostic status, the literature consistently shows that patients with schizophrenia-spectrum disorders have cognitive deficits that are thought to share pathophysiological mechanisms with schizophrenia (Buchanan et al., 2005; Goldberg & Green, 2002). In the case of crystallized verbal intelligence (as measured by the WRAT), the literature suggests that, although commonly used neuropsychological tests measure some independent cognitive variation, they also often load onto a latent "g" or intelligence-like factor (Dickinson & Harvey, 2009). Given that these two variables may be significantly related to performance on the current study's cognitive tasks and NRG1, they were included as covariates to determine whether they might mediate any potential NRG1 x age interactions on cognition.

In most cases, previously significant interactions that were reassessed with these variables as covariates became non-significant, however the effects were not equally strong for both covariates. Except in the domain of Emotional Processing, spectrum diagnosis was a largely non-significant covariate, suggesting that it did not mediate NRG1 x age interactions outside of this domain. Intelligence, however, was a significant covariate for most analyses, suggesting that intelligence-related variance may partially mediate some of the SNP x age interactions on cognitive performance in this study.

Because verbal intelligence appeared to be involved in NRG1 x age interactions on multiple cognitive domains when included as a covariate, its own relationship with NRG1 and age was explored. Intelligence (as measured by the raw, non-age-standardized WRAT scores)

was significantly genetically correlated with schizophrenia and highly heritable in the current sample. However, there were no significant main effects of age or NRG1 SNPs on intelligence. Interestingly, there were only two nominally significant interactions between two non-target NRG1 SNPs (rs6992642, rs3735781) and age in the prediction of intelligence, neither of which was significant after modified Bonferroni correction ($p<.0024$). These findings suggest that although intelligence is genetically correlated with schizophrenia, and thus potentially provides information about the pathogenesis of the disorder, this is likely independent of NRG1, as it is not associated with the SNPs in the current sample.

As expected, the cognitive domains were also significantly intercorrelated with one another, thus exploratory factor analysis was used to better understand the relationships among the domains. This analysis found that one factor explained approximately 42% of the total variance. This factor was significantly heritable, but interestingly, was not genetically correlated with schizophrenia. A significant quadratic relationship between age and factor score was evident in which performance improved until approximately 47 years of age, at which time it began to decline. However, there were no significant main effects of NRG1 SNPs. There was only one significant interaction with age (rs726908) that was no longer significant after Bonferroni correction ($p<.0032$). Overall, this suggests that there are few significant SNP x age interactions on the common variation shared among domains, and furthermore, that this common variation is not genetically related to schizophrenia. Instead, it appears that the unique elements of the cognitive tests show more interactions and are more likely to be associated with the etiology of schizophrenia.

4.1.5 Summary of Major Findings: SNP-wise Patterns

Overall, there were few main effects of individual NRG1 SNPs on individual cognitive domains (see Table 19). Nominally significant SNP effects were seen across eight SNPs, with rs10954855 being the SNP with the most effects across domains (two).

Although there were relatively few SNP main effects, there were many more SNP-by-age interactions across the domains. In total, 24 individual SNPs had at least one nominally significant total interaction effect and/or specific interaction component. Overall, the SNPs with the most significant interaction findings were SNP8NRG221533/rs35753505 (target SNP; 4 significant effects in Abstraction/Mental Flexibility and Emotional Processing), rs776401 (target; 5 significant effects in Emotional Processing and Sensorimotor Dexterity), and rs1473438 (non-target; 6 significant effects in Spatial Processing, Emotional Processing, and Sensorimotor Dexterity). All three of these SNPs are upstream of the first exon, between 31,474,141 and 31,831,015bp on the gene. The functions of these SNPs are unknown at this time, although SNP8NRG221533/rs35753505 and rs776401 have been associated with schizophrenia previously (Benzel et al., 2007; Stefansson et al., 2002).

4.2 DOMAIN-WISE FINDINGS AND COMPARISONS TO CURRENT LITERATURE

Overall, there appear to be potentially promising interactions between NRG1 variants and age in the prediction of cognition in several domains. To better understand the findings of the current study, we will discuss the domains with the greatest number of nominally significant SNP x age interactions in greater detail, beginning with the most significant target domain. For a summary of the results in these domains, see Table 41.

Table 41. Summary of important results for the most significant domains

	R_g (p)	h^2 (p)	Age of Slope Change	Significant SNP x Age Interactions	Target/ Non-Target	p-value^	Minor Allele Effect	Approximate Age of Divergence Among Genotypes	SNP x Age: Significant after IQ Covariation?
Emotional Processing (Target)	-0.358 (0.0542)	0.394 (3.0×10^{-5})	22.61	SNP8NRG221533/rs35753505	T	0.005	+/-	25, 50	N
				rs4298458	T	0.130	+/-	25, 55	N
				rs4566990	NT	0.009	+	30, 75	N
				rs1354334	NT	0.005	+	30, 60	N
				rs776401	T	0.002	+/-	30, 70	N
				rs1473438	NT	0.003	+/-	25, 70	Y
				rs10954821	NT	0.045	+/-	25, 65	N
				rs723811	NT	0.042	+	<15, 60	N
				rs4262285	T	0.017	+/-	<15, 40	N
Abstraction & Mental Flexibility (Target)	-0.604 (0.0047)	0.172 (0.059)	25.75	SNP8NRG221533/rs35753505	T	0.009	+	50	Y
				rs726908	NT	0.047	+/-	30	Y
				rs3924999	T	0.049	+	<15, 70	N
				rs2466058	T	0.037	+/-	40	N
				rs2466049	T	0.030	+	40	N
Attention (Target)	-0.552 (0.0147)	0.169 (0.023)	36.42	rs4566990	NT	0.034	+	<15, 30	N
				rs2439272	T	0.040	+	30	N
				rs6468121	NT	0.011	-	20, 60	N
				rs6988339	T	0.043	+	30	Y
				rs3735782	NT	0.054	-	<15, 30	Y
Sensorimotor Dexterity (Non-target)	-0.143 (0.5587)	0.187 (0.0278)	20, 55	SNP8NRG221132	T	0.0001	-	<15, 40, 60	N
				SNP8NRG241930	T	0.001	-	50	N
				rs1354336	NT	0.022	-	<15, 40, 70	N
				rs776401	T	0.000000	-	<15, 40, 70	Y
				rs1473438	NT	0.0000008	-	<15, 40, 65	Y
				rs1462893	NT	0.015	+	45	N
				rs726908	NT	0.001	+	25	Y
				rs3924999	T	0.043	+/-	<15, 50, 80	N
				rs2439272	T	0.011	+	50	N
				rs6468121	NT	0.023	-	55	N
				rs2466058	T	0.042	-	<15, 30, 70	N
				rs2466049	T	0.038	-	<15, 30, 70	N
				rs2976525	T	0.003	-	<15, 40, 75	N

Note. P-value listed for R_g is the difference from zero significance level. Age of slope change is estimated for sensorimotor dexterity. Interactions listed include those significant at the -2LL and/or the specific component levels. ^P-value reported is the most significant p-value across all interactions between the specific domain and SNP when multiple significant interactions or specific components are significant. Minor allele effect on performance: minor allele is advantageous (+), disadvantageous (-), or mixed (+/-) across the lifespan. Significant after IQ covariation: interaction remains significant (yes; Y) or is non-significant (no; N) after covariation with intelligence. T: target SNP; NT: non-target SNP.

4.2.1 Target Domain: Emotional Processing

The target domain of Emotional Processing showed significant genetic correlation with affected status, significant heritability, and a quadratic pattern in which performance rose and then began to decline at approximately 23 years of age (see Table 41 and Figure 4). There were 15 significant interaction effects: seven total interaction effects and eight interaction components across nine SNPs (see Table 41), although none were significant after correction for multiple comparisons. The effect of the minor allele in this domain was inconsistent across SNPs, often

changing from being advantageous during one part of the lifespan to being detrimental in another, or vice versa (see Figure 9). The age at which performance-allele patterns began to diverge was typically split due to the shifting effect of the minor allele, with a divergence early in life (before age 30) and then later in life (at/after age 50). To our knowledge, no other studies have assessed NRG1 in relation to emotional processing performance.

4.2.2 Target Domain: Abstraction/Mental Flexibility

The target domain of Abstraction/Mental Flexibility also showed significant genetic correlation with affected status, significant heritability, and a quadratic pattern where the age at which performance began to decline was approximately 26 years (see Table 41 and Figure 4). There were six nominally significant SNP x age interaction effects: one of which was a total interaction effect and five that were interaction components across five SNPs. The effect of the minor alleles in this domain was generally advantageous to performance, especially at mid-life or later; however, two SNPs showed a pattern in which they were advantageous and disadvantageous at different parts of the lifespan (see Figure 6). Performance-allele patterns generally begin to diverge between the ages of 30 and 50 years.

To our knowledge, only one other study has addressed the relationship between abstraction and mental flexibility tasks and NRG1. Kircher et al. (2009) found that the minor allele of SNP8NRG221533/rs35753505 was related to poorer semantic verbal fluency (often considered to be related to abstraction/mental flexibility skills) compared to the major allele in a sample of healthy individuals. In the current study, Abstraction/Mental Flexibility performance showed a significant age interaction with rs35753505 at both the total interaction effect and specific component levels. In contrast to Kircher et al. (2009), there was no significant main

effect of this SNP on Abstraction/Mental Flexibility performance and the minor allele was advantageous to performance in the SNP x age interaction.

4.2.3 Target Domain: Attention

The target domain of Attention showed significant genetic correlation with affected status, significant heritability, and a quadratic pattern in which performance began to decline at approximately 36 years of age (see Table 41 and Figure 4). There were six significant interaction effects: one which was a total interaction effect and five that were interaction components across five SNPs. The effects of the minor alleles in this domain were inconsistent; they conveyed an advantage to performance in three interactions but were detrimental in two others (see Figure 7). The age at which performance-allele patterns began to diverge was also inconsistent, with some SNPs showing divergence at 20 years and others at 60 years.

To our knowledge, one other study has investigated the relationship between NRG1 and attention. Stefanis et al. (2007) found that sustained attention was associated with SNP8NRG221533/rs35753505 and microsatellite 433E1006, whereby the minor allele of SNP8NRG221533/rs35753505 and the G allele of the microsatellite were associated with poorer performance in a sample of Greek military conscripts. This SNP lacked both main effects and SNP x age interactions in the domain of attention in the current sample, and microsatellites were not genotyped in this study.

4.2.4 Non-Target Domain: Sensorimotor Dexterity

Surprisingly, the non-target domain of Sensorimotor Dexterity showed the highest number of significant interactions and was the only domain that had interactions that survived correction for multiple comparisons. Performance in this domain was significantly heritable, but was not

significantly genetically correlated with schizophrenia (see Table 41), thus these findings are unlikely to be related to the pathogenesis of this disorder and are more likely to apply to sensorimotor function in the general population.

This domain showed a cubic pattern where the performance declined until the age of approximately 20 years, stabilized, and then began declining again at the age of 55 (see Figure 5). There were 22 significant interaction effects: 11 total interaction effects and 11 interaction components across 13 SNPs. The effect of the minor allele in this domain was often detrimental to performance, with only four SNPs showing an advantage during some part of the lifespan (see Figure 11). Divergence of performance-allele patterns occurred typically during early life, mid-life, and late life due to the cubic relationship that this domain had with age. To our knowledge, no other study has assessed the relationship between NRG1 and sensorimotor dexterity performance.

4.3 AGE PATTERNS IN RELATION TO SCHIZOPHRENIA AGE OF ONSET

In terms of the main effects of age, for most quadratic and cubic domains, the age at which cognitive performance began to worsen was near the end of the peak age of onset for schizophrenia. More specifically, Abstraction/Mental Flexibility, Emotional Processing, Facial Memory, and Sensorimotor Dexterity had performance patterns that showed declines between 20-28 years of age, while Attention and Spatial Processing showed a worsening of cognition during the late 30s and early 40s (see Figures 4 and 5). Studies have commonly shown that fluid intelligence, and frontal cortical function more generally, plateaus or begins to decline in early adulthood (Craik & Bialystok, 2006), which is generally consistent with the findings of the current study.

However, in the analyses of SNP x age interactions, changes in genotype-performance patterns did not typically occur near the peak age of onset in most cases, although very different patterns were evident in the effects of the minor allele across the lifespan and across different cognitive domains. Across the total 34 nominally significant interactions, the majority of the interactions showed very complex patterns in which SNP-performance patterns diverged multiple times across the lifespan (n=22), while a few prototypical examples of early (n=1), middle (n=2), and late (n=9) divergence were apparent and are discussed in more detail below. The timing of divergence indicates the time at which genotype effects became differentiated, and thus may be of importance in terms of understanding the contribution that NRG1 SNPs may make to the onset of schizophrenia. As such, interactions that display early (earlier than age 20; before the period of greatest risk on onset), middle (between 20-30; during the period of greatest risk), and late (after age 30; in the post-risk period) divergence will be the focus of the following qualitative descriptions. In these discussions, we will focus solely on the pattern of divergence and ignore (1) the pattern of the minor allele homozygote because there are typically far fewer participants in this genotype group, and (2) the direction of the minor allele effect.

4.3.1 Prototypical Performance by Genotype Patterns: Early Divergence

There was only one interaction that showed an early divergence. In the domain of Abstraction/Mental Flexibility (see Figure 6), rs726908 showed a pattern in which the major allele homozygote and heterozygote are divergent before the age of 15. These genotype groups converge near the age of 45, at which time Abstraction/Mental Flexibility performance between them remains largely similar throughout the remainder of life. One hypothesis is that divergence of genotype effects may occur early, in this pre-onset period and may serve to time the onset of

schizophrenia; however, this sample provides only a snapshot of these effects due to the fact that all participants were 15 years or older at the time of study participation, thus limiting our ability to assess this hypothesis.

4.3.2 Prototypical Performance by Genotype Patterns: Middle Divergence

There were two interactions that showed prominent genotype effects in the risk period. As seen in Figure 7, rs2439272 and rs6988339 both show a pattern in which Attention performance is largely similar between the major allele homozygote and the heterozygote until the age of approximately 25-30, at which time the groups diverge for the rest of the lifespan. This divergence occurs at the end of the traditional risk period for schizophrenia, and thus may have some importance for age of onset, as hypothesized.

Multiple patterns of change in this age period could support the hypothesis that these SNP effects may be related to onset of the disorder, including (1) a parallel pattern in which genotype differences emerge at the time of greatest risk and remain stable across the lifespan; (2) an amplification pattern in which the differences emerge and then increase throughout life; or (3) an effect that is only temporary near the onset period where genotype patterns converge again after the risk period. The findings in the Attention domain are consistent with the first, parallel-type pattern.

4.3.3 Prototypical Performance by Genotype Patterns: Late Divergence

Somewhat surprisingly, the most common prototypical pattern (n=9) was one where divergence between genotype groups occurred past the typical risk period for schizophrenia. This pattern was seen across multiple domains and is most clearly illustrated in the domains of Abstraction/Mental Flexibility, Emotional Processing, and Sensorimotor Dexterity.

In the domain of Abstraction/Mental Flexibility (see Figure 6), markers rs35753505, rs2466058, and rs2466049 all show a pattern in which the genotype groups perform relatively similarly until the age of 40-55, at which time the groups diverge for the rest of the lifespan. In Emotional Processing (see Figure 9), rs4262285 and rs4298458 diverge at approximately 40 and 60 years of age, respectively, and remain separate throughout the rest of the lifespan. In the domain of Sensorimotor Dexterity (see Figure 11), SNP8NRG241930, rs1462893, rs2439272, and rs6468121 show a pattern in which the genotype groups are very similar until approximately the ages of 35-55 at which time the groups diverge throughout the rest of the lifespan.

Interestingly, in each of these examples, the genotype effects begin after the risk period and increase in magnitude as age increases. This suggests that it is not a static change that is maintained between genotypes across the lifespan, but rather that the genotype effects become exaggerated with continued aging. This pattern is consistent with the hypothesis that NRG1 SNPs play an important role in the continued function of the nervous system and cognitive performance throughout life. But this pattern is inconsistent with the idea that changes in genotype-performance patterns near the peak age of onset could serve to time the onset of the disorder.

This pattern of persisting, cumulative SNP effects over the lifespan may be consistent with the hypothesized lack of age-related down-regulation of neurodevelopmental genes generally in schizophrenia (Torkamani et al., 2010), in which the effects accumulate over the lifespan due to continued expression of these genes over the lifespan, as well as the findings of overexpression of NRG1 specifically (Chong et al., 2008; Hashimoto et al., 2004; Law et al., 2006). Although it would be expected that this over-expression would be related to the age of onset, and thus occur during the period of greatest risk for the disorder, a growing literature

suggests that there are molecular as well as progressive neurodevelopmental changes in this disorder (Archer, 2010; Torkamani et al., 2010). While the results of the current study do not suggest that NRG1 effects are particularly strong at the typical age of onset of schizophrenia, it may be that the accumulated effects of NRG1 over the lifespan are related to some of the progressive changes in brain structure that occur after schizophrenia onset (for a review, see Archer, 2010).

It is also interesting when considering the findings of a recent study that employed both linkage and association methods to better understand the genetic causes of Alzheimer's disease with psychosis (ADP) in families multiplex for ADP (Go et al., 2005). This study found a significant linkage peak on chromosome 8p that encompassed the NRG1 gene, a significant association between NRG1 SNP rs3924999 and affected status, and an association that trended toward significance for the NRG1 haplotype of rs3924999, SNP8NRG221533/rs35753505, and SNP8NRG243177. However, a study utilizing a mixed sample of Alzheimer's disease patients with and without psychosis failed to replicate these findings (Middle et al., 2010). Taken together, the findings by Go et al. (2005) and the current study may suggest that NRG1 has continuing effects on cognition over the lifespan in individuals at risk for psychosis due to a family history of schizophrenia-spectrum disorders or Alzheimer's disease with psychosis.

Another consideration of these patterns is the increased interindividual variation in cognition that is believed to occur later in life. A number of studies have found increased interindividual variation in cognitive functioning in older participants when compared to younger ones (Christensen et al., 1994); however, other studies have failed to replicate these findings (for a review, see Christensen et al., 1994). Such increased individual differences are believed to result from changes in genetic and/or environmental contributions to cognitive performance

associated with aging (Finkel, Pedersen, Plomin, & McClearn, 1998). For example, age-related increases or decreases in genetic expression, as well as the accumulation of both genetic effects and environmental experiences and insults throughout the lifespan, may all contribute to greater interindividual variation in older age. This is consistent with the findings of the current study that suggest that the accumulation of NRG1 SNP effects, as well as other factors, may impact cognition late in life. However, given that the most common genotype pattern was complex, rather than one of the prototypical patterns, it may be that NRG1's genotype patterns are generally complex across the lifespan and that no simple, clear pattern actually exists.

4.4 SPECULATIONS AND FUTURE HYPOTHESES

Although the findings of the current study are largely not significant after correction for multiple comparisons and thus may not replicate, these preliminary findings warrant further speculation and investigation as they may hint at previously unknown relationships between NRG1 and the progressive changes associated with schizophrenia.

The idea of a progressive neurodevelopmental disorder is not inconsistent with the aforementioned early and late neurodevelopmental hypotheses of schizophrenia. Rather, it suggests that molecular changes continue over the lifespan in individuals with schizophrenia due to genetic, environmental, and gene x environment influences. The most well-replicated finding of this progression is a reduction in brain volume that is present at the time of disorder onset, but continues over the lifespan in at least some patients. Overall cerebral volume is typically seen with a corresponding increase in cerebrospinal fluid, with specific areas of tissue reduction evident in frontal and temporal white and grey matter regions (for a review, see Dutt et al., 2009,

and Andreasen et al., 2011). At present, there are relatively few studies of the genetic effects that are related to these structural changes, however.

One hypothesis emergent from the current study is that there may be a genetic association between NRG1 variants and these structural changes due to the numerous roles that NRG1 plays in neurodevelopment and the continued function of the brain throughout the lifespan. Indeed, there is some evidence for this idea. Two recent studies showed that the NRG1 markers were genetically associated with reduced hippocampal volume (Gruber et al., 2008) and enlarged lateral ventricles (Mata et al., 2009) in patients with schizophrenia; however, these findings were not supported by a more recent study (Dutt et al., 2009). In the only known study (Andreasen et al., 2011) of epistatic, or interactional, genetic effects of NRG1 and progressive brain changes, Andreasen et al. (2011) found an interaction between variants in NRG1 and Reelin that was associated with small increases, rather than decreases, in the volume of the caudate and putamen in a longitudinally followed schizophrenia sample.

Overall, these findings suggest that NRG1 may have a role in the progressive brain changes seen in patients with schizophrenia, which is consistent with the findings in the current study that showed increasing genotypic effects of the gene late in life in multiple cognitive domains. However, the mechanisms behind these changes have yet to be identified. It may be that the roles that NRG1 originally plays in the early development of the CNS are ongoing in the brains of patients with schizophrenia, impacting processes such as synaptic maintenance later in life in patients compared to healthy individuals and resulting in progressive changes. Such questions may be answered once the ways in which NRG1 SNPs regulate NRG1 expression or are related to other downstream biological changes have been identified.

4.5 STRENGTHS AND LIMITATIONS

To our knowledge, this is the first study of its kind, as previous studies on this topic have relied on gene expression analyses in post-mortem samples without antemortem cognitive assessment or have neglected to take into account age effects across adolescence – late adulthood when assessing genotype-phenotype relationships. This study is unique because of the lifespan developmental approach that was employed in the investigation of genetic effects on cognition in schizophrenia. In addition, the use of the large, multigenerational, multiplex family sample is a powerful study design and the sample had a very wide age range that captured much of the risk period for psychosis conversion and into late life. Moreover, existing studies typically assesses only a few cognitive domains and a few SNPs, while the current study used a comprehensive cognitive battery and large number of NRG1 SNPs.

Despite these strengths, the current study has some limitations. First, very few findings survived correction by a modified Bonferroni correction, suggesting that although these findings may be promising, they must be replicated in a larger, better powered sample to be considered convincing. At the nominal significance level of .05, statistical power was good (power = .80) for analyses that included both affected and unaffected individuals, being able to detect effects that accounted for approximately 1% of trait variance. However, power was reduced for analyses that included only the unaffected sample and was further reduced when correcting for multiple comparisons.

In addition, although much of the risk period for conversion to psychosis was captured by the age ranges present in this sample, the number of teenagers in the sample was relatively small, and there were no participants in the sample under the age of 15 years. It is important to increase

the number of participants aged 10-20 years old to better detect any amplification of genetic effects related to age of onset.

Moreover, all of the interpretations of the current study's findings with reference to schizophrenia depend on the presence of an etiological relationship between NRG1 and schizophrenia. Although NRG1 is a strong functional candidate for schizophrenia and many genetic studies of schizophrenia have found linkage and association to the 8p chromosomal region and to NRG1 specifically, a number have found no relationship, including multiple large genome-wide studies. In the current study, the relationship between NRG1 and schizophrenia was not specifically assessed due to the small number of schizophrenia patients in the sample. This prevented the investigation of these interactions in a patient-only sample which may have helped elucidate the relationship between NRG1 and schizophrenia further. This study also lacked a genotyped control group, preventing the assessment of these findings in a non-schizophrenia-related sample, and thus we are unable to determine whether the patterns found in the current sample also exist in the general population.

The current study also utilized a cross-sectional design that confounds age with birth cohort effects. A prospective longitudinal study design would have allowed investigation of age effects without possible birth cohort-related concerns that might be particularly strong on cognitive function and intelligence level. Although it is difficult to separate cohort effects from the effects of age-related changes in a cross-sectional design, the lack of age effects on WRAT scores suggests an absence of cohort effects on verbal intelligence, and perhaps other cognitive domains as well.

Although some of the individuals in this study who were unaffected at the time of participation may go on to develop schizophrenia, at the time of their clinical evaluation and

study participation, they had no history of this disorder and were found to be free of the disorder and the increased variance that accompanies it (e.g., due to symptoms, medication effects and side effects, and a history of prolonged hospitalization). Thus their performance reflects that of an individual at higher risk for the disorder, which was the goal of the multiplex family design.

Finally, although a literature based SNP set was utilized in this study, a more comprehensive tag SNP (tSNP) design might have further elucidated the individual NRG1 SNPs that are important in cognitive performance. However, an estimated 348 NRG1 SNPs are needed for tSNP designs due to the size and complexity of this gene.

4.6 FUTURE DIRECTIONS

Future directions aimed at resolving the limitations in the current study include replicating this study with a well-powered relative sample that includes a large teenage cohort. In addition, it may be informative to replicate these findings in a schizophrenia sample and healthy control sample. Of particular interest would be replications within the domains of Emotional Processing, Abstraction/Mental Flexibility, Attention, and Sensorimotor Dexterity and the markers SNP8NRG221132, SNP8NRG221533/rs35753505, rs776401, and rs1473438, as these domains and SNPs showed the most number of nominally and/or Bonferroni-corrected significant interactions. These replications would help evaluate, and possibly support, the findings presented in the current study, and would allow a better understanding of the role that these interactions play in the teenage years, as well as in patients and the general population. Importantly, if these results were replicated in a healthy sample, that would suggest that NRG1 x age interactions on cognitive performance are present in the general population.

Other directions in answering the question of NRG1 x age interactions with reference to cognition include studying additional markers within NRG1, such as a tSNP set or the microsatellites previously associated with schizophrenia (T. Li et al., 2004; Stefansson et al., 2003; Stefansson et al., 2002). Additionally, the use of methods and models that incorporate epistatic and environmental influences that might play a moderating role in these interactions may be helpful in better understanding these relationships. There is evidence that variations in both NRG1 and the ErbB receptor tyrosine kinase family may be associated with an elevated risk for schizophrenia (Benzel et al., 2007; Norton et al., 2006), thus studies of the NRG1-ErbB signaling pathway may further elucidate the pathophysiology of schizophrenia-related cognitive dysfunction as it pertains to NRG1 and age. Finally, incorporation of NRG1 genetic studies into longitudinal studies of the progressive brain changes related to psychosis may also further elucidate the late emerging genotype effects seen in the current study.

4.7 CONCLUSIONS

Overall, the current study found a number of nominally significant NRG1 SNP x age interactions across several cognitive domains and individual genetic markers, although very few withstood correction for multiple comparisons, and thus it may be the case that these nominally significant findings represent alpha error.

However, there were a number of nominally significant findings present in several the domains of Emotional Processing, Abstraction/Mental Flexibility, Attention, and Sensorimotor Dexterity, perhaps suggesting that these domains do show truly significant relationships with NRG1 SNPs and age that would be replicated with a larger sample. The patterns seen here suggest that NRG1's effects are generally consistent across early and mid-life, and as such, may

have no special role in explaining schizophrenia's peak age of onset. There were, however, some interesting patterns found later in life. Although these are likely not informative for schizophrenia onset, they may suggest that NRG1 SNP effects are important for the hypothesis that schizophrenia is a neurodevelopmental disorder characterized by progressive brain and molecular changes throughout the lifespan. The findings of the current study may suggest a role for NRG1 and its related signaling networks in these ongoing processes.

In addition, these SNP effects may be important for understanding cognition throughout the lifespan outside of the context of schizophrenia. This seems particularly true for Sensorimotor Dexterity performance, which although having the most interactions and the only interactions that withstood correction for multiple comparisons, was not significantly genetically correlated with schizophrenia. This may suggest that NRG1 x age interactions are relevant to cognitive function in the general population, especially in this domain.

BIBLIOGRAPHY

Addington, A. M., Gornick, M. C., Shaw, P., Seal, J., Gogtay, N., Greenstein, D., et al. (2007). Neuregulin 1 (8p12) and childhood-onset schizophrenia: susceptibility haplotypes for diagnosis and brain developmental trajectories. *Mol Psychiatry, 12*(2), 195-205.

Allen, N., Bagade, S., McQueen, M., Ioannidis, J., Kavvoura, F., Khoury, M., et al. (2008). Systematic Meta-Analyses and Field Synopsis of Genetic Association Studies in Schizophrenia: The SzGene Database. *Nat Genet, 40*(7), 827-834.

Almasy, L., & Blangero, J. (1998). Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet, 62*(5), 1198-1211.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders-IV-TR*. Arlington, VA: American Psychiatric Association.

Andreasen, N. C., Wilcox, M. A., Ho, B. C., Epping, E., Ziebell, S., Zeien, E., et al. (2011). Statistical epistasis and progressive brain change in schizophrenia: an approach for examining the relationships between multiple genes. *Mol Psychiatry*.

Archer, T. (2010). Neurodegeneration in schizophrenia. *Expert Rev Neurother, 10*(7), 1131-1141.

Badner, J. A., & Gershon, E. S. (2002). Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry, 7*(4), 405-411.

Barros, C. S., Calabrese, B., Chamero, P., Roberts, A. J., Korzus, E., Lloyd, K., et al. (2009). Impaired maturation of dendritic spines without disorganization of cortical cell layers in mice lacking NRG1/ErbB signaling in the central nervous system. *Proc Natl Acad Sci U S A, 106*(11), 4507-4512.

Bennett, M. (2008). Dual constraints on synapse formation and regression in schizophrenia: neuregulin, neuroligin, dysbindin, DISC1, MuSK and agrin. *Aust N Z J Psychiatry, 42*(8), 662-677.

Bennett, M. (2009). Positive and negative symptoms in schizophrenia: the NMDA receptor hypofunction hypothesis, neuregulin/ErbB4 and synapse regression. *Aust N Z J Psychiatry, 43*(8), 711-721.

Benton, A. L., Varney, N. R., & Hamsher, K. S. (1975). *Judgment of Line Orientation, Form V.* Iowa City, IA: University of Iowa Hospitals.

Benzel, I., Bansal, A., Browning, B. L., Galwey, N. W., Maycox, P. R., McGinnis, R., et al. (2007). Interactions among genes in the ErbB-Neuregulin signalling network are associated with increased susceptibility to schizophrenia. *Behav Brain Funct, 3*, 31.

Bertram, I., Bernstein, H. G., Lendeckel, U., Bukowska, A., Dobrowolny, H., Keilhoff, G., et al. (2007). Immunohistochemical evidence for impaired neuregulin-1 signaling in the prefrontal cortex in schizophrenia and in unipolar depression. *Ann N Y Acad Sci, 1096*, 147-156.

Blakemore, S. J., Burnett, S., & Dahl, R. E. (2010). The role of puberty in the developing adolescent brain. *Hum Brain Mapp, 31*(6), 926-933.

Boer, S., Berk, M., & Dean, B. (2009). Levels of neuregulin 1 and 3 proteins in Brodmann's area 46 from subjects with schizophrenia and bipolar disorder. *Neurosci Lett, 466*(1), 27-29.

Braff, D. L., Freedman, R., Schork, N. J., & Gottesman, II. (2007). Deconstructing schizophrenia: an overview of the use of endophenotypes in order to understand a complex disorder. *Schizophr Bull, 33*(1), 21-32.

Braff, D. L., Schork, N. J., & Gottesman, II. (2007). Endophenotyping schizophrenia. *Am J Psychiatry, 164*(5), 705-707.

Brown, G. W., & Birley, J. L. (1968). Crises and life changes and the onset of schizophrenia. *J Health Soc Behav, 9*(3), 203-214.

Buchanan, R. W., Davis, M., Goff, D., Green, M. F., Keefe, R. S., Leon, A. C., et al. (2005). A summary of the FDA-NIMH-MATRICS workshop on clinical trial design for neurocognitive drugs for schizophrenia. *Schizophr Bull, 31*(1), 5-19.

Buonanno, A. (2010). The neuregulin signaling pathway and schizophrenia: from genes to synapses and neural circuits. *Brain Res Bull, 83*(3-4), 122-131.

Cardno, A. G., & Gottesman, II. (2000). Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. *Am J Med Genet, 97*(1), 12-17.

Carter, C. J. (2006). Schizophrenia susceptibility genes converge on interlinked pathways related to glutamatergic transmission and long-term potentiation, oxidative stress and oligodendrocyte viability. *Schizophr Res, 86*(1-3), 1-14.

Chadwick, R. B., Conrad, M. P., McGinnis, M. D., Johnston-Dow, L., Spurgeon, S. L., & Kronick, M. N. (1996). Heterozygote and mutation detection by direct automated fluorescent DNA sequencing using a mutant Taq DNA polymerase. *Biotechniques, 20*(4), 676-683.

Chong, V. Z., Thompson, M., Beltaifa, S., Webster, M. J., Law, A. J., & Weickert, C. S. (2008). Elevated neuregulin-1 and ErbB4 protein in the prefrontal cortex of schizophrenic patients. *Schizophr Res, 100*(1-3), 270-280.

Christensen, H., Mackinnon, A., Jorm, A. F., Henderson, A. S., Scott, L. R., & Korten, A. E. (1994). Age differences and interindividual variation in cognition in community-dwelling elderly. *Psychol Aging, 9*(3), 381-390.

Colantuoni, C., Hyde, T. M., Mitkus, S., Joseph, A., Sartorius, L., Aguirre, C., et al. (2008). Age-related changes in the expression of schizophrenia susceptibility genes in the human prefrontal cortex. *Brain Struct Funct, 213*(1-2), 255-271.

Compton, M. T., & Walker, E. F. (2009). Physical manifestations of neurodevelopmental disruption: are minor physical anomalies part of the syndrome of schizophrenia? *Schizophr Bull, 35*(2), 425-436.

Conneely, K. N., & Boehnke, M. (2007). So Many Correlated Tests, So Little Time! Rapid Adjustment of P Values for Multiple Correlated Tests. *Am J Hum Genet, 81*(6).

Corfas, G., Roy, K., & Buxbaum, J. D. (2004). Neuregulin 1-erbB signaling and the molecular/cellular basis of schizophrenia. *Nat Neurosci, 7*(6), 575-580.

Craik, F. I., & Bialystok, E. (2006). Cognition through the lifespan: mechanisms of change. *Trends Cogn Sci, 10*(3), 131-138.

Crowley, J. J., Keefe, R. S., Perkins, D. O., Stroup, T. S., Lieberman, J. A., & Sullivan, P. F. (2008). The neuregulin 1 promoter polymorphism rs6994992 is not associated with chronic schizophrenia or neurocognition. *Am J Med Genet B Neuropsychiatr Genet, 147B*(7), 1298-1300.

Crown, J. (1993). *A Developmental Behavior Genetic Approach to Normal Development from Adolescence to Young Adulthood.* Unpublished Specialty Paper, University of Pittsburgh, Pittsburgh.

Dickinson, D., & Harvey, P. D. (2009). Systemic hypotheses for generalized cognitive deficits in schizophrenia: a new take on an old problem. *Schizophr Bull, 35*(2), 403-414.

Dutt, A., McDonald, C., Dempster, E., Prata, D., Shaikh, M., Williams, I., et al. (2009). The effect of COMT, BDNF, 5-HTT, NRG1 and DTNBP1 genes on hippocampal and lateral ventricular volume in psychosis. *Psychol Med, 39*(11), 1783-1797.

Esper, R. M., Pankonin, M. S., & Loeb, J. A. (2006). Neuregulins: versatile growth and differentiation factors in nervous system development and human disease. *Brain Res Rev, 51*(2), 161-175.

Farber, N. B. (2003). The NMDA receptor hypofunction model of psychosis. *Ann N Y Acad Sci, 1003*, 119-130.

Fazzari, P., Paternain, A. V., Valiente, M., Pla, R., Lujan, R., Lloyd, K., et al. (2010). Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. *Nature, 464*(7293), 1376-1380.

Feinberg, I. (1982a). Schizophrenia and Late Maturational Brain Changes in Man. *Psychopharmacology Bulletin, 18*(3), 29-31.

Feinberg, I. (1982b). Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *J Psychiatr Res, 17*(4), 319-334.

Finkel, D., Pedersen, N. L., Plomin, R., & McClearn, G. E. (1998). Longitudinal and cross-sectional twin data on cognitive abilities in adulthood: the Swedish Adoption/Twin Study of Aging. *Dev Psychol, 34*(6), 1400-1413.

Fischbach, G. D. (2006). Schizophrenia: signals from the other side. *Nat Med, 12*(7), 734-735.

Fischbach, G. D. (2007). NRG1 and synaptic function in the CNS. *Neuron, 54*(4), 495-497.

Glahn, D. C., Almasy, L., Blangero, J., Burk, G. M., Estrada, J., Peralta, J. M., et al. (2007). Adjudicating neurocognitive endophenotypes for schizophrenia. *Am J Med Genet B Neuropsychiatr Genet, 144B*(2), 242-249.

Glahn, D. C., Gur, R. C., Ragland, J. D., Censits, D. M., & Gur, R. E. (1997). Reliability, performance characteristics, construct validity, and an initial clinical application of a visual object learning test (VOLT). *Neuropsychology, 11*(4), 602-612.

Go, R. C., Perry, R. T., Wiener, H., Bassett, S. S., Blacker, D., Devlin, B., et al. (2005). Neuregulin-1 polymorphism in late onset Alzheimer's disease families with psychoses. *Am J Med Genet B Neuropsychiatr Genet, 139B*(1), 28-32.

Goldberg, T. E., & Green, M. F. (2002). Neurocognitive Functioning in Patients with Schizophrenia: An Overview. In K. L. Davis, D. Charney, J. T. Coyle & C. Nemeroff (Eds.), *Neuropsychopharmacology: The Fifth Generation of Progress* (pp. 657-669): Lippincott Williams & Wilkins.

Gottesman, II, & Gould, T. D. (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry, 160*(4), 636-645.

Gottesman, II, & Shields, J. (1967). A polygenic theory of schizophrenia. *Proc Natl Acad Sci U S A, 58*(1), 199-205.

Greenwood, T. A., Braff, D. L., Light, G. A., Cadenhead, K. S., Calkins, M. E., Dobie, D. J., et al. (2007). Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. *Arch Gen Psychiatry, 64*(11), 1242-1250.

Gruber, O., Falkai, P., Schneider-Axmann, T., Schwab, S. G., Wagner, M., & Maier, W. (2008). Neuregulin-1 haplotype HAP(ICE) is associated with lower hippocampal volumes in schizophrenic patients and in non-affected family members. *J Psychiatr Res, 43*(1), 1-6.

Gu, Z., Jiang, Q., Fu, A. K., Ip, N. Y., & Yan, Z. (2005). Regulation of NMDA receptors by neuregulin signaling in prefrontal cortex. *J Neurosci, 25*(20), 4974-4984.

Gur, R. C., Jaggi, J. L., Ragland, J. D., Resnick, S. M., Shtasel, D., Muenz, L., et al. (1993). Effects of memory processing on regional brain activation: cerebral blood flow in normal subjects. *Int J Neurosci, 72*(1-2), 31-44.

Gur, R. C., Ragland, J. D., Moberg, P. J., Bilker, W. B., Kohler, C., Siegel, S. J., et al. (2001). Computerized neurocognitive scanning: II. The profile of schizophrenia. *Neuropsychopharmacology, 25*(5), 777-788.

Gur, R. C., Ragland, J. D., Moberg, P. J., Turner, T. H., Bilker, W. B., Kohler, C., et al. (2001). Computerized neurocognitive scanning: I. Methodology and validation in healthy people. *Neuropsychopharmacology, 25*(5), 766-776.

Gur, R. E., Cowell, P. E., Latshaw, A., Turetsky, B. I., Grossman, R. I., Arnold, S. E., et al. (2000). Reduced dorsal and orbital prefrontal gray matter volumes in schizophrenia. *Arch Gen Psychiatry, 57*(8), 761-768.

Gur, R. E., Kohler, C. G., Ragland, J. D., Siegel, S. J., Lesko, K., Bilker, W. B., et al. (2006). Flat affect in schizophrenia: relation to emotion processing and neurocognitive measures. *Schizophr Bull, 32*(2), 279-287.

Hahn, C. G., Wang, H. Y., Cho, D. S., Talbot, K., Gur, R. E., Berrettini, W. H., et al. (2006). Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia. *Nat Med, 12*(7), 824-828.

Hall, J., Whalley, H. C., Job, D. E., Baig, B. J., McIntosh, A. M., Evans, K. L., et al. (2006). A neuregulin 1 variant associated with abnormal cortical function and psychotic symptoms. *Nat Neurosci, 9*(12), 1477-1478.

Hare, E., Gur, R. C., Pogue-Geile, M. F., Calkins, M. E., Peralta, J. M., Prasad, K., et al. (Unpublished data). Genetic Correlations between Neurocognitive Measures in a Multiplex Multigenerational Study of Schizophrenia.

Harris, L. W., Lockstone, H. E., Khaitovich, P., Weickert, C. S., Webster, M. J., & Bahn, S. (2009). Gene expression in the prefrontal cortex during adolescence: implications for the onset of schizophrenia. *BMC Med Genomics, 2*, 28.

Harrison, P. J., & Law, A. J. (2006). Neuregulin 1 and schizophrenia: genetics, gene expression, and neurobiology. *Biol Psychiatry, 60*(2), 132-140.

Hashimoto, R., Straub, R. E., Weickert, C. S., Hyde, T. M., Kleinman, J. E., & Weinberger, D. R. (2004). Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. *Mol Psychiatry, 9*(3), 299-307.

Heydebrand, G. (2006). Cognitive deficits in the families of patients with schizophrenia. *Curr Opin Psychiatry, 19*(3), 277-281.

Holmans, P. A., Riley, B., Pulver, A. E., Owen, M. J., Wildenauer, D. B., Gejman, P. V., et al. (2009). Genomewide linkage scan of schizophrenia in a large multicenter pedigree sample using single nucleotide polymorphisms. *Mol Psychiatry, 14*(8), 786-795.

Honea, R., Crow, T. J., Passingham, D., & Mackay, C. E. (2005). Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. *Am J Psychiatry, 162*(12), 2233-2245.

Husted, J. A., Lim, S., Chow, E. W., Greenwood, C., & Bassett, A. S. (2009). Heritability of neurocognitive traits in familial schizophrenia. *Am J Med Genet B Neuropsychiatr Genet, 150B*(6), 845-853.

Huttenlocher, P. R. (1979). Synaptic density in human frontal cortex - developmental changes and effects of aging. *Brain Res, 163*(2), 195-205.

Jaaro-Peled, H., Hayashi-Takagi, A., Seshadri, S., Kamiya, A., Brandon, N. J., & Sawa, A. (2009). Neurodevelopmental mechanisms of schizophrenia: understanding disturbed postnatal brain maturation through neuregulin-1-ErbB4 and DISC1. *Trends Neurosci, 32*(9), 485-495.

Kircher, T., Krug, A., Markov, V., Whitney, C., Krach, S., Zerres, K., et al. (2009). Genetic variation in the schizophrenia-risk gene neuregulin 1 correlates with brain activation and impaired speech production in a verbal fluency task in healthy individuals. *Hum Brain Mapp, 30*(10), 3406-3416.

Kircher, T., Thienel, R., Wagner, M., Reske, M., Habel, U., Kellermann, T., et al. (2008). Neuregulin 1 ICE-single nucleotide polymorphism in first episode schizophrenia correlates with cerebral activation in fronto-temporal areas. *Eur Arch Psychiatry Clin Neurosci*.

Kristiansen, L. V., Huerta, I., Beneyto, M., & Meador-Woodruff, J. H. (2007). NMDA receptors and schizophrenia. *Curr Opin Pharmacol, 7*(1), 48-55.

Krivosheya, D., Tapia, L., Levinson, J. N., Huang, K., Kang, Y., Hines, R., et al. (2008). ErbB4-neuregulin signaling modulates synapse development and dendritic arborization through distinct mechanisms. *J Biol Chem, 283*(47), 32944-32956.

Krug, A., Markov, V., Eggermann, T., Krach, S., Zerres, K., Stocker, T., et al. (2008). Genetic variation in the schizophrenia-risk gene neuregulin1 correlates with differences in frontal brain activation in a working memory task in healthy individuals. *Neuroimage, 42*(4), 1569-1576.

Kurtz, M. M., Ragland, J. D., Bilker, W., Gur, R. C., & Gur, R. E. (2001). Comparison of the continuous performance test with and without working memory demands in healthy controls and patients with schizophrenia. *Schizophr Res, 48*(2-3), 307-316.

Kurtz, M. M., Ragland, J. D., Moberg, P. J., & Gur, R. C. (2004). The Penn Conditional Exclusion Test: a new measure of executive-function with alternate forms of repeat administration. *Arch Clin Neuropsychol, 19*(2), 191-201.

Lachman, M. E. (2004). Development in midlife. *Annu Rev Psychol, 55*, 305-331.

Lau, C. G., & Zukin, R. S. (2007). NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nat Rev Neurosci, 8*(6), 413-426.

Law, A. J., Lipska, B. K., Weickert, C. S., Hyde, T. M., Straub, R. E., Hashimoto, R., et al. (2006). Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5' SNPs associated with the disease. *Proc Natl Acad Sci U S A, 103*(17), 6747-6752.

Law, A. J., Shannon Weickert, C., Hyde, T. M., Kleinman, J. E., & Harrison, P. J. (2004). Neuregulin-1 (NRG-1) mRNA and protein in the adult human brain. *Neuroscience, 127*(1), 125-136.

Lewis, C. M., Levinson, D. F., Wise, L. H., DeLisi, L. E., Straub, R. E., Hovatta, I., et al. (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet, 73*(1), 34-48.

Li, B., Woo, R. S., Mei, L., & Malinow, R. (2007). The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity. *Neuron, 54*(4), 583-597.

Li, D., Collier, D. A., & He, L. (2006). Meta-analysis shows strong positive association of the neuregulin 1 (NRG1) gene with schizophrenia. *Hum Mol Genet, 15*(12), 1995-2002.

Li, T., Stefansson, H., Gudfinnsson, E., Cai, G., Liu, X., Murray, R. M., et al. (2004). Identification of a novel neuregulin 1 at-risk haplotype in Han schizophrenia Chinese patients, but no association with the Icelandic/Scottish risk haplotype. *Mol Psychiatry, 9*(7), 698-704.

Luna, B., & Sweeney, J. A. (2001). Studies of brain and cognitive maturation through childhood and adolescence: a strategy for testing neurodevelopmental hypotheses. *Schizophr Bull, 27*(3), 443-455.

Lysaker, P. H., Outcalt, S. D., & Ringer, J. M. (2010). Clinical and psychosocial significance of trauma history in schizophrenia spectrum disorders. *Expert Rev Neurother, 10*(7), 1143-1151.

MacDonald, A. W., 3rd, & Chafee, M. V. (2006). Translational and developmental perspective on N-methyl-D-aspartate synaptic deficits in schizophrenia. *Dev Psychopathol, 18*(3), 853-876.

Mata, I., Perez-Iglesias, R., Roiz-Santianez, R., Tordesillas-Gutierrez, D., Gonzalez-Mandly, A., Vazquez-Barquero, J. L., et al. (2009). A neuregulin 1 variant is associated with increased lateral ventricle volume in patients with first-episode schizophrenia. *Biol Psychiatry, 65*(6), 535-540.

Maxwell, M. (1992). Manual for the family interview for genetic studies (FIGS). Bethesda, MD: National Institute of Mental Health.

McEwen, B. S. (2010). Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. *Ann N Y Acad Sci, 1204 Suppl*, E38-59.

Meador-Woodruff, J. H., & Kleinman, J. E. (2002). Neurochemistry of Schizophrenia: Glutamatergic Abnormalities. In K. L. Davis, D. Charney, J. T. Coyle & C. Nemeroff (Eds.), *Neuropsychopharmacology: The Fifth Generation of Progress* (pp. 717-725): Lippincott Williams & Wilkins.

Mei, L., & Xiong, W. C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat Rev Neurosci, 9*(6), 437-452.

Middle, F., Pritchard, A. L., Handoko, H., Haque, S., Holder, R., Bentham, P., et al. (2010). No association between neuregulin 1 and psychotic symptoms in Alzheimer's disease patients. *J Alzheimers Dis, 20*(2), 561-567.

Murray, R. M., & Lewis, S. W. (1987). Is schizophrenia a neurodevelopmental disorder? *Br Med J (Clin Res Ed), 295*(6600), 681-682.

Murray, R. M., Sham, P., Van Os, J., Zanelli, J., Cannon, M., & McDonald, C. (2004). A developmental model for similarities and dissimilarities between schizophrenia and bipolar disorder. *Schizophr Res, 71*(2-3), 405-416.

Norton, N., Moskvina, V., Morris, D. W., Bray, N. J., Zammit, S., Williams, N. M., et al. (2006). Evidence that interaction between neuregulin 1 and its receptor erbB4 increases susceptibility to schizophrenia. *Am J Med Genet B Neuropsychiatr Genet, 141*(1), 96-101.

Nurnberger, J. I., Jr., Blehar, M. C., Kaufmann, C. A., York-Cooler, C., Simpson, S. G., Harkavy-Friedman, J., et al. (1994). Diagnostic interview for genetic studies. Rationale,

unique features, and training. NIMH Genetics Initiative. *Arch Gen Psychiatry, 51*(11), 849-859; discussion 863-844.

O'Connell, J. R., & Weeks, D. E. (1998). PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet, 63*(1), 259-266.

Olney, J. W., & Farber, N. B. (1995a). Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry, 52*(12), 998-1007.

Olney, J. W., & Farber, N. B. (1995b). NMDA antagonists as neurotherapeutic drugs, psychotogens, neurotoxins, and research tools for studying schizophrenia. *Neuropsychopharmacology, 13*(4), 335-345.

Pankonin, M. S., Sohi, J., Kamholz, J., & Loeb, J. A. (2009). Differential distribution of neuregulin in human brain and spinal fluid. *Brain Res, 1258*, 1-11.

Petryshen, T. L., Middleton, F. A., Kirby, A., Aldinger, K. A., Purcell, S., Tahl, A. R., et al. (2005). Support for involvement of neuregulin 1 in schizophrenia pathophysiology. *Mol Psychiatry, 10*(4), 366-374, 328.

Pogue-Geile, M. F. (1991). The Development of Liability to Schizophrenia: Early and Late Developmental Models. In E. F. Walker (Ed.), *Schizophrenia: A Life-Course Developmental Perspective*. Boston: Academic Press.

Pogue-Geile, M. F. (1997). Developmental aspects of schizophrenia. In M. S. Keshavan & R. M. Murray (Eds.), *Neurodevelopment and Adult Psychopathology*. New York: Cambridge University Press.

Prevot, V., Rio, C., Cho, G. J., Lomniczi, A., Heger, S., Neville, C. M., et al. (2003). Normal female sexual development requires neuregulin-erbB receptor signaling in hypothalamic astrocytes. *J Neurosci, 23*(1), 230-239.

Pulver, A. E., Lasseter, V. K., Kasch, L., Wolyniec, P., Nestadt, G., Blouin, J. L., et al. (1995). Schizophrenia: a genome scan targets chromosomes 3p and 8p as potential sites of susceptibility genes. *Am J Med Genet, 60*(3), 252-260.

Purcell, S. M., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., Sullivan, P. F., et al. (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature, 460*(7256), 748-752.

Radley, J. J., & Morrison, J. H. (2005). Repeated stress and structural plasticity in the brain. *Ageing Res Rev, 4*(2), 271-287.

Rajji, T. K., Ismail, Z., & Mulsant, B. H. (2009). Age at onset and cognition in schizophrenia: meta-analysis. *Br J Psychiatry, 195*(4), 286-293.

Rapoport, J. L., Addington, A. M., Frangou, S., & Psych, M. R. (2005). The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry, 10*(5), 434-449.

Ripke, S., Sanders, A. R., Kendler, K. S., Levinson, D. F., Sklar, P., Holmans, P. A., et al. (2012). Genome-wide association study identifies five new schizophrenia loci. *Nat Genet, 43*(10), 969-976.

Saugstad, L. F. (1989). Age at puberty and mental illness. Towards a neurodevelopmental aetiology of Kraepelin's endogenous psychoses. *Br J Psychiatry, 155*, 536-544.

Schmidt-Kastner, R., van Os, J., Steinbusch, H., & Schmitz, C. (2006). Gene regulation by hypoxia and the neurodevelopmental origin of schizophrenia. *Schizophr Res, 84*(2-3), 253-271.

Scolnick, E. M., Petryshen, T., & Sklar, P. (2006). Schizophrenia: do the genetics and neurobiology of neuregulin provide a pathogenesis model? *Harv Rev Psychiatry, 14*(2), 64-77.

Sei, Y., Ren-Patterson, R., Li, Z., Tunbridge, E. M., Egan, M. F., Kolachana, B. S., et al. (2007). Neuregulin1-induced cell migration is impaired in schizophrenia: association with neuregulin1 and catechol-o-methyltransferase gene polymorphisms. *Mol Psychiatry, 12*(10), 946-957.

Shi, J., Levinson, D. F., Duan, J., Sanders, A. R., Zheng, Y., Pe'er, I., et al. (2009). Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature, 460*(7256), 753-757.

Simon, A. E., Cattapan-Ludewig, K., Zmilacher, S., Arbach, D., Gruber, K., Dvorsky, D. N., et al. (2007). Cognitive Functioning in the Schizophrenia Prodrome. *Schizophr Bull*.

Snitz, B. E., MacDonald, A. W., 3rd, & Carter, C. S. (2006). Cognitive deficits in unaffected first-degree relatives of schizophrenia patients: a meta-analytic review of putative endophenotypes. *Schizophr Bull, 32*(1), 179-194.

Stefanis, N. C., Trikalinos, T. A., Avramopoulos, D., Smyrnis, N., Evdokimidis, I., Ntzani, E. E., et al. (2007). Impact of schizophrenia candidate genes on schizotypy and cognitive endophenotypes at the population level. *Biol Psychiatry, 62*(7), 784-792.

Stefansson, H., Ophoff, R. A., Steinberg, S., Andreassen, O. A., Cichon, S., Rujescu, D., et al. (2009). Common variants conferring risk of schizophrenia. *Nature, 460*(7256), 744-747.

Stefansson, H., Sarginson, J., Kong, A., Yates, P., Steinthorsdottir, V., Gudfinnsson, E., et al. (2003). Association of neuregulin 1 with schizophrenia confirmed in a Scottish population. *Am J Hum Genet, 72*(1), 83-87.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., et al. (2002). Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet, 71*(4), 877-892.

Steinthorsdottir, V., Stefansson, H., Ghosh, S., Birgisdottir, B., Bjornsdottir, S., Fasquel, A. C., et al. (2004). Multiple novel transcription initiation sites for NRG1. *Gene, 342*(1), 97-105.

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry, 60*(12), 1187-1192.

Tabares-Seisdedos, R., & Rubenstein, J. L. (2009). Chromosome 8p as a potential hub for developmental neuropsychiatric disorders: implications for schizophrenia, autism and cancer. *Mol Psychiatry, 14*(6), 563-589.

Talmage, D. A. (2008). Mechanisms of neuregulin action. *Novartis Found Symp, 289*, 74-84; discussion 84-93.

Tarbox, S. I., & Pogue-Geile, M. F. (2008). Development of social functioning in preschizophrenia children and adolescents: a systematic review. *Psychol Bull, 134*(4), 561-583.

Thompson, J. L., Watson, J. R., Steinhauer, S. R., Goldstein, G., & Pogue-Geile, M. F. (2005). Indicators of genetic liability to schizophrenia: a sibling study of neuropsychological performance. *Schizophr Bull, 31*(1), 85-96.

Torkamani, A., Dean, B., Schork, N. J., & Thomas, E. A. (2010). Coexpression network analysis of neural tissue reveals perturbations in developmental processes in schizophrenia. *Genome Res, 20*(4), 403-412.

Tosato, S., Dazzan, P., & Collier, D. (2005). Association between the neuregulin 1 gene and schizophrenia: a systematic review. *Schizophr Bull, 31*(3), 613-617.

Toulopoulou, T., Picchioni, M., Rijsdijk, F., Hua-Hall, M., Ettinger, U., Sham, P., et al. (2007). Substantial genetic overlap between neurocognition and schizophrenia: genetic modeling in twin samples. *Arch Gen Psychiatry, 64*(12), 1348-1355.

Tuulio-Henriksson, A., Haukka, J., Partonen, T., Varilo, T., Paunio, T., Ekelund, J., et al. (2002). Heritability and number of quantitative trait loci of neurocognitive functions in families with schizophrenia. *Am J Med Genet, 114*(5), 483-490.

Weinberger, D. R. (1987). Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry, 44*(7), 660-669.

Weinberger, D. R., & Marenco, S. (2003). Schizophrenia as a neurodevelopmental disorder. In S. W. Hirsch & D. R. Weinberger (Eds.), *Schizophrenia.* (pp. 326-348). New York: Oxford.

Wolpowitz, D., Mason, T. B., Dietrich, P., Mendelsohn, M., Talmage, D. A., & Role, L. W. (2000). Cysteine-rich domain isoforms of the neuregulin-1 gene are required for maintenance of peripheral synapses. *Neuron, 25*(1), 79-91.

Yokley, J. L., Prasad, K. M., Chowdari, K. V., Talkowski, M. E., Wood, J., Gur, R. C., et al. (2012). Genetic associations between neuregulin-1 SNPs and neurocognitive function in multigenerational, multiplex schizophrenia families. *Psychiatr Genet, 22*(2), 70-81.

Zhang, H. X., Zhao, J. P., Lv, L. X., Li, W. Q., Xu, L., Ouyang, X., et al. (2008). Explorative study on the expression of neuregulin-1 gene in peripheral blood of schizophrenia. *Neurosci Lett, 438*(1), 1-5