GENETIC ASSOCIATION BETWEEN SCHIZOPHRENIA & NEUREGULIN-1: A STRATEGY OF CONVERGENT VALIDITY WITH COGNITION

by

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Recent work shows potentially promising associations between schizophrenia and polymorphisms in Neuregulin-1 (NRG1). A large literature has also found strong familial relationships between schizophrenia and cognitive deficits. Given the role of NRG1 in glutamate-NMDAR regulation, we hypothesize that cognitive deficits may be related to sequence variation within NRG1, thus providing a possible mechanism by which NRG1 could act as a susceptibility gene for schizophrenia.

This study examined the associations between NRG1, cognition, and schizophrenia using a multigenerational multiplex family sample (419 individuals from 40 families), including 58 affected participants (schizophrenia or schizoaffective disorder, depressed type) and their 361 unaffected relatives. In total, three samples were created from this participant pool: 1) the total sample, including affected and unaffected participants; 2) an unaffected subsample, consisting of only unaffected participants; and 3) the "No Diagnosis" subsample, consisting of participants with no diagnosis on the DIGS. In addition, a control group (N=199) was included for standardization of the cognitive data, but was not genotyped. Pedigree participants were genotyped using the SNPlex procedure for 40 NRG1 single nucleotide polymorphisms (SNPs). All participants completed structured diagnostic interviews and were administered a previously validated computerized neurocognitive battery that assessed eight cognitive

domains, including: abstraction/mental flexibility, attention, verbal/facial/spatial memory, spatial/emotional processing, and sensorimotor dexterity.

Pedigree-based variance component quantitative trait analyses using SOLAR were performed to test for associations between individual NRG1 SNPs and cognitive performance. Although the specific associations that were significant differed somewhat among subsamples, each sample had between 14-25 significant ($p \leq 0.05$) associations, encompassing multiple SNPs and multiple cognitive domains. The numerous significant findings in progressively diagnostically "cleaner" sub-samples may suggest that variation in NRG1 is more directly associated with cognition and is not solely secondary to an effect of schizophrenia or psychopathology. These associations may suggest a role for NRG1 in cognition that is a mediating risk factor for schizophrenia and/or psychopathology more generally. It is also possible that these associations with cognition are irrelevant to schizophrenia, but the literature on NRG1 and schizophrenia argues against this possibility.

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PREFACE

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1.0 INTRODUCTION

Schizophrenia is a disorder that is often characterized by hallucinations, delusions, and thought disorder in its acute phase. In the chronic phase of the illness, patients frequently suffer from negative symptoms, including blunted affect, loss of motivation, and poverty of speech. Despite these core similarities, patients vary considerably in their symptom profiles.

1.1 REVIEW OF GENETIC FINDINGS IN SCHIZOPHRENIA

Despite a great deal of research on the disorder, the details of its etiology remain largely unknown. Family, twin, and adoption studies have consistently suggested that genetic variation is the most important overall factor, with estimates of heritability (h^2) in the range of 0.80-0.85 (Cardno & Gottesman, 2000; Sullivan, Kendler, & Neale, 2003). Despite this knowledge, the genetic architecture of schizophrenia presents many complications, including a complex, polygenic transmission (Gottesman & Shields, 1967), reduced penetrance (Risch, 1990), and possible gene-environment and epistatic interactions. This complexity has made progress difficult and has slowed the elucidation of the molecular genetics of schizophrenia.

One method of identifying candidate genes for genetic disorders is linkage studies. Although the regions identified through linkage may be quite large and power may be quite low without very large sample sizes, more than twenty genome-wide linkage studies of schizophrenia have been published to date. Combined, they implicate a large percentage of the human genome

in the pathogenesis of the disorder, although many of the findings have failed upon replication. Two recent meta-analyses have reassessed these inconsistencies through the utilization of more stringent statistical methods, resulting in a significant reduction in the number of potential positional candidate regions. Only two loci were identified by both studies: 8p and 22q (Badner & Gershon, 2002; C. M. Lewis et al., 2003).

These difficulties with weak linkages have led researchers to test specific variants within or near a gene of interest using genetic association methods. Association studies correlate the frequency of certain alleles or haplotype combinations with phenotypic individual differences. Findings of association, however, are not necessarily causal. Instead, it may be that the observed variant and the unknown causal allele are in linkage disequilibrium (LD) with each other (Scolnick, Petryshen, & Sklar, 2006).

As with linkage analyses, many polymorphisms have been reported to be associated with schizophrenia and yet have failed to replicate upon further study. Only a handful of genes have been commonly replicated, however, including: neuregulin-1 (NRG1), catechol-O-methyl transferase (COMT), dysbindin (DTNBP1), regulator of G-protein signaling 4 (RGS4), metabotropic glutamate receptor 3 (GRM3), disrupted-in-schizophrenia 1 (DISC1), and others (see Harrison & Weinberger, 2005 for a review). In all, linkage and association studies have identified over 130 potential susceptibility genes, each with small effect sizes and inconsistent replication attempts (Carter, 2006).

Some of the variation in results across studies may be due to variation in allele frequency across samples. The power to detect associations between genes and disease phenotypes depends on allele frequencies, so some differences among studies may reflect allele frequency variation

between populations. In fact, important associations may be missed if the frequency of the allele in question is low in the population (Gardner et al., 2006).

Another puzzling aspect of these studies is that several have found that the same loci confers risk to schizophrenia, but some find that the risk allele is an Adenine, for example, while the others find that the risk allele is a Guanine. There are several possible explanations for this phenomenon, including differences in population substructure or differences in the haplotype background (Duan et al., 2005). However, it has also been theorized that these findings are proof of the null hypothesis, suggesting that disparate findings prove that there is no association between the gene and trait of interest.

1.2 NEUREGULIN-1 & SCHIZOPHRENIA

Based on a history of positive linkage to chromosome 8 and significant association findings, NRG1 is one of the strongest positional and functional candidates for schizophrenia, among a large number of putative susceptibility genes that have been suggested (Harrison & Law, 2006).

The neuregulin family of genes is a complex one, however. There are four members within the gene family, all of which are structurally-related glycoproteins that function as ligands for receptor tyrosine kinases of the ErbB family (Scolnick et al., 2006; Wolpowitz et al., 2000). NRG1 is a large gene, encompassing 1.3 million bases and including at least 21 exons (Steinthorsdottir et al., 2004). Approximately 0.3% of the gene codes for protein (Scolnick et al., 2006), and there are at least nine alternative promoters and 16 alternative splicing isoforms (Steinthorsdottir et al., 2004).

NRG1 was initially identified as a candidate gene for schizophrenia by a linkage study of Icelandic multiplex families with the disorder (Stefansson et al., 2003). Previous studies had

identified the 8p region, specifically 8p22-p11, as possessing one or more genes related to schizophrenia (Pulver et al., 1995). Careful mapping of this locus by Stefansson, et al. (2002) identified NRG1 as a specific candidate gene in that region. Using haplotype association and transmission disequilibrium tests (TDT), the group identified a risk haplotype in the 5' end of the gene. This combination of variants is referred to as the HapICE haplotype.

This initial study was subsequently replicated by the same research group in a Scottish population (Stefansson et al., 2003) and to date, nearly 50 replications have been conducted, with most of them finding evidence of an association between schizophrenia and NRG1 (SchizophreniaGene, 2009). Specifically, with case-control (C-C) association designs, 17/30 studies found a positive association between NRG1 and schizophrenia, while 10/19 family-based association tests (FBAT) found positive results. Overall, the estimates of relative risk (RR) lie between 1.0 and 2.2 for specific variants and haplotypes (Tosato, Dazzan, & Collier, 2005).

In addition, these associations have not been limited to schizophrenia in any one population (SchizophreniaGene, 2009). In studies using Caucasian samples only, 10/17 C-C and 4/10 FBAT studies found positive associations between NRG1 variants and schizophrenia. In Asian samples, 6/10 C-C and 4/5 FBAT studies found positive results. In less frequently studied populations, the results varied somewhat (African American samples: 1/1 C-C studies found positive results; Hispanic samples: 0/1 FBAT studies found positive results; mixed ethnicity samples: 0/2 positive C-C findings and 2/3 positive FBAT studies).

An alternate explanation of these findings, as for all association studies, is that the variants and haplotypes suggested to be associated with schizophrenia are actually in LD with other causative variants in chromosome 8 (Scolnick et al., 2006). Although this remains a concern for all association studies, there have been no other genes identified within 500kb of

either the 5' or 3' ends of NRG1 (Li, Collier, & He, 2006). This relative isolation makes it less likely that the association of this region with schizophrenia is due to LD with another causal gene. In the case of NRG1, Gardner et al. (2006) found that there are extreme differences across different populations in the frequencies of specific variant alleles and haplotypes. They suggest that these findings show clear evidence of haplotype clustering according to population and continental region, and that local selective forces may be influencing the gene. Nevertheless, although the specific loci that are significant between patients and controls vary somewhat between studies, most have found that some variants within NRG1 do associate with schizophrenia, regardless of population (SchizophreniaGene, 2009).

1.3 HYPOTHESIZED ROLE OF NRG1 IN SCHIZOPHRENIA

NRG1 plays an integral role in the development, organization, and function of the central nervous system (CNS). In general, it is described as a pleiotropic growth factor (Li et al., 2006). At least twelve functions have been identified for this gene, including hormonal control of puberty, modulation of long-term potentiation (LTP), regulation of N-methyl-D-aspartic acid (NMDA), $GABA_A$, and nicotinic receptors, and control of neuronal migration and differentiation (Harrison & Law, 2006). Because of this gene's role in the organization and development of basic CNS structures, as well as its role in the regulation of NMDA glutamate receptors throughout the lifespan, this gene may also be of functional importance in schizophrenia (Harrison & Law, 2006).

The potential relevance of these functions to schizophrenia is clear. There are several common neurobiological findings in this area: patients have decreased brain volume and enlarged ventricles (R. E. Gur et al., 2000; Honea, Crow, Passingham, & Mackay, 2005),

widespread abnormalities in cerebral blood flow (Brewer et al., 2007; Emri et al., 2006), and significant differences in neuronal density and migration ((Sei et al., 2007); Weinberger & Marenco, 2003) when compared to control groups. Variation in NRG1 may lead to incorrect CNS organization during development, in addition to any effects it has on synaptic "remodeling" in response to stressful experiences of the individual (Stefansson, Steinthorsdottir, Thorgeirsson, Gulcher, & Stefansson, 2004) or due to learning.

In addition, although the focus of much of the research on the biological basis of schizophrenia has been on dopamine receptors, the role of other neurotransmitters, specifically glutamate, cannot be ignored. The postulated biological relevance of NRG1 in schizophrenia is based on the glutamate hypothesis which arose from the phencyclidine (PCP) model of schizophrenia. Luby et al. (1959) found that when normal controls were given a subanesthetic dose of PCP, they displayed many of the symptoms that patients with schizophrenia suffered from, including psychosis, thought disorder, cognitive impairment, and apathy. The chemical cascade of PCP was later discovered and suggested that psychosis induced by this substance was the result of a non-competitive blockage of NMDA glutamate receptors (Javitt & Zukin, 1991; D. M. Thompson & Moerschbaecher, 1984; D. M. Thompson, Winsauer, & Mastropaolo, 1987).

Additional studies of NMDA antagonists, such as ketamine and MK-801, confirmed the role of glutamate in the manifestation of psychotic symptoms in normal controls, as well as the exacerbation of these symptoms in patients with schizophrenia (Carlsson, Waters, & Carlsson, 1999; Stefansson et al., 2003). In humans, studies of postmortem tissue have shown significant changes to glutamate receptor protein expression, binding, and transcription between patients with schizophrenia and controls (Konradi & Heckers, 2003; D. A. Lewis & Moghaddam, 2006). These findings resulted in the NMDA-glutamate hypothesis of schizophrenia, and suggested that

a hypoglutamatergic state was present in at least some regions of the brain. This does not imply that other neurotransmitter systems, such as dopamine, do not have a role in the disorder; it simply makes the further evaluation of glutamate in schizophrenia very important.

The putative role of NRG1 in this process lies in the influence NRG1 has on the expression of NMDA receptors through the activation of ErbB receptors. NRG1's product is brought to the synapse through vesicle support where it diffuses across the synaptic cleft to bind to ErbB receptors (Stefansson et al., 2004). Activation of ErbB receptors by this protein results in the addition of an extra phosphate group to the ErbB receptors that are co-localized with NMDA receptors at post-synaptic density protein 95 (PSD95; Fischbach, 2006). This signal is then transduced by other kinases that regulate NMDA kinetic properties, leading to a reduction in the phosphorylation of the NMDA receptors upon the binding of NMDA or glutamate (Gu, Jiang, Fu, Ip, & Yan, 2005; Hahn et al., 2006).

A recent study by Hahn et al. (2006) found that the levels of phosphorylation of activated NMDA receptors were reduced in brains from patients compared to controls. The authors suggest that enhanced endogenous NRG1-ErbB4 signaling may lead to a hypofunction of NMDA receptors, and thus a hypoglutamatergic state, such as that which is hypothesized in schizophrenia. Accordingly, a change in NRG1 may lead to an increased level of phosphorylation of ErbB4, which would result in an even stronger interaction between ErbB4 and PSD95. This interaction could lead to hypofunctionality of the NMDA receptors during acute periods, ultimately leading to a hypoglutamatergic state (Fischbach, 2006). Thus, variations within NRG1 may contribute to hypoglutamatergic states, potentially resulting in psychotic symptomology and schizophrenia.

1.4 COGNITION & SCHIZOPHRENIA

Despite the varied presentation and course of patients' symptoms, one symptom that is extremely common in schizophrenia is cognitive dysfunction. These deficits are relatively independent of illness state, and are usually global (Heydebrand, 2006). The association between cognitive deficits and schizophrenia is very strong. Even more striking is that similar deficits have also been identified in both persons who are at high-risk for developing the disorder, as well as the unaffected biological relatives of patients (Simon et al., 2007; Snitz, MacDonald, & Carter, 2006; J. L. Thompson, Watson, Steinhauer, Goldstein, & Pogue-Geile, 2005). The familiality of this dysfunction in even unaffected relatives suggests that cognitive impairment is actually a more sensitive measure of liability than symptoms alone (Snitz et al., 2006; J. L. Thompson et al., 2005).

Just as schizophrenia likely involves multiple neurotransmitter systems, the control of cognition also involves many factors, including multiple neurotransmitters. Of particular interest in this study is the effect that glutamate has on cognition. Glutamate is the major excitatory neurotransmitter in the brain and is integral to the development of long-term potentiation (LTP) (Robbins & Murphy, 2006; Tamminga, 2006).

LTP is one of the mechanisms that controls neuronal plasticity, and is important for learning and memory. Previous studies have shown that blockages of NMDA-glutamate receptors by NMDA antagonists inhibit the development of LTP, impairing several cognitive abilities. Specific deficits due to NMDAR blockage have been documented in rodents and include: spatial working memory in the Morris water-maze escape task (Morris, Anderson, Lynch, & Baudry, 1986), object recognition (Winters & Bussey, 2005), memory encoding during a flavor-place association task (Day, Langston, & Morris, 2003), as well as attentional deficits

and a failure to habituate (Nilsson, Waters, Waters, Carlsson, & Carlsson, 2001). Glutamatergic antagonist administration in humans has also been shown to have an effect on multiple cognitive domains, including impairments in verbal and nonverbal memory (Newcomer et al., 1999; Parwani et al., 2005), proverbs interpretation, working memory and set-shifting, and increased distractability (Krystal et al., 1999). These findings suggest that multiple domains of cognitive functioning are significantly impaired when the level of glutamate is low.

The results from these studies strongly suggest that NMDA-glutamate receptor integrity is important to normal functioning in several cognitive domains. Given the relationship between NRG1 and glutamate, it can be hypothesized that variation within NRG1 could potentially lead to significant differences in cognitive functioning in patients and their relatives.

1.5 CONVERGENT VALIDITY

The results of NRG1 studies of schizophrenia generally suggest that NRG1 is a potential susceptibility gene for this disorder. There is a large literature supporting the positional relevance of the gene, as well as new information which makes it an intriguing functional candidate, suggesting that the relationship between NRG1 and schizophrenia should be more thoroughly evaluated. Furthermore, because NRG1 is thought to decrease glutamate levels in the brain, and because glutamate is important in cognitive abilities, a very common deficit in patients and their relatives, evaluating the relationship of NRG1 and cognition is essential to understanding NRG1's potential role in schizophrenia.

In order to identify true susceptibility genes and variants rather than false positives, methods that attempt to take into account the relationship between genes and correlates of

schizophrenia may be helpful. One strategy, convergent validity, may increase the understanding of the small and inconsistent findings of previous molecular studies in this way.

Convergent validity is a technique which utilizes a secondary measure to elaborate the relationship between two variables (Campbell & Fiske, 1959). This type of validity is confirmed by the correlation between the primary and secondary measurements. Thus, if A is correlated with B, and B is correlated with C, a hypothesis of convergent validity would expect A to also be correlated with C. For example, in order to evaluate convergent validity for a candidate gene that may be associated with schizophrenia, the gene is correlated with another characteristic that is an important associate of the diagnosis. Given the aim of better understanding genetic effects in schizophrenia, this secondary phenotype should be a strong familial correlate of the disorder.

In particular, we utilized this strategy to assess the relationship between one of the strongest candidate genes in schizophrenia, NRG1, and one of the strongest familial correlates of the disorder, cognitive deficits. Studies have consistently found that patients with schizophrenia have pervasive deficits in cognition, largely independent of symptom state, chronicity, and cognitive domain being tested (Heinrichs, Ruttan, Zakzanis, & Case, 1997; Snitz et al., 2006). Studies of unaffected relatives of patients also show significant cognitive impairments (Cannon et al., 1994; Grove et al., 1991; Snitz et al., 2006). As with patients, these deficits seem to be general across tasks, although such hypotheses are difficult to evaluate. Together, these findings strongly suggest that cognitive impairment is a strong familial correlate of schizophrenia.

In addition to NRG1 and cognitive functioning both being important correlates of schizophrenia, the potential effects of NRG1 variation on glutamate function and glutamate's role in cognition provide a potential mechanism by which cognitive deficits may also be related to variation within NRG1, and possibly offering a way by which NRG1 acts as a susceptibility

gene for schizophrenia. Pairing cognition with NRG1 can serve as a method of convergent validity when assessed within families affected by schizophrenia.

1.6 REVIEW OF PREVIOUS STUDIES OF NRG1 & COGNITION

To specifically test the role NRG1 may have on cognition through its role in glutamate and NDMAR regulation, researchers have developed transmembrane hypomorphic mutant mice. Such studies have found that these mice are hyperactive and have moderate prepulse inhibition deficits, both of which are reversible by the antipsychotic medication clozapine (Stefansson et al., 2002). Subsequent studies also found that these mice had habituation-exploration deficits (O'Tuathaigh, Babovic, O'Meara et al., 2007) and impaired regulation of reciprocal social interactions (O'Tuathaigh et al., 2008). However, negative studies of spatial learning and working memory with regard to NRG1 variation have also been published (O'Tuathaigh, Babovic, O'Sullivan et al., 2007).

Six previous studies assessing specific NRG1 variants and cognition in humans have found mixed results. The most commonly assessed NRG1 variant in studies of cognition was SNP8NRG221533 (renamed: rs35753505). In normal controls, SNP8NRG221533/ rs35753505 was found to have no effect on working memory tasks (Krug et al., 2008). The same SNP was tested in patients with schizophrenia, finding significant effects on the blood flow in several regions, including the hippocampus, cerebellum, and both the posterior and anterior cingulate cortices (Kircher et al., 2008). However, that SNP was not associated with task performance in the same sample (Kircher et al., 2008). This SNP was also found to be associated with sustained attention in a sample of Greek male military conscripts (Stefanis et al., 2007).

Another NRG1 variant commonly studied in the context of cognition was SNP8NRG243177 (renamed: rs6994992). One study found no association between SNP SNP8NRG243177/rs6994992 and premorbid IQ on a composite measure of general neurocognitive performance in patients (Crowley et al., 2008), while another found an association between the same marker and lower premorbid IQ and lower fronto-temporal activation in patients (Hall et al., 2006), as well as a significant effect on verbal IQ and brain activation in verbal fluency tasks in participants at high-risk for developing the disorder (Hall et al., 2006). A third study found that SNP8NRG243177/rs6994992 was moderately associated with spatial working memory in a sample of Greek male military conscripts (Stefanis et al., 2007).

Two other variants, rs10503929 and microsatellite 433E1006, have also been assessed in the context of cognition. Hong et al. (2008) analyzed the association between rs10503929 and prepulse inhibition (PPI) in individuals with schizophrenia and healthy controls, finding that the marker was not associated with either PPI or schizophrenia in that sample. Microsatellite 433E1006 was 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).

1.7 AIMS & RATIONALE

The specific questions that this study aimed to address were:

1) Is cognition heritable in this multiplex family sample of schizophrenia? Trait heritability is a prerequisite for performing genetic association analyses.

2) Is variation in NRG1 associated with cognitive performance in patients and relatives of schizophrenic patients? If so, that would suggest that NRG1's mechanism of increasing risk for schizophrenia may be due, in part, to its relationship to cognition. If, however, the variation within NRG1 is not associated with cognitive performance, this would suggest that NRG1's putative mechanism of increasing risk for schizophrenia is unrelated to cognition.
3) Assuming a significant relationship between cognition and NRG1 exists, do these associations remain in diagnostically cleaner sub-samples of the participant pool? If the relationship remains in samples without psychopathology, this may imply that cognition may mediate the association between NRG1 and schizophrenia. It may also be the case that NRG1-cognition associations are unrelated to schizophrenia, but findings of association between NRG1 and schizophrenia suggest against this idea.
4) Is NRG1 associated with schizophrenia in this study? Despite the fact that our patient sample is small, we wanted to determine if there was a positive association between our gene of interest and the disorder in this study to assess generalizability.

2.0 METHODS

2.1 PARTICIPANTS

Written informed consent was obtained after the study procedures had been fully explained in accordance with the Institutional Review Boards of the University of Pittsburgh (PITT), University of Pennsylvania (PENN), and the Southwest Foundation for Biomedical Research. For participants younger than the age of 18, both the participant's and parents' consents were obtained.

2.1.1 Recruitment & Inclusion Criteria

Probands and their family members were identified 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 substance use, effects of 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. If, however, relatives met diagnostic criteria for schizophrenia or schizoaffective disorder-depressed type, but did not meet inclusion criteria, blood samples, but no neurocognitive testing, was obtained.

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. 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. In addition, potential control participants were matched as a group to index family members on age, sex, and ethnic background. All participants who passed the telephone screen and matching criteria were consented for the study and interviewed with the Diagnostic Interview for Genetic Studies, administered a computerized neurocognitive battery, and had blood drawn for DNA collection. Finally, consensus diagnosis was carried out for all PITT controls (see Procedures for more information).

Recruitment for PENN controls was done through convenience sampling and word of mouth. A screening interview was used to detect psychotic or cognitive disorders and potential participants were group-matched with index family members on age, sex, and ethnic background. 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 psychotic or cognitive disorders. They also completed the neurocognitive battery and blood draw. 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.

2.1.2 Description of the Sample

In total, there were 1608 pedigree members (43 index probands and 1565 relatives) available for recruitment and 230 unrelated controls. As can be seen in Table 1, 675 pedigree members and 230 normal controls of these individuals were 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 and 139 controls provided DNA. Thus, the final sample for the present study consisted of those remaining in the participant pool whose NRG1 genotypes were also successfully measured (see below for quality control measures), resulting in 419 pedigree members (23 index probands and 396 relatives) from 40 multiplex, multigenerational families.

Table 1. Attrition of Participants

	Pedigree Sample				Controls	Total Sample
	Families	Probands	Relatives	Total Pedigree Members		
Total Available Sample	43	43	1565	1608	230	1838
Enrolled in Study	43	43	632	675	230	905
Enrolled + Diagnosis	43	42	603	645	218	863
Enrolled + Diagnosis + CNB	42	30	538	568	199	767
Enrolled + Diagnosis + CNB + Blood drawn	42	30	523	553	139	692
Enrolled + Diagnosis + CNB + Blood drawn + NRG1	40	23	396	419	0	419

Note. Computerized neurocognitive battery abbreviated CNB. Neuregulin-1 abbreviated NRG1.

As shown in Tables 2 and 3, family size within this final sample ranged from one to 38 members (average members per family = 10.48, SD = 1.36), and the number of affected participants (with schizophrenia or schizoaffective disorder, depressed) per family in the final sample ranged from zero to four (average affected individuals per family = 1.45, SD = 5.61).

Table 2. Frequency Distribution of Family Size in Final Sample

Members in family*	Count of Families
1	3
2	3
3	0
4	5
5	3
6	1
7	4
8	4
9	1
10	3
11	1
12	2
13	0
14	1
15	0
16	1
17	0
18	1
19	0
20	0
21	1
22	1
23	0
24	1
25	1
26	1
27	0
28	0
29	0
30	0
31	1
32	0
33	0
34	0
35	0
36	0
37	0
38	1
Mean	10.48
SD	1.36

*Includes only those family members who completed all parts of the present study and are in the final sample

Table 3. Frequency of Affected Members per Family in the Final Sample

Members with schizophrenia or schizoaffective disorder, depressed	Count of Families
0	7
1	16
2	11
3	4
4	2
Mean	1.45
SD	5.61

*Includes only those members who completed all parts of the present study and are in the final sample

As shown in Table 4, there were 86 first-degree relatives of the index proband, 88 second-degree relatives, 97 third-degree relatives, 93 biological relatives extended past the third-degree, and 32 non-biological relatives within the final sample. No controls were genotyped for NRG1, 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 to whether DNA was collected.

Table 4. Demographics and Genetic Relationships in the Final Sample

	N	%Male	Mean Age (SD)	Age range	Mean Education (SD)	Education range	%Right Handed	%Pitt
Probands	**23**	**69.6%**	**42.3 (10.0)**	**22-54**	**12.5 (2.1)**	**9-17**	**91.3%**	**43.5%**
Relatives	**396**	**49.2%**	**44.9 (16.9)**	**15-84**	**13.3 (2.9)**	**6-20**	**85.9%**	**43.4%**
First-degree relatives	*86*	*47.7%*	*47.7 (14.6)*	*16-84*	*12.6 (3.0)*	*7-20*	*84.9%*	*48.8%*
Parent of proband	20	40.0%	62.8 (11.2)	43-84	11.8 (3.1)	7-18	80.0%	65.0%
Sibling of proband	56	50.0%	46.7 (9.3)	21-68	12.8 (3.0)	7-20	85.7%	41.1%
Child of proband	10	50.0%	23.7 (9.1)	16-47	12.6 (2.8)	9-17	90.0%	60.0%
Second-degree relatives	*88*	*55.7%*	*46.9 (20.9)*	*15-82*	*12.9 (2.9)*	*6-20*	*80.7%*	*42.1%*
Grandparent of proband	3	66.7%	71.0 (1.0)	70-72	12.7 (3.5)	9-16	100.0%	100.0%
Aunt/Uncle of proband	42	57.1%	64.4 (10.9)	42-82	12.6 (2.8)	8-18	78.6%	40.5%
Half-sibling of proband	2	50.0%	31.5 (2.1)	30-33	11.0 (1.4)	10-12	100.0%	50.0%
Niece/Nephew of proband	41	51.2%	27.9 (9.4)	15-56	13.2 (3.1)	6-20	80.5%	39.0%
Third-degree relatives	*97*	*49.5%*	*44.7 (12.5)*	*17-74*	*13.9 (2.9)*	*9-20*	*88.6%*	*55.7%*
1st cousin of proband	97	49.5%	44.7 (12.5)	17-74	13.9 (2.9)	9-20	88.6%	55.7%
Extended relatives	*93*	*45.7%*	*36.7 (16.6)*	*16-82*	*13.4 (2.7)*	*8-19*	*90.3%*	*40.9%*
Non-biological relatives	*32*	*43.8%*	*55.9 (11.6)*	*26-77*	*14.1 (2.9)*	*10-20*	*90.6%*	*3.1%*
Controls	**199**	**42.7%**	**47.3 (19.1)**	**18-84**	**14.9 (2.8)**	**8-20**	**87.9%**	**44.2%**

The clinical composition of the final sample is shown in Table 5. One hundred percent of the probands were diagnosed with schizophrenia. In addition, 30.4% of the proband sample had a comorbid diagnosis of a substance-related disorder. Among relatives, 8.1% were diagnosed with schizophrenia and 0.75% were diagnosed as schizoaffective disorder-depressed type. In addition, 9.1% were diagnosed with schizoaffective disorder, bipolar type, bipolar I or II, major depression with psychotic features, other organic or nonorganic psychosis, or cluster A personality disorder. In terms of non-psychotic affective disorders, 17.7% of relatives were

diagnosed with either major depression without psychotic features or another mood disorder (including mood disorders due to substance use or general medical conditions, and mood disorder NOS). Finally, 23.7% of relatives were diagnosed with a substance-related disorder. Among controls, 14.1% were diagnosed with major depressive disorder without psychotic features or another mood disorder, while 8.0% were diagnosed with a substance-related disorder.

Table 5. Clinical composition of the Final Sample

	Proband	**Relatives**	**Controls**
N	23	396	199
Schizophrenia	23	32	0
Schizoaffective Disorder, Depressed	0	3	0
Schizoaffective Disorder, Bipolar	0	2	0
Bipolar I & II	0	5	0
MDD with psychotic features	0	2	0
Other Psychosis	0	9	0
Cluster A Personality Disorder	0	18	0
MDD without psychotic features	0	70	24
Other Mood Disorder	0	43	4
Substance-related Disorder	7	94	16
Cognitive Disorder	0	0	0

Pedigree members were further classified into two mutually exclusive groups, affected or unaffected, as shown in Tables 6 and 7. The affected group consisted of 58 individuals diagnosed with schizophrenia or schizoaffective disorder, depressed. The unaffected group consisted of individuals diagnosed with any disorder other than schizophrenia or schizoaffective disorder, depressed, and those with no diagnoses on the DIGS (N=361). This group included those with spectrum diagnoses (schizoaffective disorder, bipolar type, bipolar I or II, major depression with psychotic features, other psychoses, or a cluster A personality disorder; N=36), non-spectrum diagnoses (major depression without psychotic features, other mood disorder, or substance-related disorder; N=147), and those with no diagnosis on the DIGS (N=178). In addition, 178 participants had no diagnoses on the DIGS, creating a "No Diagnosis" group.

Table 6. Demographic Information of the Final Pedigree Sample by Alternate Diagnostic Categories

	Affected^	Unaffected*^	No Diagnosis^
N	58	361	178
%Male	67.20%	47.70%	40.50%
Mean Age (SD)	43.7 (9.5)	44.9 (17.4)	45.9 (18.7)
Age range	22-59	15-84	15-82
Mean Education (SD)	12.5 (2.7)	13.4 (2.9)	13.5 (2.9)
Education range	7-20	6-20	8-20
%Pitt	41.40%	43.80%	46.10%
Index Probands (%)	23 (100.00%)	0 (0.00%)	0 (0.00%)
First-degree relatives (%)	28 (80.00%)	58 (16.10%)	27 (15.20%)
Second-degree relatives (%)	2 (5.70%)	86 (23.80%)	49 (27.50%)
Third-degree relatives (%)	2 (5.70%)	95 (26.30%)	48 (26.90%)
Extended biological relatives (%)	3 (8.60%)	90 (24.90%)	37 (20.80%)
Non-biological relatives (%)	0 (0.00%)	32 (8.90%)	17 (9.60%)

*Affected and unaffected are mutually exclusive groups. Within the unaffected group, there is a "no diagnosis" subgroup.

^The affected group includes all participants with schizophrenia or schizoaffective disorder-depressed. The unaffected group includes all participants with diagnoses other than schizophrenia or schizoaffective disorder-depressed, including those with no diagnoses on the DIGS.

Table 7. Clinical composition of the Final Pedigree Sample by Alternate Diagnostic Categories

	Affected*^	Unaffected*^	No Diagnosis
N	58	361	178
Schizophreniaº	55	0	0
Schizoaffective Disorder, Depressedº	3	0	0
Schizoaffective Disorder, Bipolarº	0	2	0
Bipolar I & IIº	0	5	0
MDD with psychotic featuresº	0	2	0
Other Psychosisº	0	9	0
Cluster A Personality Disorderº	0	18	0
MDD without psychotic featuresº	0	70	0
Other Mood Disorderº	6	37	0
Substance-related Disorderº	19	82	0
Cognitive Disorderº	0	0	0

*Affected and unaffected are mutually exclusive groups. Within the unaffected group, there is a "no diagnosis" subgroup.

^The affected group includes all participants with schizophrenia or schizoaffective disorder-depressed. The unaffected group includes all participants with diagnoses other than schizophrenia or schizoaffective disorder-depressed, including those with no diagnoses on the DIGS.

ºThe following categories are mutually exclusive: schizophrenia, schizoaffective disorder-depressed, schizoaffective disorder-bipolar, Bipolar I & II, MDD with and without psychotic features

2.1.3 Attrition Analyses

Table 8 displays the demographics and genetic relationships of participants who were enrolled in the overall study, but did not complete one or more portions of the present study, and thus are not included in the final sample. The total pool of participants lost to attrition consisted of 287 individuals (20 index probands, 236 relatives, and 31 normal controls). Diagnostic information on this sample can be found in Table 9.

Table 8. Demographics and Genetic Relationships in the Attritional Sample

	N	%Male	Mean Age (SD)	Age range	Mean Education (SD)	Education range	%Pitt
Probands	**20**	**47.4%**	**51.5 (14.9)**	**20-82**	**12.0 (2.5)**	**8-16**	**36.8%**
Relatives	**236**	**43.6%**	**47.3 (19.8)**	**15-97**	**13.0 (3.2)**	**4-20**	**35.6%**
First-degree relatives	*70*	*44.3%*	*54.1 (17.9)*	*24-89*	*13.3 (3.1)*	*8-20*	*40.0%*
Parent of proband	23	30.4%	71.5 (11.5)	40-89	12.6 (3.4)	8-20	30.4%
Sibling of proband	42	50.0%	47.9 (13.9)	24-84	13.8 (3.1)	8-20	45.2%
Child of proband	5	60.0%	30.0 (5.8)	25-39	13.2 (1.6)	12-16	40.0%
Second-degree relatives	*54*	*44.4%*	*47.2 (22.8)*	*15-97*	*12.5 (3.9)*	*4-20*	*35.2%*
Grandparent of proband	3	33.3%	82.0 (14.1)	69-97	7.0 (4.4)	4-12	33.3%
Aunt/Uncle of proband	17	41.2%	69.7 (13.3)	41-87	11.3 (3.5)	6-19	35.2%
Half-sibling of proband	2	50.0%	26.5 (7.8)	21-32	10.5 (2.1)	9-12	50.0%
Niece/Nephew of proband	32	43.8%	33.3 (12.5)	15-53	14.0 (3.3)	9-20	34.4%
Third-degree relatives	*30*	*56.7%*	*44.4 (16.3)*	*15-77*	*13.4 (2.6)*	*8-18*	*46.7%*
1st cousin of proband	30	56.7%	44.4 (16.3)	15-77	13.4 (2.6)	8-18	46.7%
Extended relatives	*58*	*36.2%*	*36.8 (16.7)*	*15-86*	*12.4 (2.7)*	*6-20*	*39.7%*
Non-biological relatives	*24*	*45.8%*	*56.9 (16.7)*	*28-87*	*14.3 (3.1)*	*9-20*	*0.0%*
Controls	**31**	**45.2%**	**30.7 (9.4)**	**19-53**	**NA**	**NA**	**0.0%**

*No education information was available for controls in the complement sample

Table 9. Clinical Composition of the Attritional Sample

	Proband	Relatives	Controls		
N	20	207	31		
Schizophrenia	19	25	0		
Schizoaffective Disorder, Depressed	1	6	0		
Schizoaffective Disorder, Bipolar	0	2	0		
Bipolar I & II	0	3	0		
MDD with psychotic features	0	2	0		
Other Psychosis	0	8	0		
Cluster A Personality Disorder	0	7	0		
MDD without psychotic features	0	47	0		
Other Mood Disorder	2	16	0		
Substance-related Disorder	4	59	0		
Cognitive Disorder	0	2	0		
* The following categories are mutually exclusive: schizophrenia, schizoaffective disorder-depressed, schizoaffective disorder-bipolar, Bipolar I & II, MDD without psychotic features, and MDD with psychotic features.					
**29 relatives in the attritional sample have missing diagnostic information					

Comparisons were made between the final and attrition samples to determine the representativeness of the final sample. Probands in the final sample were significantly younger than probands lost to attrition (t=2.39, df=40, p=0.022), but no significant differences were found in sex, personal education, or parental education. No significant differences were detected for any demographic variables between the final and attritional relative samples. Finally, the controls in the final sample were significantly older than those in the attritional sample (t=-7.63, df=75.6,

p=0.000). Differences in personal and parental education could not be tested due to the absence of education information for the controls lost to attrition.

Finally, comparisons between diagnoses present in the final and attritional sample were made to further determine the representativeness of the final sample. There were no significant differences between the frequency of specific diagnoses in the final and attritional sample of probands. Significantly fewer relatives had a diagnosis of schizoaffective disorder-depressed type, in the final sample when compared to the attritional sample (χ^2=4.24, df=1, p<0.05). In addition, significantly more controls had a diagnosis of major depressive disorder, without psychotic features, in the final sample when compared to the attritional sample (χ^2=4.17, df=1, p<0.05).

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

2.2.2.1 Abstraction and 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 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).

2.2.2.4 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).

2.2.2.5 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).

2.2.2.6 Spatial Processing. Judgment of Line Orientation (A.L. Benton, N.R. Varney, & K.S. 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).

2.2.2.7 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).

2.2.2.8 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).

2.2.2.9 Outcome. To help ensure the validity of the cognitive data, participants with missing data on 10 or more tasks in the battery were excluded from analyses (N=27; 5 probands, 3 relatives, and 19 controls). 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 participant's behavior during testing or non-standard testing conditions. In addition, controls recruited prior to the current study had higher rates of missing data due to tests added to the battery at a later time. Averaging over all of the tests in the computerized neurocognitive battery, the rate of missing data per test in the final sample was 4.7% (SD = 0.04), 2.1% (SD = 0.02), and 4.2% (SD = 0.06) for probands, relatives, and controls, respectively. As shown in Table 10, average rates of missing data per person were 1.17, 0.52, and 1.11 domain scores for probands, relatives, and controls, respectively, out of 24 total domain scores.

Table 10. Frequency of missing CNB data in the Final Sample by Group

Count of Missing Domain Scores	Probands	Relatives	Controls
0	16	341	155
1	0	0	0
2	0	0	0
3	6	45	24
4	0	0	1
5	0	1	0
6	0	5	4
7	0	0	6
8	0	0	3
9	1	4	6
Mean	1.17	0.52	1.11

2.2.3 Selection of SNPs & Primer Design

Fifty-four SNPs were chosen for this study to fit one of several categories: primary, haplotype, other, and redundant primary. All SNPs had a minor allele frequency (MAF) of at least 5% in European American populations according to Ensembl (release 43), dbSNP (build 127), and HapMap (release 21a).

"Primary" SNPs were those that were positively associated with schizophrenia by at least one study, resulting in 23 SNPs. Next, SNPs were chosen for coverage based on the haplotype blocks (e.g. HapICE, HapIRE, etc.). As many of the original haplotype markers were microsatellites, SNPs located near those microsatellites were chosen to attempt to capture that variation. All of the SNPs within these haplotype block areas were entered into HClust/R program to determine LD. This led to 19 non-redundant ($r^2 \leq 0.80$) "haplotype" SNPs. SNPs not listed in HapMap, and thus impossible to run through HClust, were included by default. The "other" category consists of SNPs from within exons or untranslated regions (UTR). Upon

proposal of the study, HapMap's HapMart program (release 22) listed four nonsynonymous exonic SNPs (one of which was already a primary SNP) and one 5' UTR, all of which were included in the SNP pool. By the conclusion of the study, newly published information on the structure of NRG1 resulted in HapMap's HapMart program (release 23a) changing the categorization of two of the original four exonic SNPs to intronic, and listing 21 UTR SNPs, seven of which are included in the final SNP pool. No other designations of SNP-type changed.

The remaining SNPs were chosen for redundancy purposes in the "redundant primary" category. These SNPs were chosen based on their LD value ($r^2 \geq 0.80$) with the primary SNPs, leading to eight additional SNPs. This step built redundancy into the design for several of the primary SNPs to be used in the event that one of the primary SNPs could not be genotyped.

After choosing the SNPs for the study, the list 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 Oligo Pool Assay (OPA). The design system checked for a noncompetitive reaction, deleterious pooling, and small pooling within the proposed pool. A total of six designs were submitted before a combination of 48 SNPs cleared the algorithm as being able to function appropriately within one OPA pool. Primary SNP SNP8NRG243177/rs6994992 failed the algorithm in each design that was submitted due to small pooling, and thus could not be included in the final OPA. In addition, primary SNP rs32016134 was excluded due to a genome error, as this is a mouse SNP, but was labeled as a human NRG1 SNP associated with schizophrenia in Munafo et al. (2006). Finally, rs776365, rs1566778, rs2466084, and rs3735775 were excluded to reduce the size of the SNP pool to the required 48. These particular SNPs were chosen for exclusion because they were redundant for primary and haplotype SNPs that already had at least one redundant in the pool.

As seen in Tables 11 and Figure 1, the final design contained 21 primary, 19 haplotype, 4 other, and 4 redundant primary SNPs.

Table 11. Proposed List of SNPs

Genome Location (bp)	Marker	Type	Category
31593281	SNP8NRG221132	Upstream	Primary
31593682	SNP8NRG221533/rs35753505	Upstream	Primary
31595198	rs10096573	Upstream	Redundant Primary
31604412	rs4298458	Upstream	Primary
31613876	SNP8NRG241930	Intron	Primary
31619806	rs1081062	Intron	Primary
31653120	rs13274954	Intron	Redundant Primary
31693237	rs4566990	Intron	Haplotype
31760521	rs1354335	Intron	Haplotype
31764413	rs1354336	Intron	Haplotype
31798512	rs13256173	Intron	Redundant Primary
31799612	rs1354334	Intron	Primary
31817937	SNP8NRG444511/rs13268724	Intron	Primary
31822702	SNP8NRG449280	Intron	Haplotype
31836504	rs776401	Intron	Haplotype
31853301	rs1473438	Intron	Primary
31878179	rs776382	Intron	Haplotype
31899469	rs1481438	Intron	Haplotype
31902481	rs800501	Intron	Haplotype
31950557	rs1462893	Intron	Haplotype
32018532	rs10954821	Intron	Haplotype
32178170	rs726908	Intron	Haplotype
32184904	rs1481747	Intron	Haplotype
32501778	rs10954855	Intron	Haplotype
32545133	rs2439306	Intron	Haplotype
32562632	rs2466062	Intron	Haplotype
32572900	rs3924999	Exon (R/Q AA change)	Primary
32592113	rs5890668	Intron	Primary
32592302	rs6150532	Intron	Primary
32595233	rs2466060	Intron	Haplotype
32612634	rs2439272	Intron	Primary
32620351	rs6468121	Intron	Primary
32621168	rs2466044	Intron	Redundant Primary
32626691	rs2466058	Intron	Primary
32634458	rs2466049	Intron	Primary
32646823	rs723811	Intron	Haplotype
32665458	rs6988339	Intron	Primary
32667621	rs10691392	Intron	Haplotype
32671731	rs2975498	Intron	Primary
32680307	rs2919382	Intron	Primary
32692525	rs2976525	Intron	Primary
32702243	rs4262285	Intron	Primary
32704976	rs3735776	Intron	Primary
32727416	rs4512342	Intron	Haplotype
32733525	rs10503929	Exon (M/T AA change)	Other
32743929	rs6992642	Downstream	Other
32744370	rs3735781	Downstream	Other
32744399	rs3735782	Downstream	Other

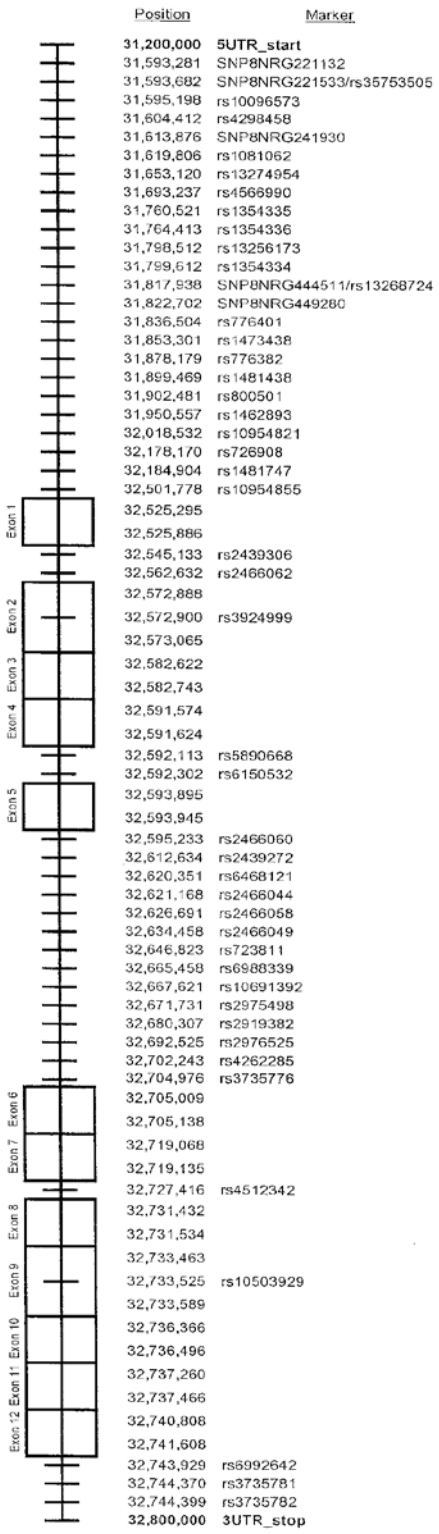

Figure 1. Line Diagram of NRG1 with SNP Locations

2.2.4 Genotyping Methods

Blood was collected in ethylenediamine tetraacetic acid (EDTA) tubes and frozen at either -70°C or -140°C 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 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. Individual analysis helped to control for 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 in a "clean-up" stage using the procedures outlined above. Genotyping was attempted one final time on those samples that continued to be problematic during the initial clean-up stage using the original mainstock samples. Samples that failed initial genotyping and both clean-up stages were excluded from all of the subsequent analyses (N=40). Next, all samples that passed the quality control standards, but failed on ten or more individual SNPs, were excluded from analysis to control for poor sample quality, contamination, and/or procedural error (N=4). In addition, all participants with one or more Mendelian Errors (N=6), as assessed by PedCheck (O'Connell & Weeks, 1998), were excluded. One individual had

homozygous genotypes at every SNP, suggesting poor sample quality or procedural error, and was excluded. In addition, five samples failed the first analysis and a clean-up analysis using the plated DNA, but were missing mainstock vials and could not be run in a second clean-up stage. Finally, 78 samples collected at the PENN site were not plated at the PITT site at the time of analysis and could not be included. Overall, this resulted in the loss of 134 participants who were enrolled, completed the diagnostic and neuropsychological portions of the study, and whose DNA was collected, but who were not successfully genotyped for NRG1 SNPs.

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 by hand independently by three of the investigators (JLY, KP, and MET) and results were checked for fidelity.

On the level of SNP performance, three SNPs (rs10691392, rs5890668, rs615032) were excluded for being insertion/deletion polymorphisms rather than true SNPs. In addition, one SNP (rs1481438) was excluded from analyses as, although it was listed as a NRG1 SNP by Munafo et al. (2006), it is actually located within a gene on chromosome six. Three SNPs (rs1481747, rs776382, rs800501) were also excluded for high failure rates (>20%). One SNP (SNP8NRG449280) was monomorphic in the sample and was excluded. After removing these SNPs, the total SNP pool for analysis contained 40 SNPs (19 primary, 13 haplotype, 4 other, and 4 redundant primary), as shown in Table 12. Every SNP was in Hardy-Weinberg Equilibrium,

except rs2919382 (p=0.0173), as calculated by SOLAR, with between 0% and 12.65% genotyping failure per SNP (mean failure = 1.09%, SD = 2.71).

Table 12. List of Successful SNPs

SNP	Location	Major Allele	Minor Allele	Minor Allele Frequency	HWE (p)	Type	Category	Successful Genotyping Rate, N (% Failure)
SNP8NRG221132	31593281	G	A	0.1047	0.092	Upstream	Primary	419 (0.00%)
SNP8NRG221533	31593682	T	C	0.3357	0.8267	Upstream	Primary	415 (0.95%)
rs10096573	31595198	T	G	0.4248	0.8138	Upstream	Redundant Primary	419 (0.00%)
rs4298458	31604412	G	C	0.4273	0.7365	Upstream	Primary	419 (0.00%)
SNP8NRG241930	31613876	G	T	0.323	0.9984	Intron	Primary	413 (1.43%)
rs1081062	31619806	T	C	0.2624	0.5541	Intron	Primary	417 (0.48%)
rs13274954	31653120	T	A	0.2499	0.7163	Intron	Redundant Primary	419 (0.00%)
rs4566990	31693237	G	A	0.3602	0.8488	Intron	Haplotype	419 (0.00%)
rs1354335	31760521	C	G	0.1818	0.5845	Intron	Haplotype	410 (2.15%)
rs1354336	31764413	T	C	0.2504	0.4461	Intron	Haplotype	414 (1.19%)
rs13256173	31798512	A	G	0.1821	0.3825	Intron	Redundant Primary	419 (0.00%)
rs1354334	31799612	G	T	0.3806	0.612	Intron	Primary	419 (0.00%)
SNP8NRG444511	31817937	T	A	0.1821	0.3825	Intron	Primary	419 (0.00%)
rs776401	31836504	T	C	0.3677	0.7222	Intron	Haplotype	417 (0.48%)
rs1473438	31853301	A	G	0.3741	0.8179	Intron	Primary	416 (0.72%)
rs1462893	31950557	C	G	0.2064	0.7706	Intron	Haplotype	417 (0.48%)
rs10954821	32018532	G	A	0.3049	0.6254	Intron	Haplotype	419 (0.00%)
rs726908	32178170	A	G	0.4833	0.4034	Intron	Haplotype	416 (0.72%)
rs10954855	32501778	T	A	0.2292	0.6433	Intron	Haplotype	419 (0.00%)
rs2439306	32545133	G	A	0.2172	0.7888	Intron	Haplotype	397 (5.25%)
rs2466062	32562632	A	G	0.2714	0.1154	Intron	Haplotype	418 (0.24%)
rs3924999	32572900	C	T	0.3857	0.8048	Exon (R/Q change)	Primary	417 (0.48%)
rs2466060	32595233	G	A	0.4882	0.4585	Intron	Haplotype	374 (10.74%)
rs2439272	32612634	C	T	0.44	0.5747	Intron	Primary	418 (0.24%)
rs6468121	32620351	G	T	0.453	0.1228	Intron	Primary	415 (0.95%)
rs2466044	32621168	T	C	0.0831	0.8241	Intron	Redundant Primary	417 (0.48%)
rs2466058	32626691	G	A	0.0862	0.7073	Intron	Primary	419 (0.00%)
rs2466049	32634458	C	T	0.0809	0.6305	Intron	Primary	416 (0.72%)
rs723811	32646823	T	C	0.0883	0.8834	Intron	Haplotype	419 (0.00%)
rs6988339	32665458	A	G	0.4153	0.8957	Intron	Primary	419 (0.00%)
rs2975498	32671731	A	G	0.1794	0.1645	Intron	Primary	419 (0.00%)
rs2919382	32680307	T	C	0.167	0.0173	Intron	Primary	419 (0.00%)
rs2976525	32692525	A	C	0.0879	0.8411	Intron	Primary	419 (0.00%)
rs4262285	32702243	C	T	0.043	0.4804	Intron	Primary	419 (0.00%)
rs3735776	32704976	C	A	0.1553	0.9587	Intron	Primary	366 (12.65%)
rs4512342	32727416	T	G	0.1037	0.7385	Intron	Haplotype	417 (0.48%)
rs10503929	32733525	T	C	0.1814	0.5694	Exon (M/T change)	Other	419 (0.00%)
rs6992642	32743929	T	C	0.4059	0.6583	Downstream	Other	419 (0.00%)
rs3735781	32744370	A	G	0.418	0.5275	Downstream	Other	416 (0.72%)
rs3735782	32744399	C	A	0.4827	0.1678	Downstream	Other	418 (0.24%)

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, with the exception of a few high LD SNPs that were built into the pool for redundancy.

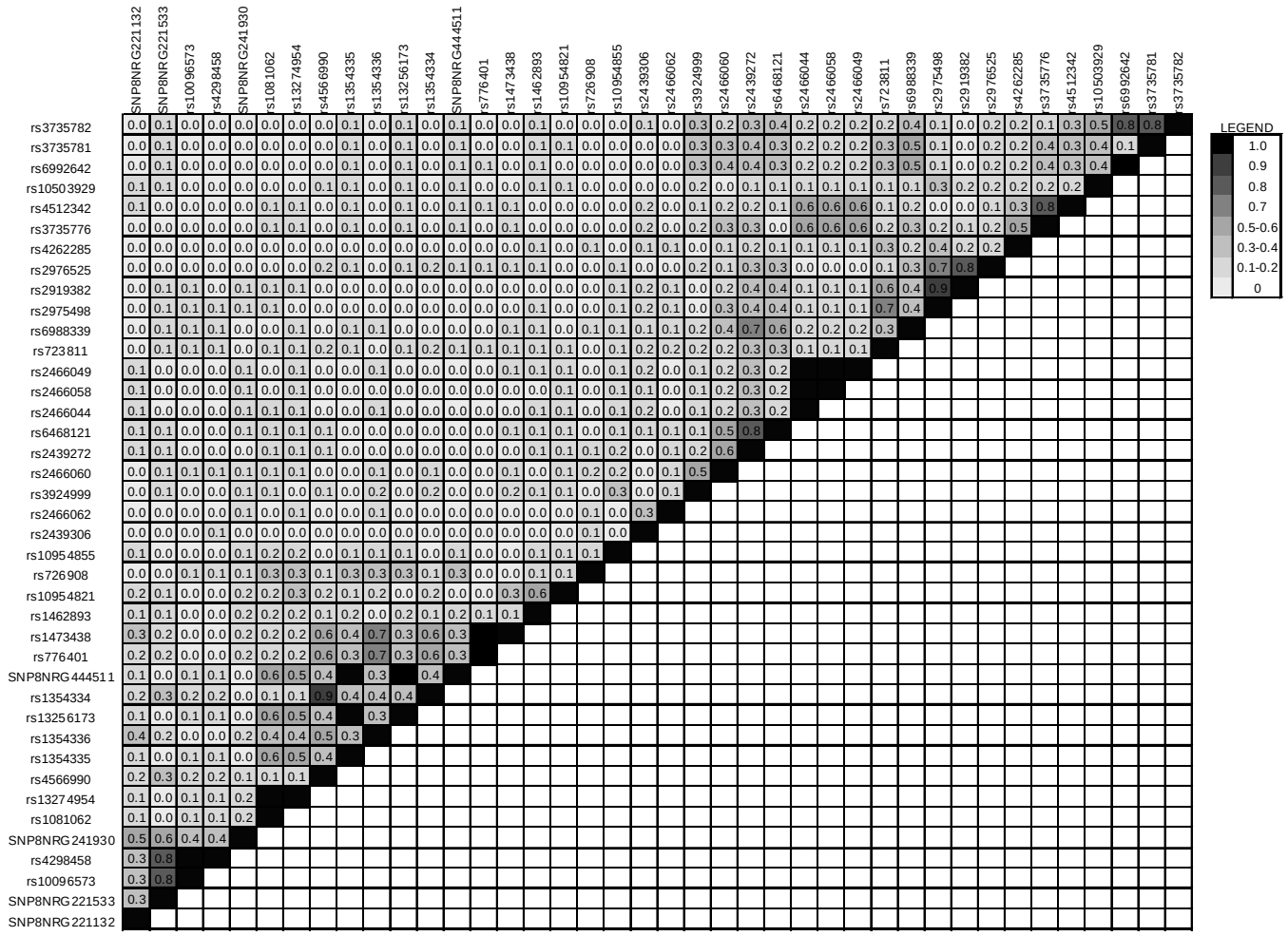

Figure 2. Linkage Disequilibrium (rho) Values

3.0 RESULTS

3.1 DEMOGRAPHIC COMPARISONS

Comparisons between the final proband and final control samples revealed that there were significantly more females in the control sample (t=-2.58, df=27.96, p=0.016) and that controls had significantly more personal education than probands in this sample (t=-5.03, df=218, p=0.000; see Table 4). In addition, both age and paternal education showed trends towards significance, with controls being older (t=-1.99, df=43.33, p=0.052) and having more paternal education (t=-1.91, df=211, p=0.058) than probands.

Comparisons between the final relative and final control samples revealed that controls had significantly more personal (t=-7.85, df=480.3, p=0.000), maternal (t=-5.09, df=520, p=0.000), and paternal (t=-4.79, df=509, p=0.000) education than relatives, but were not significantly different on age or sex.

When the final sample was broken down into affected, unaffected, and control participants (Table 6), comparisons between the affected and control participants showed significantly more females in the control sample (t=-3.43, df=96.45, p=0.001) and significantly more personal education for controls than affected participants in this sample (t=-5.91, df=255, p=0.000). In addition, control participants had greater paternal education than affected participants (t=-1.93, df=236, p=0.55).

Comparisons between unaffected pedigree members and controls found that control participants had significantly more personal (t=-6.16, df=558, p=0.000), maternal (t=-5.14,

df=491, p=0.000), and paternal (t=-5.17, df=485, p=0.000) education than unaffected participants, but were not significantly different on age or sex.

3.2 NEUROCOGNITIVE BATTERY

The cognitive data for the final control and pedigree samples were checked for outliers by box plot analysis collapsed over group. Two domain scores had one extreme outlier each. These outliers were each more than six standard deviations from the next most extreme score, and were Winsorized to reduce the amount of effect the data point would exert on the distribution as a whole. Next, all participants' domain scores were converted to z-scores, with the present study's final control group data serving as the standard. As seen in Tables 13-15, this standardization resulted in the control group's mean being zero (SD=1) for every domain. As expected, there was a significant difference in performance (as measured by efficiency) between affected participants and controls for every CNB domain (p=0.000). The performance of unaffected relatives was also poorer than controls for every domain, except for attention and abstraction and mental flexibility's speed scores. In addition, most domains had significant skew for all groups on all domain categories (efficiency, accuracy, and speed).

Table 13. Neurocognitive Battery Descriptive Data (Efficiency)

	Affected Relatives & Probands				Unaffected Relatives				Controls			
	N	Mean	Std. Deviation	Skew ness	N	Mean	Std. Deviation	Skew ness	N	Mean	Std. Deviation	Skew ness
Abstraction & Mental Flexibility	55	-1.227	1.1671	0.846	348	-0.169	1.1077	-0.515	199	0	1	-0.8292
Attention	55	-1.805	2.0679	-1.426	344	-0.344	1.4281	-2.04	159	0	1	-2.3109
Verbal Memory	58	-1.209	1.3732	-0.358	360	-0.331	1.0963	-0.802	185	0	1	-0.4262
Facial Memory	58	-0.924	0.9303	-0.04	360	-0.257	1.0034	-0.605	188	0	1	-0.3713
Spatial Memory	57	-0.789	1.0051	0.073	359	-0.175	0.9539	-0.074	199	0	1	-0.097
Spatial Processing	52	-1.125	1.4523	-0.346	353	-0.211	1.1041	-0.874	195	0	1	-0.5398
Sensorimotor Dexterity	54	-1.397	1.7499	-1.234	345	-0.235	1.2738	-4.136	191	0	1	-2.4976
Emotional Processing	57	-1.554	1.2852	-0.136	359	-0.29	1.1571	-0.748	194	0	1	-0.8743

Standard error of the skew ness for affected relatives and probands ranged betw een 0.3140-0.3250; SE for unaffected relatives ranged betw een 0.1290-0.1310; SE for controls ranged betw een 0.1723-0.1925.

Table 14. Neurocognitive Battery Descriptive Data (Accuracy)

	Affected Relatives & Probands				Unaffected Relatives				Controls			
	N	Mean	Std. Deviation	Skewness	N	Mean	Std. Deviation	Skewness	N	Mean	Std. Deviation	Skewness
Abstraction & Mental Flexibility	55	-1.23	1.2174	0.806	348	-0.17	1.1106	-0.577	199	0	1	-0.9255
Attention	55	-1.74	2.1278	-1.609	344	-0.39	1.443	-2.288	159	0	1	-2.9889
Verbal Memory	58	-1.26	1.5838	-0.642	360	-0.31	1.1803	-1.125	195	0	1	-0.6453
Facial Memory	58	-0.97	1.00156	-0.086	360	-0.23	1.01552	-0.69	188	0	1	-0.6646
Spatial Memory	57	-0.81	1.1216	0.017	359	-0.15	0.9749	-0.166	199	0	1	-0.1457
Spatial Processing	53	-1.16	1.633	-0.436	353	-0.19	1.1891	-0.936	198	0	1	-0.6003
Sensorimotor Dexterity	54	-1.18	2.4003	-2.124	345	-0.09	1.6107	-7.056	191	0	1	-4.8296
Emotional Processing	57	-1.53	1.4126	-0.272	359	-0.26	1.1557	-0.845	194	0	1	-1.434

Standard error of the skewness for affected relatives and probands ranged between 0.3140-0.3270; SE for unaffected relatives ranged between 0.1290-0.1310; SE for controls ranged between 0.1723-0.1925.

Table 15. Neurocognitive Battery Descriptive Data (Speed)

	Affected Relatives & Probands				Unaffected Relatives				Controls			
	N	Mean	Std. Deviation	Skewness	N	Mean	Std. Deviation	Skewness	N	Mean	Std. Deviation	Skewness
Abstraction & Mental Flexibility	55	-0.47	0.9704	-1.029	348	0.076	0.8953	-2.726	199	0	1	-3.0384
Attention	55	-0.92	1.2665	-0.84	344	0.031	1.0084	-0.75	159	0	1	-0.7978
Verbal Memory	58	-0.84	1.7162	-2.575	360	-0.32	1.51	-2.728	195	0	1	-1.3268
Facial Memory	58	-0.24	1.0668	-1.909	360	-0.32	1.6285	-3.403	188	0	1	-1.5394
Spatial Memory	57	-0.21	1.2636	-2.455	359	-0.17	1.1166	-2.004	199	0	1	-2.2258
Spatial Processing	52	-1.4	2.7318	-2.555	353	-0.21	1.3287	-3.028	198	0	1	-1.6117
Sensorimotor Dexterity	54	-1.37	1.4406	-0.414	345	-0.32	1.2955	-2.581	191	0	1	-2.5124
Emotional Processing	57	-0.4	1.1085	-1.511	359	-0.11	1.1577	-3.375	194	0	1	-1.4521

Standard error of the skewness for affected relatives and probands ranged between 0.3140-0.3300; SE for unaffected relatives ranged between 0.1290-0.1310; SE for controls ranged between 0.1723-0.1925.

3.2.1 Heritability (h^2)

Heritability was estimated in SOLAR for each cognitive domain. Potential covariates included in the polygenic model for heritability estimates and associations included: age, sex, age^2, age-by-sex, age^2-by-sex, and handedness. Each covariate was screened separately and retained in the model if it was significant at $p<0.10$. The retained covariates and heritability estimates for every cognitive domain are shown in Table 16, with values closely matching the heritability levels found by Gur et al. (2007). All of the heritability estimates were significant and ranged from 0.23 to 0.57 (efficiency), 0.24-0.52 (accuracy), and 0.07-0.54 (speed), indicating the importance of total genetic variation for all of the cognitive domains.

Table 16. Heritability Estimates for CNB Domains (Accuracy, Time, & Efficiency) in the Final Sample

		Abstraction & Mental Flexibility	Attention	Verbal Memory	Facial Memory	Spatial Memory	Spatial Processing	Sensorimotor Dexterity	Emotional Processing
	N	402	398	417	417	415	405	398	415
Efficiency	**h^2**	0.3737	0.2961*	0.5327*	0.3963	0.4428	0.5682	0.2324*	0.4517
	p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000
	Covariates	age, sex, age^2	age, age^2, age^2*sex	age, sex	age, sex, age^2	age	age, sex, age^2, age^2*sex, handedness	age	age, sex, age^2
Accuracy	**h^2**	0.3441	0.2406*	0.4906*	0.3805	0.4145	0.5176*	Not applicable^	0.4273*
	p-value	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000		0.0000
	Covariates	age, sex, age^2	age, age2, age^2*sex	age, sex	age, sex, age2	age	sex, age*sex		age, sex, age^2
Speed	**h^2**	0.1311*	0.5397*	0.1896*	0.3506*	0.1040*	0.1127*	0.2477*	0.0700*
	p-value	0.0201	0.0000	0.0103	0.0000	0.0655	0.0970	0.0008	0.1725
	Covariates	age, age^2	age, age^2	age, sex	age, age^2	age, sex, handedness	age, sex, age^2, age^2*sex	age	age, age^2

3.3 ASSOCIATIONS BETWEEN CNB DOMAINS AND NRG1 SNPS

SNPs were tested for association with cognitive variation by quantitative trait linkage disequilibrium (QTLD), unless there was evidence of stratification of the trait. In this case, quantitative transmission disequilibrium (QTDT) was used to measure associations. QTLD is a more powerful measure of association than QTDT, but is less robust to population stratification (Havill, Dyer, Richardson, Mahaney, & Blangero, 2005). QTDT is more conservative, but more appropriate when stratification is present.

In each analysis, at least one association was significant for QTDT while having significant ($p \leq 0.05$) stratification , as defined as $\beta_{between} \neq \beta_{within}$ (Fulker, Cherny, Sham, & Hewitt, 1999). In addition, most associations with significant stratification had opposite signed beta weights for the within- and between-family SNP regression coefficients, indicating that the

association was one direction within families and the opposite direction between families. Such associations are noted below.

3.3.1 Associations in the Final Sample

Twenty-two significant ($p \leq 0.05$) associations between the efficiency measures of each CNB domain and NRG1 SNPs were detected in the final sample, which consisted of both affected and unaffected participants. As seen in Table 17, every domain, except spatial memory, had at least one significant association with a NRG1 SNP (range: 0-10 associations per domain; mean of 2.75). These associations encompassed 13 SNPs (range: 0-4 associations per SNP; mean of 0.55), and were spread across the gene, including UTR's, introns, and exons. The domain of attention had the highest number of significant associations (n=10) and the largest number of highly significant ($p \leq 0.01$) associations (n=3). All associations in this sample were measured by QTLD, except for nine total QTDT associations in the domains of attention (rs1354335, rs1354336, rs13256173, SNP8NRG444511/rs13268724, rs776401, rs1473438, and rs10503929), facial memory (rs6468121), and verbal memory (rs10503929), due to significant levels of stratification. Eight of the nine QTDT associations had opposite between- and within-family beta weights; the QTDT association between attention and rs10503929 had negative between and within-family betas.

Table 17. Significant Associations (p-values; $p \leq 0.05$) by SNP and Efficiency Domain in the Final Sample

SNP	Abstraction & Mental Flexibility	Attention	Verbal Memory	Facial Memory	Spatial Memory	Spatial Processing	Sensorimotor Dexterity	Emotional Processing
SNP8NRG221132								
SNP8NRG221533								
rs10096573								
rs4298458								
SNP8NRG241930								
rs1081062								
rs13274954								
rs4566990								
rs1354335		0.0175*^						
rs1354336		**0.0021*^**						
rs13256173		0.0291*^						
rs1354334								
SNP8NRG444511		0.0291*^						
rs776401		0.0440*^						
rs1473438		0.0324*^						
rs1462893								
rs10954821								
rs726908								
rs10954855			0.0127					
rs2439306			0.0411					
rs2466062								
rs3924999		0.031						0.0235
rs2466060		**0.0044**						0.0262
rs2439272		0.0152		0.0306		0.0458		0.0356
rs6468121				0.0485*^				0.0502
rs2466044								
rs2466058								
rs2466049								
rs723811								
rs6988339								
rs2975498								
rs2919382								
rs2976525								
rs4262285								
rs3735776								
rs4512342								
rs10503929	0.0318	0.0094*	0.0287*^				0.047	
rs6992642								
rs3735781								
rs3735782								

See Table 16 for covariates included in the association model for each cognitive domain.
Associations significant after Bonferroni correction ($p \leq 0.0051$) are bolded.
*All p-values reflect QTLD results, except the following QTDT results in the domain of attention (rs1354335, rs1354336, rs13256173, SNP8NRG444511, rs776401, rs1473438, and rs10503929), facial memory (rs6468121), and verbal memory (rs10503929).
^Associations with significant stratification and opposite signs for between- and within-family beta weights

3.3.1.1 Determining the Number of Effective SNPs. As previously mentioned, several "redundant" SNPs in high LD with "primary" ones were included in the genotyping pool. These redundant SNPs were included in the association analyses and support the genotyping and association results of the primary SNPs. As shown in Table 18, the results of the redundant and primary SNPs are highly concordant, confirming our genotyping and association findings.

Table 18. Association Findings between SNPs in High LD

Pair-wise LD	SNP	Abstraction & Mental Flexibility	Attention	Verbal Memory	Facial Memory	Spatial Memory	Spatial Processing	Sensorimotor Dexterity	Emotional Processing
LD = 1.0	rs776401 rs1473438		0.0440* 0.0324*						
LD = 0.99	rs10096573 rs4298458								
LD = 0.95	rs1081062 rs13274954								
LD = 0.98	rs1354335 SNP8NRG444511		0.0175* 0.0291*						
LD = 1.0	rs13256173 SNP8NRG444511		0.0291* 0.0291*						
LD = 0.98	rs13256173 rs1354335		0.0291* 0.0175*						
LD = 0.98	rs2466044 rs2466058								
LD = 0.99	rs2466058 rs2466049								
LD = 0.99	rs2466044 rs2466049								
LD = 0.99	rs6992642 rs3735781								

*Significant stratification

To estimate the number of effective SNPs needed to capture the genetic heterogeneity among SNPs in the pool given the LD structure of the primer set, and to control the alpha error rate, p-values adjusted for correlated tests (p_ACT) (Conneely & Boehnke, 2007) were computed using the R-program. This analysis revealed that only 24.753 of the 40 final SNPs were necessary to explain the genetic variation in the final sample. Genotype information from 37 unrelated individuals in the final sample and 52 HapMap CEPH founders was used to estimate the number of effective SNPs. The combination of individuals from the current study and CEPH participants was used to raise the sample size to ensure the reliability of the results of the test. Individuals from the current study were chosen by the following method: if both parents of the index proband were in the final study, those individuals were included in the effective tests. If one or both parents were not in the final sample, then other individuals were chosen from the pedigree to represent the maternal and paternal sides of the family tree, working backwards from the proband. For example, if the father was not in the final sample, then the father's mother or father was used. If one of those individuals was not in the final sample, a sibling of the father was used. If a sibling was not available, then a child of the sibling was used.

Next the associations in Table 17 were corrected for multiple comparisons by using a Bonferroni adjustment ($\alpha_B = \alpha_{FW}/C$, where α_{FW} is the familywise error rate $(1-(1-\alpha)^C)$ and C is the number of comparisions; Keppel & Wickens, 2004) for comparisons between eight domains and 24.753 SNPs (C = 8*24.753 = 198.024), as suggested by the p_ACT results. This led to a new alpha level threshold of 0.0051. Using this alpha level, two associations remained significant: attention and rs1354336 (p=0.0021; QTDT), attention and rs2466060 (p=0.0044; QTLD).

3.3.1.2 Performance by Genotype. Performance by genotype, as measured by the within-family beta weights, for each significant association can be seen in Table 19. The minor allele conferred advantage in cognitive performance in 14 associations, while it conferred a detriment in eight associations. Effect sizes, as measured by the within-family beta weight, ranged between 0.1569 and 0.5169 for those associations significant in the direction of the minor allele. This indicated that each copy of the minor allele was associated with an increase in cognitive performance on the specific task by between 0.1569 and 0.5169 standard deviations. The absolute values of the effect sizes in those associations significant in the opposite direction ranged from 0.177 to 0.5691, indicating a decrease in performance by between 0.177 to 0.5691 standard deviations per copy of the minor allele.

Table 19. Significant Efficiency Associations in the Final Sample (by Genotype)

SNP	Domain	Major Allele	Minor Allele	Beta within*	Beta between*
rs1354335	Attention^	C	G	0.5169	-0.384
rs1354336	Attention^	T	C	-0.5691	0.2479
rs13256173	Attention^	A	G	0.4601	-0.3559
SNP8NRG444511	Attention^	T	A	0.4601	-0.3559
rs776401	Attention^	T	C	-0.3614	0.0399
rs1473438	Attention^	A	G	-0.3897	0.0451
rs10954855	Verbal Memory	T	A	0.3125	0.05
rs2439306	Verbal Memory	G	A	0.2296	0.267
rs3924999	Attention	C	T	0.3207	0.0703
	Emotional Processing	C	T	0.2769	0.132
rs2466060	Attention	G	A	0.4221	-0.0275
	Emotional Processing	G	A	0.1569	0.0732
rs2439272	Attention	C	T	0.2641	-0.0902
	Facial Memory	C	T	0.2329	-0.0816
	Spatial Processing	C	T	0.2159	-0.1163
	Emotional Processing	C	T	0.2219	-0.0306
rs6468121	Facial Memory^	G	T	-0.2751	0.0682
	Emotional Processing	G	T	-0.2528	-0.0431
rs10503929	Abstraction & Mental Flexibility	T	C	-0.3942	0.0364
	Attention^	T	C	-0.556	-0.0323
	Verbal Memory^	T	C	-0.4035	0.1936
	Sensorimotor Dexterity	T	C	-0.177	-0.0421

*A positive value indicates that the minor allele confers a benefit, while a negative beta weight value indicates that the minor allele confers a detriment to cognitive performance.

^ Significant stratification

3.3.1.3 The Role of Education within the Associations. In order to assess the role of education level in the association between NRG1 SNPs and cognitive function, education was entered as a covariate for each cognitive domain (see Table 20). The models were re-estimated, including the previous covariates (age, sex, age^2, age-by-sex, age^2-by-sex, and handedness), as well as education. Again, all covariates with $p<0.10$ were retained in the model. Fourteen significant associations were found in this analysis. Every domain had at least one significant association with a NRG1 SNP (range: 0-8 associations per domain; mean of 1.75), except spatial memory, spatial processing, and emotional processing. These associations included 11 SNPs (range: 0-2 associations per SNP; mean of 0.350) and were spread across the gene. All associations in this analysis were estimated by QTLD, except for three QTDT associations in the domain of attention. All three QTDT associations had opposite between- and within-family beta weights.

Table 20. Significant Associations (p-values; p≤0.05) by SNP and Efficiency Domain including Education as a Covariate

SNP	Abstraction & Mental Flexibility	Attention	Verbal Memory	Facial Memory	Spatial Memory	Spatial Processing	Sensorimotor Dexterity	Emotional Processing
SNP8NRG221132								
SNP8NRG221533								
rs10096573								
rs4298458								
SNP8NRG241930								
rs1081062								
rs13274954								
rs4566990								
rs1354335		0.0332*^						
rs1354336		**0.0030*^**						
rs13256173								
rs1354334								
SNP8NRG444511								
rs776401							0.0307	
rs1473438		0.0555*^					0.0231	
rs1462893								
rs10954821								
rs726908								
rs10954855			0.0338					
rs2439306								
rs2466062								
rs3924999								
rs2466060		0.0077						
rs2439272		0.0233		0.0436				
rs6468121								
rs2466044								
rs2466058								
rs2466049								
rs723811								
rs6988339				0.0457				
rs2975498								
rs2919382								
rs2976525								
rs4262285								
rs3735776								
rs4512342								
rs10503929	0.009	0.0079						
rs6992642		0.0377						
rs3735781		0.0477						
rs3735782								

All associations were re-estimated, including the following covariates: age, sex, age^2, age-by-sex, age^2-by-sex, handedness, and education. All covariates with p<0.10 were retained in the model

Associations significant after Bonferroni correction (p≤0.0051) are bolded.

Shaded cells correspond to significant associations in the final sample when education was not included as a covariate (see Table 17)

*QTDT results reported due to significant trait stratification.

^Associations with significant stratification and opposite signs for between- and within-family beta weights

Only eight associations in this analysis were also significant in the total final sample when education was not included as a potential covariate, as shown in Table 20, suggesting that variance in education levels may account for some of the relationship between NRG1 and cognitive functioning.

Furthermore, associations using education as the trait with the original covariate set revealed two significant associations with individual SNPs, including: rs1081062 (p=0.0063; QTLD) and rs3924999 (p=0.0350; QTDT).

3.3.1.4 Associations with Accuracy & Speed. NRG1 SNPs were then checked for association using the components of efficiency (e.g., accuracy and speed). As seen in Table 21, there were 21 significant associations found between NRG1 SNPs and accuracy over the seven cognitive domains. Sensorimotor dexterity accuracy could not be examined due to low variance in performance. Every domain, except spatial memory, had at least one significant association with an individual SNP (range: 0-8 associations per domain; mean of 3.000). These associations encompassed 12 SNPs (range: 0-4 associations per SNP; mean of 0.525) across the gene. All associations in this analysis were measured by QTLD, except for eight associations total across the domains of attention, verbal memory, and spatial processing. All eight QTDT findings were of opposite beta weight signs, except the association between attention and rs10503929. When compared to the associations found in the total final sample, 16 associations remained significant, while five new associations were found, and five previous associations were lost.

Table 21. Significant Associations (p-values; p≤0.05) by SNP and Accuracy Domain

SNP	Abstraction & Mental Flexibility	Attention	Verbal Memory	Facial Memory	Spatial Memory	Spatial Processing	Sensorimotor Dexterity	Emotional Processing
SNP8NRG221132			0.0309				NA	
SNP8NRG221533	0.0497						NA	
rs10096573							NA	
rs4298458							NA	
SNP8NRG241930							NA	
rs1081062							NA	
rs13274954							NA	
rs4566990							NA	
rs1354335		0.0340*^					NA	
rs1354336		0.0034*^					NA	
rs13256173		0.0437*^					NA	
rs1354334							NA	
SNP8NRG444511		0.0437*^					NA	
rs776401							NA	
rs1473438							NA	
rs1462893							NA	
rs10954821							NA	
rs726908							NA	
rs10954855			0.0082	0.0448			NA	
rs2439306			0.02				NA	
rs2466062							NA	
rs3924999		0.0334					NA	0.0126
rs2466060		0.0438*^				0.0446	NA	0.0181
rs2439272		0.0284				0.0171	NA	0.0432
rs6468121							NA	
rs2466044							NA	
rs2466058							NA	
rs2466049							NA	
rs723811							NA	
rs6988339							NA	
rs2975498							NA	
rs2919382							NA	
rs2976525							NA	
rs4262285							NA	
rs3735776							NA	
rs4512342							NA	
rs10503929	0.0324	0.0130*	0.0318*^			0.0180*^	NA	
rs6992642							NA	
rs3735781							NA	
rs3735782							NA	

All associations were re-estimated, including the following covariates: age, sex, age^2, age-by-sex, age^2-by-sex, and handedness.
All covariates with p<0.10 were retained in the model
NA: Accuracy in the sensorimotor dexterity domain could not be estimated due to low variance in the variable.
Associations significant after Bonferroni correction (p≤0.0051) are bolded.
Shaded cells correspond to significant associations in the final sample when education was not included as a covariate (Table 17)
*QTDT results reported due to significant trait stratification.
^Associations with significant stratification and opposite signs for between- and within-family beta weights

As seen in Table 22, there were 17 significant associations found between NRG1 SNPs and speed over the eight cognitive domains. Every domain, except emotional processing, had at least one significant association with an individual SNP (range: 0-6 associations per domain; mean of 2.125). These associations encompassed 14 SNPs (range: 0-3 associations per SNP; mean of 0.425) across the gene. All associations in this analysis were measured by QTLD, except for seven associations total in the domains of attention and spatial memory. All QTDT findings were of opposite beta weight signs. When compared to the associations found in the total final sample, five associations remained significant, while 12 new associations were found and 17 previous associations were lost.

Table 22. Significant Associations (p-values; p≤0.05) by SNP and Speed Domain

SNP	Abstraction & Mental Flexibility	Attention	Verbal Memory	Facial Memory	Spatial Memory	Spatial Processing	Sensorimotor Dexterity	Emotional Processing
SNP8NRG221132								
SNP8NRG221533								
rs10096573								
rs4298458								
SNP8NRG241930								
rs1081062								
rs13274954			0.0349					
rs4566990								
rs1354335		0.0362*^						
rs1354336	0.051	0.0328*^						
rs13256173		0.0427*^						
rs1354334								
SNP8NRG444511		0.0427*^						
rs776401							0.0505	
rs1473438	0.0509							
rs1462893								
rs10954821	0.0165							
rs726908					0.0335			
rs10954855								
rs2439306								
rs2466062		0.0032*^						
rs3924999								
rs2466060								
rs2439272								
rs6468121	0.0411							
rs2466044								
rs2466058								
rs2466049								
rs723811								
rs6988339								
rs2975498								
rs2919382								
rs2976525								
rs4262285						0.0409		
rs3735776				0.0248	0.0027*^	0.0379		
rs4512342								
rs10503929		0.0288*^						
rs6992642								
rs3735781								
rs3735782								

All associations were re-estimated, including the following covariates: age, sex, age^2, age-by-sex, age^2-by-sex, and handedness.
All covariates with p<0.10 were retained in the model
Associations significant after Bonferroni correction (p≤0.0051) are bolded.
Shaded cells correspond to significant associations in the final sample when education was not included as a covariate (Table 17)
*QTDT results reported due to significant trait stratification.
^Associations with significant stratification and opposite signs for between- and within-family beta weights

When comparing the accuracy and speed results with the associations with efficiency, most (72.72%) of the SNPs with significant efficiency associations also had significant associations with accuracy. Only a few (22.72%) had significant associations with both accuracy and speed, and no SNPs were significantly associated with speed alone. Finally, six efficiency associations had no significant associations with either accuracy or speed, suggesting that the combination of the two components drove the association findings.

3.3.2 Associations in the Unaffected Sample

In order to understand better the associations between NRG1 and cognition in the context of schizophrenia, associations were re-run in progressively "cleaner" samples. As previously mentioned, there were 361 participants in the final sample who did not meet criteria for either schizophrenia or schizoaffective disorder-depressed, creating an unaffected sub-sample within the final sample. QTLD and QTDT were estimated for this subsample using the methods outlined above for model estimation and covariate screening. As seen in Table 23, 23 significant associations were found in this subsample. Every domain, except spatial processing, had at least one significant association with a NRG1 SNP (range: 0-7 associations per domain; mean of 2.875). These associations encompassed 13 SNPs (range: 0-3 associations per SNP; mean of 0.575), and were spread across the gene, including UTR's, introns, and exons. All associations in this sample were measured by QTLD, except for 13 QTDT associations total across the domains of abstraction and mental flexibility; attention; verbal, facial, and spatial memory; and sensorimotor dexterity. All QTDT associations were of opposite beta weight directions.

Table 23. Significant Associations (p-values) by SNP and Efficiency Domain for the Unaffected Sub-sample of the Final Sample

SNP	Abstraction & Mental Flexibility	Attention	Verbal Memory	Facial Memory	Spatial Memory	Spatial Processing	Sensorimotor Dexterity	Emotional Processing
SNP8NRG221132			0.0502		0.0497*^			
SNP8NRG221533								
rs10096573								
rs4298458								
SNP8NRG241930								
rs1081062		0.0299*^						
rs13274954								
rs4566990								
rs1354335		0.0059*^					0.0423*^	
rs1354336		**0.0044*^**						
rs13256173	0.0511*^	0.0212*^					0.0431*^	
rs1354334								
SNP8NRG444511	0.0511*^	0.0212*^					0.0431*^	
rs776401								
rs1473438								
rs1462893				0.0211				
rs10954821								
rs726908								
rs10954855			0.0433	0.048				
rs2439306								
rs2466062								
rs3924999								0.0251
rs2466060		0.0077						0.0168
rs2439272		0.0404		0.0185				
rs6468121				0.0341*^				
rs2466044								
rs2466058								
rs2466049								
rs723811								
rs6988339								
rs2975498								
rs2919382								
rs2976525								
rs4262285								
rs3735776								
rs4512342								
rs10503929	0.0534		0.0514*^					
rs6992642								
rs3735781								
rs3735782								

Associations significant after Bonferroni correction ($p \leq 0.0051$) are bolded.

Shaded cells correspond to significant associations in the final sample when education was not a covariate (Table 17)

*QTDT results reported due to significant trait stratification.

^Associations with significant stratification and opposite signs for between- and within-family beta weights

When compared to the associations found in the total final sample, thirteen associations remained significant, while ten new associations were found in this sample and nine previous associations were lost. When these results were adjusted by Bonferroni correction, as described above, one association remained significant: attention and rs1354336 (p=0.0044; QTDT). This association was also significant in the total final sample after Bonferroni correction.

3.3.3 Associations in the "No Diagnosis" Sample

Finally, associations were estimated in the sub-sample of individuals who had no diagnosis on the DIGS (N=178) using the methods described above. As seen in Table 24, 25 associations were found in this sample. Every domain had at least one significant association with a NRG1 SNP (range: 1-7 associations per domain; mean of 3.125). These associations encompassed 16 SNPs (range: 0-4 associations per SNP; mean of 0.625), and were spread across the gene. All associations in this sample were measured by QTLD, except for six QTDT associations total across the domains of attention; facial and spatial memory; and spatial processing. Four of the six QTDT associations had opposite signed beta weights. The associations between spatial memory and SNPs rs1081062 and rs13274954 had positive values for between- and within-family betas.

Table 24. Significant Associations (p-values) by SNP and Efficiency Domain for the No Diagnosis Sub-sample of the Final Sample

SNP	Abstraction & Mental Flexibility	Attention	Verbal Memory	Facial Memory	Spatial Memory	Spatial Processing	Sensorimotor Dexterity	Emotional Processing
SNP8NRG221132			0.0152					
SNP8NRG221533	0.0178							
rs10096573								
rs4298458								
SNP8NRG241930								
rs1081062				0.0217*^	**0.0022***			
rs13274954				0.0088*^	**0.0018***			
rs4566990								
rs1354335					0.0514			
rs1354336		0.0354*^						
rs13256173								
rs1354334								
SNP8NRG444511								
rs776401								
rs1473438								
rs1462893								
rs10954821								
rs726908								
rs10954855		0.0396	0.0258	0.0152		**0.0018*^**		
rs2439306								
rs2466062								
rs3924999		0.0303						
rs2466060		0.0072				0.0372		
rs2439272						0.0234		
rs6468121								
rs2466044				0.0537				
rs2466058				0.0343				
rs2466049				0.0349				
rs723811								
rs6988339								
rs2975498								
rs2919382								
rs2976525						0.0501		0.049
rs4262285								
rs3735776			0.0347	0.0212				
rs4512342								
rs10503929	0.0354						0.0439	
rs6992642								
rs3735781								
rs3735782								

Associations significant after Bonferroni correction ($p \leq 0.0051$) are bolded.

Shaded cells correspond to significant associations in the final sample when education was not a covariate (Table 17)

*QTDT results reported due to significant trait stratification.

^Associations with significant stratification and opposite signs for between- and within-family beta weights

When compared to the associations found in the total final sample, seven associations remained significant, while eighteen new associations were found in this sample and eleven previous associations were lost. When these results were adjusted by Bonferroni correction, as described previously, three associations remain significant: spatial memory and rs1081062 (p=0.0022; QTDT), spatial memory and rs13274954 (0.0018; QTDT), and spatial processing and rs10954855 (p=0.0018; QTDT). None of these associations were significant in the final sample or the unaffected sample.

3.4 ASSOCIATION BETWEEN NRG1 VARIATION AND SCHIZOPHRENIA

Finally, associations between NRG1 SNPs and affected status (e.g., diagnosis of schizophrenia or schizoaffective disorder-depressed) were calculated to determine whether NRG1 was associated with schizophrenia in the final sample. One significant association was found: rs1081062 (p=0.0514; QTDT). The lack of findings here is likely influenced by the small number of affected participants in the final sample.

4.0 DISCUSSION

4.1 SUMMARY OF FINDINGS

As expected, we found that cognition is heritable in this schizophrenia family sample, helping to confirm the generalizability of our results. In addition, this study found evidence that variation in NRG1 is significantly associated with cognitive performance in the total affected and unaffected sample, as well as in the unaffected and "No Diagnosis" subsamples; albeit with some differences in the associations found in each sample. This evidence suggests NRG1's putative mechanism of increasing risk for schizophrenia may be due, in part, to its relationship to cognition.

Associations were re-analyzed in progressively "cleaner" samples to better understand the associations between NRG1 and cognition in the context of schizophrenia, and to control for potential effects of psychopathology. Although the specific associations varied by sample, each sample had significant associations, even after correction for multiple comparisons was made. Over all of the samples, the domain of attention consistently had the most, or nearly the most, significant findings when compared to other cognitive areas. This may suggest that NRG1 plays an especially important role in attention.

There was also one significant association between NRG1 SNPs and the diagnosis of schizophrenia or schizoaffective disorder-depressed in the current study, although it was non-significant after correcting for multiple comparisons using a Bonferroni ($p_B = 0.0290$) correction.

This may suggest that the current NRG1 SNP set is not associated with schizophrenia; however, it may also be due to the small sample of affected participants leading to low power to detect associations between the disorder and individual SNPs. Given the large literature showing a positive association between NRG1 and schizophrenia, the latter seems more likely.

To better understand the significant associations in the current study, we will discuss the following SNPs in greater detail: rs1081062, rs13274954, rs1354336, rs10954855, rs2466060, and rs10503929. The LD within this group of SNPs was less than or equal to 0.2, except for the following: rs1354336 and rs1081062 (LD = 0.4), rs1354336 and rs13274954 (LD = 0.4), and rs1081062 and rs13274954 (LD = 1.0). As seen in Table 25, these SNPs were significant after Bonferroni correction ($p_B = 0.0051$) in any given cognitive domain and subsample, and/or were significant at the $p \leq 0.05$ level for all of the subsamples in a specific domain. They are discussed in order of basepair (5' to 3').

Table 25. SNPs Significant after Bonferroni Correction ($p \leq 0.0051$) and/or Significant ($p \leq 0.05$) in All Subsamples

Marker	Domain(s)	Sample(s)	p-value	Beta within^	Beta between^	Effect of the Minor Allele
rs1081062	Spatial Memory	"No Diagnosis"	**0.0022***	0.5769	0.0598	Benefit
rs13274954	Spatial Memory	"No Diagnosis"	**0.0018***	0.6066	0.0835	Benefit
rs1354336	Attention	Final (without education)	**0.0021***	-0.5691	0.2479	Detriment
		Final (with education as a covariate)	**0.0030***	-0.5624	0.2929	
		Unaffected	**0.0044***	-0.5171	0.2714	
		"No Diagnosis"	0.0354*	-0.5001	0.2957	
rs10954855	Spatial Processing	"No Diagnosis"	**0.0018***	0.7375	-0.0759	
	Verbal Memory	Final (without education)	0.0127	0.3125	0.05	Benefit
		Final (with education as a covariate)	0.0338	0.2923	0.0179	
		Unaffected	0.0433	0.1953	0.0524	
		"No Diagnosis"	0.0258	0.264	0.1688	
rs2466060	Attention	Final (without education)	**0.0044***	0.4221	-0.0275	Benefit
		Final (with education as a covariate)	0.0077	0.4334	0.0235	
		Unaffected	0.0077	0.3218	-0.0787	
		"No Diagnosis"	0.0072	0.5098	-0.0945	
rs10503929	Abstraction & Mental Flexibility	Final (without education)	0.0318	-0.3942	0.0364	Detriment
		Final (with education as a covariate)	0.0090	-0.4041	-0.0046	
		Unaffected	0.0534	-0.3363	-0.0596	
		"No Diagnosis"	0.0354	-0.5115	-0.0851	

Associations significant after Bonferroni correction are bolded.

*QTDT results reported due to significant trait stratification

^A positive value indicates that the minor allele confers a benefit, while a negative beta weight value indicates that the minor allele confers a detriment to cognitive performance.

4.1.1 SNP-wise Findings & Comparison to Current Literature

4.1.1.1 Marker rs1081062. SNP rs1081062 was found to be significant in the domain of spatial memory in the "No Diagnosis" sample, even after correction for multiple comparisons. Although there was significant stratification in this analysis, both the between- and within-family

betas had positive signs. In this association, the minor allele (C) was associated with better performance in spatial memory than the major allele (T). This SNP was originally associated with schizophrenia by Fukui et al. (2006) in a Japanese population, where homozygotes of the minor allele (genotype CC) had a significantly increased risk of schizophrenia compared to the heterozygote and major allele homozygotes (TT). However, two other recently published studies failed to find an association between this marker and schizophrenia, including a replication attempt in a Japanese sample (Shiota et al., 2008) and a study utilizing a Bulgarian sample (Georgieva et al., 2008). To our knowledge, no studies of rs1081062 and cognition exist in patient or control samples.

4.1.1.2 Marker rs13274954. As expected based on its LD with rs1080162 (LD=1, see above), marker rs13274954 was found to be significant in spatial memory in the "No Diagnosis" sample, even after Bonferroni correction. Stratification was significant in this association, but both between- and within-family beta weights were positive. The minor allele (A) was associated with better spatial memory than the major allele (T). To our knowledge, no studies of rs13274954 and cognition exist in patient or control samples.

4.1.1.3 Marker rs1354336. SNP rs1354336 was significantly associated with attention in all samples of the current study. Significant stratification was detected in this association for all samples; in each, the within-family beta was negative, while the between-family beta was positive. Specifically, the major allele (T) was associated with better efficiency in attention in all samples compared to the minor allele (C). This SNP is near several of the microsatellites (specifically marker 420M9-1395) within the original haplotype blocks associated with schizophrenia in a European sample by Stefansson, et al. (2002, 2003) and others. To our knowledge, no studies of rs1354336 and cognition exist in patient or control samples.

4.1.1.4 Marker rs10954855. In the current study, rs10954855 was found to be significantly associated with spatial processing in the "No Diagnosis" sample, even after Bonferroni correction. Significant stratification was detected in this association; the within-family beta was positive, while the between-family beta was negative. In addition, it was also significantly associated with verbal memory in all samples of the study, albeit not always surviving Bonferroni correction. No significant stratification was detected in these associations.

In both spatial processing and verbal memory, the minor allele (A) was associated with better performance compared to the major allele (T). This SNP lies near microsatellite 317J8-2123, which was associated with schizophrenia in a Chinese sample by Li et al. (2004). To our knowledge, no studies of rs10954855 and cognition exist in patient or control samples.

4.1.1.5 Marker rs2466060. Marker rs2466060 was significantly associated with attention in all samples of the current study, but did not always survive Bonferroni correction. Significant stratification was detected in this association in the final sample; the within-family beta was positive, while the between-family beta was negative. The minor allele (A) was associated with better attention in each sample. It is located near microsatellite 317J8-4858 which was associated with schizophrenia in a Chinese sample by Li et al. (2004). Unlike the other highly significant markers whose genotyping failure rate ranged from 0.00-1.19%, this SNP had a failure rate of 10.74%. Thus despite its consistent significance in all samples at the $p \leq 0.05$ level, its high failure rate requires us to take caution in our interpretation of this marker. To our knowledge, no studies of rs2466060 and cognition exist, in patient or control samples.

4.1.1.6 Marker rs10503929. Marker rs10503929 was significantly associated with abstraction and mental flexibility in all samples of this study. No significant stratification was detected in any of the samples studied. The major allele (T) was associated with better

abstraction and mental flexibility than the minor allele (C). Importantly, this is a missense non-synonymous SNP that leads to the substitution of a methionine (M) to threonine (T) amino acid if a T to C allele change occurs. Although no published reports have suggested what the effect of this substitution might be, M is a much larger molecule than T (149.21 g mol^{-1} compared to 119.12 g mol^{-1}) (Nelson & Cox, 2000) and has a different secondary structure (helical-favoring compared to C-beta-branched extended-favoring), although both are polar and neutral amino acids (Nelson & Cox, 2000). This SNP has been associated with schizophrenia in several studies, including Schwab, et al. (2006; Australian sample), Baines, et al. (2008; Costa Rican sample), and Rosa, et al. (2007; Spanish sample). In the Rosa study, there was a significant over-transmission of the C allele compared to the T allele in the families with patients with psychosis. To our knowledge, one study of rs10503929 and cognition has been published (Hong, Wonodi, Stine, Mitchell, & Thaker, 2008) that found no association between this marker and pre-pulse inhibition in individuals with schizophrenia or healthy controls.

4.1.1.7 Other NRG1 Markers Associated to Cognition in Previous Literature. Despite significant associations between SNP8NRG243177/rs6994992 and cognition in previous studies, this marker failed the SNPlex algorithm in multiple design submissions and could not be analyzed in the current study. SNP8NRG221533/rs13268724 was found to be associated to sustained attention in one previous study (Stefanis et al., 2007). In the current study, it was associated with accuracy in abstraction and mental flexibility in the total final sample ($p=0.0497$) and efficiency in abstraction and mental flexibility in the "no diagnosis" sample ($p=0.0178$), but not with attention in any sample. Finally, microsatellites were not tested in the current study, so 433E1006's association with sustained attention and verbal working memory could not be assessed.

4.1.2 Stratification Findings

Many of the significant associations between NRG1 and cognition had significant stratification, including stratification where the sign of the beta weight differed between- and within-families, indicating that one allele conferred cognitive advantage within families, while the alternative allele conferred benefit between families. Although this was unexpected, the between-family measure is sensitive to population stratification or admixture, while the within-family measure is safe from such problems due to the family structure of the study. Such stratification is due to the combination of families across different population strata and only affects the between-family beta (Abecasis, Cardon, & Cookson, 2000; Havill et al., 2005). Abecasis et al. (2000) found that spurious associations between the marker and phenotype are contained within the between-family component, while the within-family beta estimates only the additive genetic value, even when significant stratification is present. However, stratification is generally expected to lead to greater associations between- than within-families, and thus it is unusual to have the between-family component to be less than the within-family component. In the current study, there are frequently associations in which the signs of the between- and within-family betas are opposite, which may indicate that some between-family factor is associated with the allele, leading to significant stratification in which the direction of the association is different between compared to within families.

In addition, stochastic, or random, variation cannot be ruled out as the cause of stratification in this study. However, some of the most significant associations, as defined by p-values, were highly stratified with opposite beta weight signs (see Table 25), making random error less likely of an explanation. In addition, the threshold for stratification was set to $\alpha = 0.05$ and was not corrected for multiple comparisons in order to be conservative in the use of QTLD,

given its lack of robustness to stratification. However, the high number of overall comparisons without correction make it likely that a Type I error occurred and that at least some associations with significant stratification are due to chance (Havill et al., 2005).

4.2 CONCLUSIONS

Although the specific associations that were significant differed somewhat among subsamples, each sample had between 14-25 significant ($p \leq 0.05$) associations, encompassing multiple SNPs and multiple cognitive domains. The numerous significant findings in progressively "cleaner" sub-samples may suggest that variation in NRG1 is more directly associated with cognition and is not solely secondary to an effect of schizophrenia or psychopathology. These associations may suggest a role for NRG1 in cognition that is a mediating risk factor for schizophrenia and/or psychopathology more generally. It is also possible that these associations with cognition are irrelevant to schizophrenia, but the literature on NRG1 and schizophrenia argues against this possibility.

In considering the cognitive domains separately from individual SNPs, attention consistently had the most, or nearly so, significant associations over all of the samples assessed. Spatial memory generally had the fewest significant findings. This may suggest that over all of the domains tested, NRG1 may have a more specific role in the maintenance of attention, while playing only a small role in spatial memory.

Considering only the SNPs with the most significant results (rs1081062, rs13274954, rs1354336, rs10954855, rs2466060, and rs10503929), four are in the 5' UTR, one is between exons 5-6, and one is in exon 9. LD among these SNPs is less than or equal to 0.40, except for rs1081062 and rs13274954, which are in perfect LD and share the same associations. In addition,

the low genotyping failure rates (0.00-1.19%; except for rs2466060 (10.74%)) among these SNPs allow us to be relatively confident with these findings, despite significant stratification.

Of these SNPs, the most promising associations encompass four SNPs and three cognitive domains. Specifically: rs1354336 and attention, rs10954855 and verbal memory, rs2466060 and attention, and rs10503929 and abstraction and mental flexibility. Three of these SNPs (rs1354336, rs10954855, and rs2466060) were chosen for the current study due to their close proximity to microsatellites identified as being associated with schizophrenia by previous studies, while the fourth (rs10503929) was suggested by three association studies.

Perhaps the most interesting association in the current study was between rs1354336 and attention. This association was significant in every sample studied, and survived Bonferroni correction in all but the "No Diagnosis" sample. In addition, this marker lies in close proximity to a microsatellite within the original haplotype blocks associated to schizophrenia by Stefansson et al. (2002, 2003), making it potentially useful for better understanding the association between NRG1 and cognition within the context of schizophrenia. However, rs1354336 had significant stratification with opposite signed beta weights in its associations with attention in every sample.

A more conservative approach may be to follow-up on the association of rs10503929 and abstraction and mental flexibility. This association was significant for every sample, but did not always survive Bonferroni correction. It has been associated to schizophrenia by three studies, is a missense non-synonymous variant and has intriguing functional implications, and had no significant stratification within any sample in the current study.

4.3 LIMITATIONS

Although this study provides evidence for a potentially important role of NRG1 in cognition within multiplex families with schizophrenia, it has several limitations. First, the number of participants with schizophrenia or schizoaffective disorder-depressed in the sample was small. This prevented us from testing for associations with NRG1 and cognition in the patient-only sample. In addition, the general lack of associations between NRG1 and affected status was also likely affected by this low power.

A second important limitation of this study was a lack of a genotyped control group. Although we used progressively "cleaner" diagnostic sub-groups of the main sample to estimate the effect of NRG1 and cognition within the context of schizophrenia and general psychopathology, it is difficult to determine whether the role of NRG1 in cognition is relevant to schizophrenia or is a more general effect.

4.4 FUTURE DIRECTIONS

Future directions aimed at resolving the limitations in the current study include further narrowing the SNP set and assessing the relationship of NRG1 and cognition and its specificity to schizophrenia. First, a Bayesian analysis (Blangero et al., 2005) would allow the combination of SNPs that best predicts cognitive performance to be determined. Next, a genetic cross correlation analysis would allow a better understanding of the role of NRG1 in cognition and schizophrenia. One specific question that might be assessed here is whether the genes contributing to cognitive performance are correlated with those that contribute to schizophrenia within families. Finally, the addition of a control group would allow us to assess the previous associations to determine

whether NRG1 is related to cognition in a healthy sample, thus further parsing the role of NRG1 and cognition in schizophrenia.

Other directions in answering the NRG1-cognition question include studying more markers within NRG1, specifically the microsatellites found to be associated with schizophrenia in the HapICE and HapIRE haplotypes and by Li et al. (2004), especially as SNPs in close proximity to three of those microsatellites were found to be significantly associated with cognition in this study. In addition, determining what implications the M to T amino acid change might have for SNP rs10503929 may provide clues to its role, if any, in attention.

Finally, the use of methods and models that incorporate epistatic and environmental influences that might play a moderating role in the relationship between NRG1 and cognition are necessary to better understand these associations. There is evidence that variation in both NRG1 and ErbB4, as well as the interaction between those variants, is associated with an elevated risk for schizophrenia (Norton et al., 2006). A subsequent study (Benzel et al., 2007) confirmed the interaction between ErbB4 and NRG1, in addition to finding evidence of epistasis between NRG1-NRG2 and NRG1-NRG3 in a sample of patients with schizophrenia and control participants. Another recent study found a ten-fold increase in risk for schizophrenia depending on the combination of variants in both NRG1 and Interleukin-1β (Hanninen et al., 2008). Such findings suggest that intergenic interactions between NRG1 and other genes may increase susceptibility to schizophrenia and may also help mediate the role of NRG1 in cognition.

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