SEARCHING FOR ENDOPHENOTYPES:

NEGATIVE SYMPTOMS AND SCHIZOPHRENIA

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Searching for Endophenotypes: Negative Symptoms and Schizophrenia

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Schizophrenia is a disorder affecting millions of individuals, with a prevalence rate over one percent. Although the diagnosis of schizophrenia has a high heritability, identification of individual risk variants has been difficult, due to their small individual effects on the diagnosis. Identification of useful endophenotypes - that is, features more sensitive to genetic effects than the diagnosis itself - should aid in detecting genetic variants that contribute to the disorder. The current study examined negative symptoms as a possible endophenotype for schizophrenia. Specifically, the study examined the genetic correlations between the Scale for the Assessment of Negative Symptoms (SANS) and schizophrenia among multigenerational, multiplex schizophrenia families. To examine diagnostic specificity, major depressive disorder and substance abuse were also tested for genetic correlations with negative symptoms. The total sample (493) included 43 families, 90 schizophrenia patients, 359 of their relatives, and 44 controls. The majority of SANS scales were significantly heritable (average $h^2 = 0.48$). Results suggested that even among relatives without any diagnoses, the prevalence of negative symptoms increased significantly with the degree of genetic relationship to schizophrenia (average $R_G = 0.76$) but not depression or substance abuse. This suggests the potential utility of negative symptoms as a candidate endophenotype with some diagnostic specificity in studies seeking to identify genetic risk variants contributing to schizophrenia.

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PREFACE

I would like to wholeheartedly thank my mentor and thesis committee chair Michael Pogue-Geile, PhD for his tireless work with me throughout my undergraduate career, teaching me both directly and indirectly about behavioral genetics, clinical research, and mental health. Looking back, I am amazed at how lost I would be in this process without his invaluable guidance and support. I would also like to thank my thesis committee members, Kathryn Roecklein, PhD, Elaine Walker, PhD, and Vishwajit Nimgaonkar, MD, PhD, for providing helpful suggestions on this thesis. I especially want to acknowledge and thank Susan Kuo, the final committee member, for assisting me on statistical analyses of this research, and teaching me the techniques necessary. Her patience and support as I struggled with coding programs and missing data were absolutely crucial.

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1.0 INTRODUCTION

First proposed by Emil Kraeplin (Kraepelin, 1919), schizophrenia, characterized by a severe disruption in basic human attributes such as thought, language, perception, emotion and self, affects more than one percent of the world's population, including over 3 million Americans at a cost of $62 billion a year (Knapp et al., 2004). Treatment for the disorder primarily consists of pharmacotherapies that often but not always reduce the psychotic symptoms of the disorder. In contrast, few pharmacotherapies impact the cognitive and emotional disruptions that are present, causing patients to have extreme difficulty assimilating into society, attaining employment, and maintaining social relationships. Mortality due to natural and unnatural causes is also considerable, and the projected lifespan for individuals with schizophrenia is some 15 years less than the general population, largely due to the comorbidity with drug abuse, poverty, and other health risks (Crump et al., 2013).

1.1 SCHIZOPHRENIA AND VULNERABILITY

1.1.1 Genetic Influence

Numerous epidemiological studies have shown a heritability of approximately 80% for the schizophrenia diagnosis (Cardno et al., 1999; Sullivan et al., 2003). This reflects the observation that the risk of developing schizophrenia in family members increases with the degree of genetic relatedness to the patient – greater risks are associated with higher levels of shared genes. First-degree relatives (e.g. siblings, dizygotic (DZ) twins) of patients share 50% of their genetic variation and show a risk of about 9%. Monozygotic (MZ) co-twins of schizophrenia patients share 100% of genetic variation and present risks near 50% (Fischer, 1971; Gottesman & Bertelsen, 1989).

It is also clear that this high heritability is not due to one gene solely responsible for the diagnosis; rather, it is the combined effect of many genes, each with a small effect (O'Donohue et al., 2007; O'Donovan et al., 2003; Pogue-Geile & Gottesman, 1999). For example, recently several thousand SNPs were reported to be implicated in the etiology of schizophrenia (Purcell, 2009). In addition to these multiple common genetic variants with small effects, there also appear to be rare variants with large effects (Stefansson et al., 2008).

The question of importance thus becomes one of identifying such individual genetic variants of small effect. One approach to this is through increasing study sample size, thus creating an increase in statistical power. As one example of this strategy, in 2013 a genome-wide association study identified 13 new risk loci for schizophrenia (Ripke et al., 2013). Part of this study's success was due to the sheer size of the sample: 5,001 cases and 6,243 controls, followed up with meta-analyses of another 8,000 patients. A second tactic to detect small genetic effects is

to improve the quality of measurement. A measurement that is imprecise may fail to detect the differences between two groups, while a precise measurement would reveal significant differences.

1.1.2 Endophenotype (Improving the Measurement)

One way to improve the measurement for the purpose of detecting genetic effects is through the use of "endophenotypes" (Gottesman & Gould, 2003; Gottesman & Shields, 1972). The idea of an endophenotype is that it is more sensitive to genetic effects than is the diagnosis itself. By identifying useful endophenotypes, researchers should be in a position to more easily identify the genetic variants that contribute to the disorder (Gottesman & Gould, 2003; Tsuang et al., 2000). The goal of the endophenotype is that of higher statistical power: certain genes may contribute to the diagnosis but do not usually produce clinical symptoms (Moldin, 1994; Tarbox et al., 2012). A related endophenotype issue is specificity; in other words, one must differentiate if a liability gene has been detected, or if the endophenotype is only discerning the causes of more generalized deficits (which could include the target deficit; Faraone et al., 1995).

How then should such potential endophenotypes be identified? As originally formulated, an endophenotype would occur in both monozygotic twins, even those discordant for the diagnosis (Gottesman & Shields, 1972). Such an indicator would thus be more common in the unaffected relatives of patients than the controls (who are unrelated to a proband). The observation that abnormalities are present among relatives who do not have the diagnosis suggests that such characteristics are more sensitive to risk gene variants than is the diagnosis itself. Numerous studies have demonstrated neuropsychological deficits and behavioral patterns

falling below the threshold of a clinical diagnosis among relatives of schizophrenic patients (Faraone et al., 1995; Faraone et al., 2000; Katsanis et al., 1990; Thompson et al., 2005). Additional studies have also observed such traits in children of schizophrenia parents that are similar to, but less severe than those of schizophrenia patients (Davalos et al., 2004; Davies et al., 1998; Tarbox et al., 2012; Tarbox & Pogue-Geile, 2008).

The question then becomes, "Where to look for possible endophenotypes?" It has been proposed that symptoms, in essence the same as those observed in individuals with the schizophrenia diagnosis, can also be measured at subclinical levels in individuals without schizophrenia in the general population (Tarbox et al., 2012). The concept of a disorder continuum implies that schizophrenia is not a categorical disorder, but rather the extreme expression of otherwise more or less continuously distributed traits in the population. The symptoms of schizophrenia can generally be divided into three categories: positive, negative, and cognitive, based on factor analyses.

1.1.3 Three Dimensions

Positive symptoms include hallucinations and delusions (American Psychiatric Association, DSM-5 Task Force., 2013). These symptoms are "excessive" psychotic behaviors typically not seen in healthy patients. Conversely, negative symptoms are aspects or traits that are reduced in patients with schizophrenia as compared to the general population. These include flattened affect, social withdrawal, speech poverty and anhedonia (Andreasen et al., 1994; Andreasen & Olsen, 1982; Bassett et al., 1993; Eaton et al., 1995; Lewine et al., 1983; Peralta et al., 1992; Walker & Harvey, 1986). For example, patients may have masked *facies*, with blank or restricted

emotional expressions. Lastly, cognitive impairments such as deficits in working memory and attention exist (Cannon et al., 1994; Thompson et al., 2005). However, current pharmacological treatments, such as antipsychotic medications, for schizophrenia only address the positive symptoms. Unfortunately, such antipsychotic medications – either typical or atypical – have little or no effect upon the negative or cognitive symptomology.

Negative symptoms, more than positive symptoms, have a significant association with functional outcomes and quality of life for individuals with schizophrenia. Negative symptoms also tend to be more persistent compared to the more episodic nature of positive symptoms. These negative traits have been correlated with cognitive problems, as well as being prognostic. For these reasons, negative symptoms are good candidates for a potentially useful endophenotype.

Identification of endophenotypes that correlate with familial risk is a crucial tactic for better detecting genetic variation related to the development of schizophrenia. Based on this, the current study examined negative symptoms' sensitivity to schizophrenia familial liability and explored their potential as endophenotypes. We are not the first to examine negative symptoms, although only a small number of studies have studied their presence among relatives of schizophrenia probands. Next we will review and evaluate the work of our predecessors.

1.2 LITERATURE REVIEW

"Negative symptoms" is a term that has many meanings and measurements and has been variably used from study to study, as well as over time. Such symptoms have been assessed with a variety of measures: the Brief Psychiatric Rating Scale (BPRS) anergia factor (Overall & Gorham, 1962); Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1989) and Positive and Negative Symptom Scale (PANSS) (Kay et al., 1987) for example. Here, we will restrict the literature review only to studies that have employed either the SANS or a similar behavior rating scale of negative symptoms.

1.2.1 Methodological Considerations

Throughout the literature, there are several methodological points that must be considered. First, there is often a lack of distinction in how studies group first-degree relatives; some combine parents and siblings, while others keep them separate (which is preferable). Second, only a few studies report that raters were blind to participants' status (relatives or controls). Third, often studies neglect to state from where the controls were selected; others lack details on the screening process. The exclusion criteria for the control groups have also often been stricter than for the relatives of patients, creating potentially different comparison group characteristics. Potential "healthy" controls are often screened for all psychiatric disorders, leading to the possibility that the control group is completely free of any diagnoses. This creates a problem if the relatives had any diagnosis, but were being compared to dissimilar controls, potentially leading to artifactual results. A "supernormal" control group could thus produce an inflated

effect size (Tarbox et al., 2012). In addition, diagnostic specificity is rarely investigated with few studies of negative symptoms among relatives of depressed or other diagnosed probands.

1.2.2 Review of Schizophrenia Symptoms in Relatives

Table 1 presents the seven studies of behavior ratings of negative symptoms among relatives of schizophrenic probands. The majority of the studies (six), seen in Table 1, imply a genetic correlation between negative symptoms and relatives of schizophrenia probands (Chen et al., 2009; Delawalla et al., 2006; Fanous et al., 2001; Kendler et al., 1993, 1993; Scala et al., 2012; Smith et al., 2008). The one study (Craver et al., 1999) with non-significant effects used a modified version of the SANS scale: the Avolition-Apathy, Anhedonia-Asociality, and Attention scales were excluded, and certain components of the Affective Flattening (inappropriate affect and affective nonresponsivity) and Alogia (poverty of content of speech) scales were removed. Additionally, the sample size was smaller than most of the other studies reviewed. Chen's article was perhaps the most informative, as it not only provided correlations but also estimated the heritability of negative symptoms. The study discussed the possibility of genetic components that affect both negative symptoms and certain neurocognitive deficits (Chen et al., 2009).

1.2.3 Limitations

It is worth noting that in the literature, the composition of the relatives group varies.
Three studies used first-degree relatives, with no discrimination between parents and siblings.
One study did not differentiate between degrees of relationship to the proband, while four

articles reported comparison based only on siblings. Furthermore, although most studies did match controls to relatives on age and sex to minimize group differences, only six studies included relatives of control probands, and none of the studies made use of more distant relatives as a comparison group.

Another potential limitation found in numerous articles was that of a wide age range. Although the average age of a relative was generally in the 40s and 50s, some subjects were as young as 18 – an age still within the risk for developing schizophrenia. Thus, individuals could have been misclassified; if researchers had followed up these individuals, some could have developed schizophrenia. Although only explicitly mentioned in the Bassett article, there may also have been incidences of rater bias, especially if diagnostic interviews and symptom assessments were done simultaneously or by the same individuals across relatives. Craver's study excluded portions of the SANS scale in analysis, and found that there was not a relationship between these negative symptoms and relatives (Craver & Pogue-Geile, 1999).

1.2.4 Summary of the Review

The data reviewed above generally supported the idea of a genetic correlation between negative symptoms and schizophrenia, based on comparisons between first-degree relatives of schizophrenia patients and psychiatrically healthy controls. Certain negative symptoms (i.e., anhedonia) may also have been ruled out as a potential endophenotype, but not conclusively.

1.3 AIMS OF THE CURRENT STUDY

To build upon the literature and improve upon past studies' methodological constraints, the present study examined a large, multigenerational, multiplex family sample to assess associations between behavior ratings of negative symptoms and genetic liability for schizophrenia among non-psychotic relatives of schizophrenia probands, to determine whether negative symptoms might be a potentially useful endophenotype in molecular genetic studies of schizophrenia.

For these questions, multigenerational, multiplex family samples have several advantages. To the extent that schizophrenia risk alleles are more common among relatives in multiplex families than in singleton families, statistical power is increased. An additional benefit of the current study is that the inclusion of controls is not required. As the sample includes first through fourth-degree relatives, one will be able to determine a genetic correlation simply by comparing different degrees of relatives. This avoids the difficulty of ascertaining an appropriately equivalent control group. The current study also had approximately twice as many participants as any prior study of the question and examined other diagnoses in the relatives of probands, allowing for diagnostic specificity to be evaluated.

The following hypotheses were examined:

1) Negative symptoms will be more prevalent in schizophrenia probands than non-psychotic relatives and controls.
2) Heritabilities of negative symptoms should be significantly different from zero.
3) The prevalence of negative symptoms should increase with the degree of genetic relationship to schizophrenia; in short, the genetic correlation between the negative

symptoms and the diagnosis of schizophrenia should be significant. If so, this implies some common familial causality between schizophrenia and negative symptoms.

4) The prevalence of negative symptoms should not increase with the degree of genetic relationship to major depressive disorder probands (i.e., genetic correlation with depression should not be significant).
5) The prevalence of negative symptoms should not increase with the degree of genetic relationship to substance abuse probands (i.e., genetic correlation with substance abuse should not be significant).

Additional questions were also examined regarding the measurement of negative symptoms. For each of the above questions, we examined both overall negative symptoms as well as individual negative symptoms (e.g., affective flattening, alogia, avolition) to determine which might be the most genetically correlated with schizophrenia and the other disorders. To our knowledge, no previous study has included as many relatives of schizophrenia patients or examined diagnostic specificity in this fashion.

2.0 METHODS

2.1 PARTICIPANTS

Probands and their relatives were recruited as part of a large, multisite schizophrenia multiplex family study (Gur et al., 2007; Tarbox et al., 2012). The 740 participants in the overall study included both European-American pedigree members from 43 multigenerational multiplex families and nonpsychotic control individuals demographically matched to the pedigree members. Written informed consent according to the guidelines of the University of Pittsburgh and University of Pennsylvania Institutional Review Boards was signed by all participants.

2.1.1 Index probands

Potential probands were identified through consumer and mental health organizations located in Pennsylvania, Delaware, Indiana, Kentucky, Michigan, New Jersey, Ohio, and West Virginia. Probands were at least 18 years old, European-American, met DSM-IV diagnostic criteria for schizophrenia or schizoaffective disorder, depressed type, and had at least one first-degree relative who also was at least 18 years old, European-American, met DSM-IV criteria for either schizophrenia or schizoaffective disorder, depressed type, and might be willing to participate. All diagnoses were established by consensus based on diagnostic interviews. Each proband was

additionally required to have at least 10 first- to fourth-degree relatives possibly willing to participate. Potential probands were excluded according to the following criteria: unable to provide signed informed consent, unwilling to provide consent to contact family members, psychosis due to a substance use disorder, medication, medical/neurological condition, or pervasive developmental disorder by DSM criteria, existence of a medical condition that may cause neurocognitive deficits, IQ < 70, or lack of proficiency in English. Thirty-six index probands met inclusion criteria and were enrolled in the study.

2.1.2 Index relatives

First- to fourth-degree relatives of each index proband who were 15 years of age or older at the time of recruitment and resided within the contiguous United States were eligible for participation. Potentially eligible relatives were identified by the probands and other enrolled family members and gave permission to be contacted by phone. Eligibility was established via a brief phone screening. Exclusion criteria were minimal: existence of a medical condition that may cause neurocognitive deficits, or lack of proficiency in English. In person interviews were scheduled with eligible relatives who agreed to participate.

2.1.3 Pedigree Members Diagnoses

Pedigree members meeting DSM criteria for schizophrenia or schizoaffective were considered "affected" (American Psychiatric Association, 2000). The remaining relatives were not diagnosed with either schizophrenia or schizoaffective disorder, depressed type, and thus were

"unaffected" pedigree members. The following additional hierarchical diagnostic groups were formed among the unaffected relatives: Other Psychoses; Cluster A (schizotypal personality-disorder); Major Depressive Disorder; Substance Abuse; Miscellaneous; and No Diagnosis.

2.1.4 Controls

Non-psychotic, European-American individuals age 18 to 84, who did not have a first-degree relative with a psychotic disorder, were eligible for inclusion in the control group. Recruitment procedures implemented at the University of Pittsburgh were designed to achieve a representative control group that was on average matched on age, sex, and location of residence to the index relatives enrolled in the study. Potential control individuals residing in the regions from which the majority of index probands and relatives had been recruited were initially contacted through random digit dialing. Interested potential control individuals completed a telephone screening to assess the following exclusion criteria: they or a first-degree relative had been diagnosed with a schizophrenia spectrum disorder or other psychotic disorder, recent exacerbation of non-psychotic psychiatric symptoms (e.g., psychiatric hospitalization or a dose increase of psychiatric medication in the past month), electroconvulsive therapy in the past six months, treatment for alcohol or substance disorder in the past six months, medical condition that could produce psychiatric symptoms or neurocognitive deficits (e.g., Alzheimer's disorder), history of head injury resulting in cognitive changes, or sensory or physical impairments that could interfere with completion of study measures.

2.2 ASSESSMENTS

2.2.1 Diagnostic Assessment

Lifetime, multiaxial diagnoses based on DSM-IV criteria were established by consensus conference by licensed psychiatrists and psychologists who were blind to subject identity and group status (proband, relative, control) (American Psychiatric Association, 2000). All interviews were conducted by trained interviewers with established reliability (kappa > 0.80) and under the supervision of the investigators. Reliability and training among interviewers was reviewed at semi-annual meetings. Interviewers were not blind to participant group status. All participants were administered the Diagnostic Interview for Genetic Studies 2.0 (DIGS) (Nurnberger et al., 1994) to assess current and lifetime psychiatric diagnoses and medical history. Furthermore, at least one relative of each proband was administered the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992) to gather additional diagnostic information about family members. Considerable effort was made to interview each participant in person. On the rare occasions when an in-person appointment was not feasible, interviews were conducted by phone. If available, medical records were also reviewed.

2.2.2. Scale for the Assessment of Negative Symptoms

The Scale for the Assessment of Negative Symptoms (SANS) was used to measure negative symptoms. Developed by Andreasen (1983), it is divided into five subscales: Affective Flattening, Alogia, Avolition-Apathy, Anhedonia-Asociality, and Attention (Table 2). The

ratings were made by a trained interviewer following the DIGS interview, using a six-point scale (0 = not at all to 5 = severe).

In addition to five global subscale ratings, summed subscales were also calculated by summing the individual items of each subscale (e.g., four items for Anhedonia) and dividing by the number of non-missing items for the subscale. A total global scale (average of global subscale ratings) and a total summed scale (average of summed subscale ratings) were also calculated. There was a total of 12 SANS scales analyzed: five global subscales, five summed subscales, one total global scale and one total summed scale.

2.2.3 Depression-Cognitive Score

A depression-cognitive score was also created based on the DIGS ratings for the current episode of major depression (items: guilt; worthlessness; suicidal thoughts; self-harming behaviors). This depression-cognitive scale was used as a covariate to minimize any potential confounding of negative symptoms by depression.

3.0 RESULTS

3.1 SAMPLE CHARACTERISTICS

3.1.1 Sample Selection and Attrition

The initial step was the identification of individuals who had the complete SANS ratings and individuals with missing data. Seven hundred and forty individuals had a DIGS interview of whom five hundred individuals had at least one SANS rating. Individuals who were missing 13 or more items from the SANS ratings (out of 25) were then removed.

After this step, the sample was 493 total individuals, including 449 pedigree and 44 control members. In one of the two sites, the SANS was not administered to any of the controls, explaining for the low number of available controls. Frequency distributions of each SANS rating were inspected for outliers and all scores were deemed valid.

3.1.2 Demographics and Diagnoses

A description of pedigree members with schizophrenia, pedigree members without schizophrenia, and controls in terms of sex, age, and education level is shown in Table 3. There were no significant overall differences observed among the samples on age

[F (2,490) = 2.506, p = 0.073] or sex [χ^2 (2, N = 493) = 5.357, p = .069]. Not surprisingly, there was a significant difference among the samples on education [F (2,489) = 13.730, p = 0.001]. The affected pedigree group (those with the diagnosis of schizophrenia) had significantly fewer years of education than either the unaffected pedigree or controls. The affected pedigree group also had significantly fewer years of education than the controls.

A breakdown by diagnosis is shown in Table 4. There was a total of 90 individuals diagnosed with schizophrenia. One hundred and eighty one individuals within the pedigree possessed other diagnoses; 178 individuals had no diagnoses. Among the 44 controls, 22 had diagnoses and 22 had none.

3.1.3 Correlation of SANS Scales with Demographics

The correlations of the SANS scales with age, sex, education, and depression score are reported in Table 5. Sex was correlated with nine scales ($p < 0.05$): Summed Total, Global Total, Summed Alogia, Global Alogia, Summed Avolition-Apathy, Global Avolition-Apathy, Summed Anhedonia-Asociality, Global Anhedonia-Asociality, and Global Attention with males being rated with higher negative symptoms. Age was positively associated only with the Summed Anhedonia-Asociality rating ($p < 0.05$). Education was negatively correlated with all of the scales ($p < 0.01$). The depression score was only correlated positively with the Global Affective Flattening rating ($p < 0.05$).

3.2 EXAMINATION OF SANS SCALES

3.2.1 Intercorrelations of SANS Scales

Correlations between each SANS scale in the pedigree sample are presented in Table 6. All scales were positively correlated with each of the other scale measures at a p-value of 0.01, including the global and summed scales. The Global Total and Summed Total scales were the highest correlated ($r = 0.95$). All summed scales were highly correlated with their corresponding global scales ($r = 0.85$ to 0.95).

3.2.2 Group Mean Differences on SANS Scales

A description of samples compared on each scale rating is shown in Table 7. Members with schizophrenia, pedigree members without schizophrenia, and controls were compared on each SANS scale, with age, sex, and depression score as covariates. There were significant overall differences among samples for each scale ($p < 0.0001$). The schizophrenia group had significantly higher mean scores on each scale than both the pedigree non-schizophrenia and control group and there were significant differences between the non-schizophrenia pedigree and control groups on four scales: Global Total, Global Affective Flattening, Global Alogia, and Summed Attention ($p < 0.05$).

3.2.3 Heritability of SANS Scales

All analyses of heritability and bivariate analyses of genetic correlation were completed using the SOLAR program (Sequential Oligogenic Linkage Analysis Routines) (Almasy & Blangero, 1998). All dichotomous variables were modeled as threshold traits and all continuous variables were fit to a t-distribution. Total trait variance was separated into two components: (a) a component due to additive polygenic effects, and (b) a component due to the effects of random environmental factors. SOLAR used maximum likelihood estimation to a mixed effects model, and included fixed effects (known covariates) and variance components (genetic and random environmental effects). Shared environmental influences were assumed to be linearly related to the degree of genetic relatedness. In order to prevent ascertainment bias, analyses were conditioned on the trait values of the probands. These analyses were performed first in the sample of all pedigree members, and secondly among pedigree members without any diagnoses in order to minimize potential diagnostic effects on negative symptoms.

Heritability (h^2) indicates the proportion of trait variation that is due to genetic effects. As seen in Table 8, all scales showed significant heritability ($h^2 = 0.31$ to 0.74, $p = 0.0001$) for the entire pedigree sample. In the more conservative sample of pedigree members without any diagnosis, all the scales except Global Attention and Summed Anhedonia-Asociality were significantly heritable ($h^2 = 0.26$ to 0.73, $p = 0.0001$). In general, heritabilities were roughly similar in the two samples.

3.2.4 Genetic Correlations of SANS Scales and Schizophrenia

Genetic correlations were calculated between negative symptoms and schizophrenia. Table 9 presents the genetic (R_G) correlations. Correlations were estimated controlling for covariates (age, sex, and depression score). For both the total and no diagnoses (except schizophrenia) samples, all negative symptoms displayed significant positive genetic correlations with the diagnosis of schizophrenia (R_G = 0.29 to 0.92). The scales for Avolition-Apathy, both summed and global, showed the strongest correlations (R_G = 0.75 for both, p = 0.001) in the total pedigree sample, as well as in the no diagnosis sample. In general, the genetic correlations were similar in the two samples. Such results indicate that even in a conservative sample, the more closely related to an individual with schizophrenia is, the more negative symptoms will be displayed.

3.2.5 Genetic Correlations of SANS Scales and Major Depressive Disorder

Subsequently, genetic correlations with major depressive disorder were estimated for each of the SANS scales in both the general pedigree sample and the sample of pedigree members with either diagnoses of major depressive disorder or no diagnoses. Correlations were estimated controlling for covariates (age, sex, and depression score). As reported in Table 10, unlike schizophrenia, major depressive disorder was not significantly genetically correlated with any of the SANS scales, and in fact showed non-significant trends for negative genetic correlations (R_G = -1 to 0.23) in all the scales. Similar results were obtained within the conservative sample.

As such, the results reflect that relatives who are closely related to an individual with major depressive disorder are no more likely to display negative symptoms than those who are more distantly related.

3.2.6 Genetic Correlations of SANS Scales and Substance Abuse

The genetic correlations with substance abuse were also calculated for each of the SANS scales in both the general pedigree sample and the sample of pedigree members with either diagnoses of substance abuse or no diagnoses. Correlations were estimated controlling for covariates (age, sex, and depression score). Table 11 displays the data for substance abuse genetic correlations. In the general pedigree sample, substance abuse showed a negative correlation with SANS ratings (R_G = -0.30 to 0.20), though not significant. Genetic correlations were slightly more positive in the conservative sample (R_G = 0.46 to 1.0), but still not significant. Of all the scales, within the conservative sample, Summed Attention most closely approached significance (R_G = 0.84, p = 0.03); in the larger sample, no scale neared being significantly different.

These results indicate that relatives who are closely related to an individual with substance abuse are no more likely to display negative symptoms than those who are more distantly related.

4.0 DISCUSSION

The aims of this multigenerational, multiplex study were five-fold: 1) compare the prevalence of negative symptoms among probands, non-psychotic relatives, and controls, 2) estimate the heritabilities of negative symptoms, 3) calculate genetic correlations between individual negative symptom ratings and schizophrenia, 4) calculate genetic correlations between individual negative symptom ratings and major depressive disorder, and 5) calculate genetic correlations between individual negative symptom ratings and substance abuse. The study also served to examine and compare the different components of the SANS scale. The results are summarized as follows, and are elaborated upon below:

1) Negative symptoms were more prevalent in schizophrenia probands than non-psychotic relatives and controls.
2) The majority of SANS scales, both summed and global, were highly heritable.
3) Significant positive genetic correlations were observed between negative symptoms and the diagnosis of schizophrenia.
4) Significant genetic correlations were not observed between negative symptoms and the diagnosis of major depressive disorder.
5) Significant genetic correlations were not observed between negative symptoms and the diagnosis of substance abuse.

4.1 NEGATIVE SYMPTOM PREVALENCE

As predicted, negative symptoms were more prevalent in probands and pedigree members than control individuals.

4.2 SANS SCALE SELECTION

After calculating correlations between each individual SANS scales, it was observed that all the scales were associated with each other to a high degree. This suggested that either global or summed scales are adequate and sufficient for examining negative symptoms scores. The two total scales could also be used alone, or in conjunction with other scales. For example, an individual high in Affective Flattening is likely to also have a high rating in Alogia. Additionally, such correlations could be beneficial in predicting values for incomplete or missing data. No SANS scales were uncorrelated.

4.3 GENETIC LIABILITY TO SCHIZOPHRENIA

4.3.1 Heritabilities and Genetic Correlations

The heritabilities of the SANS scales were largely significant, suggesting that the characteristics measured by the scales are influenced by genetic factors. The heritability estimates were similar to those reported in previous studies (Chen et al., 2009).

Foremost, in both the total and the conservative sample, Summed and Global Avolition-Apathy exhibited the highest genetic correlations with schizophrenia, suggesting that lack of persistence, energy, and motivation all share a percentage of their genetic effects with schizophrenia.

Though SANS scales were mainly genetically correlated with the diagnosis of schizophrenia, Summed Attention was not in the large sample ($R_G = 0.29$, $p = 0.06$) and displayed the smallest significant correlation in the restricted sample ($R_G = 0.53$, $p = 0.001$). It is unclear why this is so, considering the correlations between all of the scales.

As predicted, neither major depressive disorder nor substance abuse were significantly correlated with negative symptoms, although both displayed non-significant negative trends. This suggests that negative symptoms have a specific genetic connection to schizophrenia, rather than contributing to a more generalized association shared by numerous diagnoses.

Being both heritable and strongly genetically correlated with schizophrenia in this sample, individual SANS scores may be potentially useful in genetic studies, especially Avolition-Apathy. Overall, it seems unlikely that genes contributing to liability to major depressive disorder or substance abuse also influence SANS scale scores.

4.3.2 Endophenotype Potential

Returning to the original question, how do the results stack up to the concept and criteria of an endophenotype? In order to be beneficial in the understanding of the disorder, such phenotypes should be sensitive to specifically schizophrenia liability in particular, not a general signal of liability to psychopathology. As negative symptoms were genetically correlated with schizophrenia (and not major depressive disorder or substance abuse), the possibility as an endophenotype fits the definition well.

4.4 LIMITATIONS

As in all research, the current study did have limitations. For one, the genetic correlations with schizophrenia could potentially have been increased by the choice of multiplex pedigrees compared to simplex families. In addition, there could be a problem due to potential effects of the medications subjects were taking during data collection.

As individuals with diagnoses of major depressive disorder or substance abuse also were relatives of schizophrenia patients, such results should be reproduced in a general population sample. As always, there is a possibility of selection bias, or an error in the selection of choosing the subjects, resulting in a non-random sample. However most results relied on relatives who were not biased. Another potential limitation was that of a wide age range. Although the average age of a relative was generally in the 40s and 50s, some subjects were quite young, and at risk for developing schizophrenia or another disorder. Thus, individuals could have been

misclassified; if researchers had followed up these individuals, some could have developed schizophrenia. Such an error would inflate the results, and increase the chance of positive genetic correlations.

Finally, as the study was not an adoption study, the separation of genetic and shared environmental effects rested on the assumption that shared environmental effects are not linear across relative classes.

4.5 FUTURE DIRECTIONS

The results of this study suggest that the numerous negative symptoms scales, but especially those for Avolition-Apathy, both summed and global, may be sensitive to genetic effects that increase risk for schizophrenia. Such genetic factors could be applied to a more thorough examination, such as linkage analyses, in order to evaluate the potential of such factors to aid in identifying specific genetic variants that contribute to the etiology of schizophrenia. For example, such genetic factors could be employed in genome-wide bivariate linkage analyses of schizophrenia, allowing the value of each factor to be discerned. Furthermore, one could examine the genetic intercorrelations among the SANS scales – if present, it would be suggest a genetic homogeneity across individual negative symptoms.

Another potential avenue of research would be a comparison with positive or negative symptoms; perhaps a different dimension would demonstrate stronger genetic correlations and heritabilities, and thus serve as a better endophenotype.

5.0 CONCLUSION

The current study offered evidence of the use of negative symptoms as a potential endophenotype for schizophrenia. This method is better than the initial use of GWAS as it is prioritizing: negative symptoms seems to affect a crucial aspect of the disorder, rather than examining all genes equally. The question of if endophenotypes will find more genetic variants depends on the penetrance.

Overall, the study suggests the potential utility of negative symptoms as a candidate endophenotype with some diagnostic specificity in studies seeking to identify genetic risk variants contributing to schizophrenia.

APPENDIX

TABLES

Table 1. Studies of Negative Symptoms among Relatives of Schizophrenia Patients

Study	Year	Relatives of Schizophrenia Probands				Control Comparisons							Assessment[1]	Blind Raters	Results		Effect Size	P-value
		Relative composition	Number	Age Range	Sex (% male)	Origin of controls	Control's Relatives (Y/N)	Screening Criteria	Match to SZ relatives	Number	Age Range	Sex (% male)			Relatives (mean/SD)	Controls (mean/SD)		
Kendler et al.	1993	First-degree	314	N/a	N/a	Voter registration	Yes	a	Age, sex	183	N/a	N/a	Interview	Yes	34.60%	19.30%	0.62	0.02
Craver et al.	1999	Siblings	39	18-45	18	Local advertising	Yes	b	Age, sex	38	21-34	11	SANS	N/a	0.57/(1.15)	0.50/(0.86)	0.08	0.266
Fanous et al.	2001	First-degree	127	N/a	N/a	N/a	No	b	N/a	N/a	N/a	N/a	MSSS	Yes	0.20692	N/a	N/a	0.0002
Delawalla et al.	2006	Siblings	31	18-25	51.6	Local advertising	Yes	d	Age, sex	42	18-25	71.4	SANS	N/a	Graph	Graph	1.3	0.01
Smith et al.	2008	Siblings	34	18-25	47.1	Local advertising	Yes	c	Age, sex	56	17-23	19.6	SANS	N/a	-0.06/(0.09)[3]	-0.40/(0.05)	N/a	0.002
Chen et al.	2009	Siblings	65	9-31	41.5	Local advertising	Yes	b	Age, sex, education, ethnic	80	9-31	33.8	SANS	N/a	-0.13/(0.062)[4]	-0.36/(0.039)	2.76	0.0001
Scala et al.	2012	First-degree	55	18-60	40	Local advertising	No	c	Age, sex, education	55	29-49	40	SANS	N/a	0.56 (0.65)	0.21 (0.3)	N/a	0.0001

a No schizophenia or schizoaffective
b No life history of Axis 1 psychotic
c No life history of Axis 1 psychotic, substance abuse
d No life history of Axis 1 psychotic, substance abuse, MDD

1 Negative Symptoms Assessment Test
2 Negative schizotypy relationship with negative symptom
3 Least square means
4 Z-score

SANS Scale for the Assessment of Negative Symptoms
MSSS Major Symptoms of Schizophrenia Scale

Table 2. Scale for the Assessment of Negative Symptoms[1,2]

1	Affective Flattening or Blunting	
		1 Unchanged Facial Expression
		2 Decreased Spontaneous Movements
		3 Paucity of Expressive Gestures
		4 Poor Eye Contact
		5 Affective Nonresponsiveness
		6 Inappropriate Affect
		7 Lack of Vocal Inflections
		8 Global Rating of Affective Flattening
2	Alogia	
		9 Poverty of Speech
		10 Poverty of Content of Speech
		11 Blocking
		12 Increased Latency of Response
		13 Global Rating of Alogia
3	Avolition-Apathy	
		14 Grooming and Hygiene
		15 Impersistence at Work or School
		16 Physical Anergia
		17 Global Rating of Avolition-Apathy
4	Anhedonia-Asociality	
		18 Recreational Interests and Activities
		19 Sexual Activity
		20 Ability to Feel Intimacy and Closeness
		21 Relationships with Friends and Peers
		22 Global Rating of Anhedonia-Asociality
5	Attention	
		23 Social Inattentiveness
		24 Inattentiveness During Mental Status Testing
		25 Global Rating of Attention

[1] Andreasen (1983).
[2] Ratings are measured on a 0 – 5 scale.

Table 3. Demographics of Pedigree and Control Participants

	Number	Sex	Age		Education
		% male (number)	Mean (St. Dev)		Mean (St. Dev)
Affected Pedigree	90	58.89 (53)	46.13 (12.20)		12.37 (2.83)[a]
Unaffected Pedigree	359	45.40 (163)	44.49 (18.52)		13.48 (2.97)[b]
Control	44	45.45 (20)	50.41 (18.28)		15.16 (2.48)[c]
P-value[1]	---	0.069	0.073		0.0001

[1] P-value for the overall ANOVA or chi-square analysis.
[a, b, c] Groups not sharing superscripts are significantly different based on LSD test.

Table 4. Diagnostic Distribution among Pedigree and Control Participants

Diagnosis[1]	Pedigree	Control
Schizophrenia	90	0
Other Psychoses	21	2
ClusterA	18	0
Major Depressive Disorder	47	9
Substance Abuse	37	7
Miscellaneous	58	4
No Diagnosis	178	22
Total	449	44

[1] Diagnoses of schizophrenia, other psychoses, schizotypal personality-disorder (Cluster A), major depressive disorder, substance abuse, and miscellaneous are hierarchical.

Table 5. Correlations between Demographics and SANS Scales in the Pedigree Sample

	Sex[1]	Age	Education	Depression Score
Summed Total	-0.148 [b]	0.011	-0.322[b]	0.027
Global Total	-0.145 [b]	-0.012	-0.311[b]	0.050
Summed Affective Flattening	-0.068	-0.069	-0.302[b]	0.069
Global Affective Flattening	-0.081	-0.077	-0.263[b]	0.100 [a]
Summed Alogia	-0.095[a]	0.006	-0.258[b]	0.036
Global Alogia	-0.154[b]	-0.029	-0.243[b]	0.022
Summed Avolition-Apathy	-0.177[b]	-0.007	-0.266[b]	0.033
Global Avolition-Apathy	-0.153[b]	-0.022	-0.243[b]	0.047
Summed Anhedonia-Asociality	-0.154[b]	0.089[a]	-0.281[b]	0.018
Global Anhedonia-Asociality	-0.139[b]	0.073	-0.250[b]	0.048
Summed Attention	-0.066	0.032	-0.333[b]	0.005
Global Attention	-0.082	0.016	-0.285[b]	-0.005

[1] Sex (male = 1, female = 2).
[a] Correlation is significant at the 0.05 level (2-tailed).
[b] Correlation is significant at the 0.01 level (2-tailed).

Table 6. Intercorrelations of SANS Scales

	Summed Total	**Global Total**	Summed Affect	Global Affect	Summed Alogia	Global Alogia	Summed Avolition	Global Avolition	Summed Anhedonia	Global Anhedonia	Summed Attention	Global Attention
Summed Total		0.956[a]	0.865[a]	0.816[a]	0.832[a]	0.792[a]	0.877[a]	0.846[a]	0.871[a]	0.821[a]	0.662[a]	0.703[a]
Global Total	0.956[a]		0.823[a]	0.861[a]	0.803[a]	0.833[a]	0.842[a]	0.854[a]	0.873[a]	0.840[a]	0.703[a]	0.774[a]
Summed Affective Flattening	0.865[a]	0.823[a]		0.881[a]	0.753[a]	0.723[a]	0.688[a]	0.667[a]	0.641[a]	0.613[a]	0.525[a]	0.552[a]
Global Affective Flattening	0.816[a]	0.861[a]	0.881[a]		0.696[a]	0.725[a]	0.663[a]	0.676[a]	0.669[a]	0.648[a]	0.500[a]	0.543[a]
Summed Alogia	0.832[a]	0.803[a]	0.753[a]	0.696[a]		0.905[a]	0.610[a]	0.593[a]	0.597[a]	0.565[a]	0.617[a]	0.632[a]
Global Alogia	0.792[a]	0.833[a]	0.723[a]	0.725[a]	0.905[a]		0.594[a]	0.593[a]	0.597[a]	0.580[a]	0.595[a]	0.624[a]
Summed Avolition-Apathy	0.877[a]	0.842[a]	0.688[a]	0.663[a]	0.610[a]	0.594[a]		0.953[a]	0.707[a]	0.678[a]	0.549[a]	0.596[a]
Global Avolition-Apathy	0.846[a]	0.854[a]	0.667[a]	0.676[a]	0.593[a]	0.593[a]	0.953[a]		0.698[a]	0.688[a]	0.524[a]	0.575[a]
Summed Anhedonia-Asociality	0.871[a]	0.837[a]	0.64[a]	0.669[a]	0.597[a]	0.597[a]	0.707[a]	0.698[a]		0.932[a]	0.519[a]	0.570[a]
Global Anhedonia-Asociality	0.821[a]	0.840[a]	0.613[a]	0.648[a]	0.565[a]	0.580[a]	0.678[a]	0.688[a]	0.932[a]		0.490[a]	0.532[a]
Summed Attention	0.662[a]	0.703[a]	0.525[a]	0.500[a]	0.617[a]	0.595[a]	0.549[a]	0.521[a]	0.519[a]	0.490[a]		0.855[a]
Global Attention	0.703[a]	0.774[a]	0.552[a]	0.543[a]	0.632[a]	0.624[a]	0.596[a]	0.575[a]	0.570[a]	0.532[a]	0.855[a]	

[a] Correlation is significant at the 0.01 level (2-tailed).

Table 7. Sample Differences on SANS Scales[1]

Sample	**Summed Total**	**Global Total**	Summed Affective Flattening	Global Affective Flattening	Summed Alogia	Global Alogia	Summed Avolition-Apathy	Global Avolition-Apathy	Summed Anhedonia-Asociality	Global Anhedonia-Asociality	Summed Attention	Global Attention
	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)	mean (st. dev)
Affected Pedigree	1.46 (1.03)[a]	1.81 (1.17) [a]	1.21 (1.03) [a]	1.91 (1.32) [a]	1.00 (1.07) [a]	1.39 (1.35) [a]	1.78 (1.29) [a]	2.04 (1.47) [a]	2.09 (1.40) [a]	2.33 (1.37) [a]	1.47 (1.38) [a]	1.55 (1.43) [a]
Unaffected Pedigree	0.31 (0.53) [b]	0.50 (0.73) [b]	0.27 (0.56) [b]	0.54 (0.96) [b]	0.26 (0.56) [b]	0.43 (0.88) [b]	0.32 (0.73) [b]	0.40 (0.89) [b]	0.53 (0.89) [b]	0.69 (1.14) [b]	0.55 (0.95) [b]	0.43 (0.89) [b]
Control	0.15 (0.29) [b]	0.18 (0.37) [c]	0.11 (0.29) [b]	0.18 (0.43) [c]	0.08 (0.21) [b]	0.09 (0.26) [c]	0.16 (0.50) [b]	0.17 (0.65) [b]	0.34 (0.89) [b]	0.37 (0.92) [b]	0.11 (0.37) [c]	0.12 (0.39) [b]
P-value[2]	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[1] Covariates were sex, age and depression score.
[2] P-value for the overall ANOVA analysis.
[a, b, c] Groups not sharing superscripts are significantly different based on LSD test.

Table 8. Heritabilities of SANS Scales in the Pedigree Sample[1]

	Pedigree		Pedigree Without Diagnosis	
	Heritability	P-value	Heritability	P-value
Summed Total	0.53	0.0001	0.31	0.0001
Global Total	0.62	0.0001	0.65	0.0001
Summed Affective Flattening	0.43	0.0001	0.67	0.0001
Global Affective Flattening	0.52	0.0001	0.55	0.0001
Summed Alogia	0.56	0.0001	0.26	0.0001
Global Alogia	0.74	0.0001	0.35	0.0001
Summed Avolition-Apathy	0.45	0.0001	0.69	0.0001
Global Avolition-Apathy	0.48	0.0001	0.73	0.0001
Summed Anhedonia-Asociality	0.40	0.0001	0.36	0.5000
Global Anhedonia-Asociality	0.37	0.0001	0.28	0.0001
Summed Attention	0.66	0.0001	0.64	0.0001
Global Attention	0.31	0.0001	0.27	0.5000

[1] Covariates were sex, age and depression score.

Table 9. Genetic Correlations of SANS Scales with Schizophrenia[1] in the Pedigree Sample

	Pedigree		Pedigree without Diagnosis	
	R_G	P-value from 0	R_G	P-value from 0
Summed Total	0.61	0.001	0.84	0.001
Global Total	0.57	0.001	0.79	0.001
Summed Affective Flattening[2]	0.58	0.001	0.78	0.001
Global Affective Flattening[2]	0.55	0.001	0.77	0.001
Summed Alogia	0.46	0.003	0.72	0.001
Global Alogia	0.69	0.003	0.69	0.001
Summed Avolition-Apathy	0.75	0.001	0.87	0.001
Global Avolition-Apathy	0.75	0.001	0.92	0.001
Summed Anhedonia-Asociality[2]	0.64	0.001	0.82	0.001
Global Anhedonia-Asociality[2]	0.59	0.001	0.73	0.001
Summed Attention	0.29	0.060	0.53	0.007
Global Attention	0.47	0.003	0.67	0.001

[1] Dichotomized variable: Diagnosis/symptoms: absent = 0, present = 1.
[2] Was not evaluated with covariates (sex, age, and depression score) due to convergence failure.

Table 10. Genetic Correlation of SANS Scales with Major Depressive Disorder[1] in the Pedigree Sample

	Pedigree		Pedigree without Diagnosis	
	R_G	P-value from 0	R_G	P-value from 0
Summed Total	-0.47	0.07	-0.49	0.33
Global Total	-0.27	0.26	-0.09	0.85
Summed Affective Flattening[2]	-0.62	0.04	-0.48	0.52
Global Affective Flattening[2]	-0.34	0.22	0.01	0.99
Summed Alogia	-0.29	0.27	-0.90	0.47
Global Alogia	-0.35	0.21	-1.00	0.65
Summed Avolition-Apathy	-0.54	0.06	-0.27	0.52
Global Avolition-Apathy	-0.34	0.21	0.01	0.97
Summed Anhedonia-Asociality[2]	-0.29	0.37	-0.29	0.64
Global Anhedonia-Asociality[2]	-0.16	0.62	-0.27	0.61
Summed Attention	-0.27	0.32	-0.53	0.28
Global Attention	-0.16	0.56	0.23	0.80
Substance Abuse	-1	0.11	Convergence Failure	

[1] Dichotomized variable: Diagnosis/symptoms: absent = 0, present = 1.
[2] Was not evaluated with covariates (sex, age, and depression score) due to convergence failure.

Table 11. Genetic Correlation of SANS Scales with Substance Abuse[1] in the Pedigree Sample

	Pedigree		Pedigree without Diagnosis	
	R_G	P-value from 0	R_G	P-value from 0
Summed Total	-0.22	0.38	0.57	0.20
Global Total	-0.10	0.68	0.61	0.14
Summed Affective Flattening[2]	-0.30	0.33	1.00	0.17
Global Affective Flattening[2]	-0.13	0.66	1.00	0.06
Summed Alogia	-0.18	0.50	1.00	0.37
Global Alogia	-0.05	0.87	1.00	0.35
Summed Avolition-Apathy	-0.23	0.40	0.51	0.08
Global Avolition-Apathy	-0.18	0.52	0.58	0.08
Summed Anhedonia-Asociality[2]	-0.18	0.59	0.48	0.28
Global Anhedonia-Asociality[2]	0.03	0.92	0.46	0.23
Summed Attention	0.20	0.41	0.84	0.03
Global Attention	0.03	0.90	0.90	0.22
Major Depressive Disorder	-1	0.11	Convergence Failure	

[1] Dichotomized variable: Diagnosis/symptoms: absent = 0, present = 1.
[2] Was not evaluated with covariates (sex, age, and depression score) due to convergence failure.

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