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Association of *Sult4A1* SNPs with psychopathology and cognition in patients with schizophrenia or schizoaffective disorder

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ABSTRACT

A number of genes located on chromosome 22q11–13, including catechol-*O*-methyltransferase (COMT), are potential schizophrenia susceptibility genes. Recently, the sulfotransferase-4A1 (*Sult4A1*) locus within chromosome 22q13 was reported to be linked to schizophrenia in a family TDT study. *Sult4A1* is related to metabolism of monoamines, particularly dopamine and norepinephrine, both of which have been implicated in the pathophysiology of the psychopathology and cognitive dysfunction components of schizophrenia. An available, prospectively collected data base was interrogated to determine how three *Sult4A1* SNPs: rs138060, rs138097, and rs138110, previously shown to be associated with schizophrenia might be associated with psychopathology, cognition, and quality of life in a sample of 86 Caucasian patients with schizophrenia or schizoaffective disorder. The majority of patients met criteria for treatment resistant schizophrenia and had been drug-free for one week or longer at the time of evaluation. The major findings were: 1) patients heterozygous (T/G) for rs138060 had significantly worse Brief Psychiatric Rating Scale (BPRS) Total and anxiety/depression sub-scale scores, and higher Scale for the Assessment of Positive Symptoms (SAPS) Total scores than G/G homozygous patients; and 2) patients heterozygous (A/G) for rs138097 demonstrated significantly worse performance on neuropsychological testing, specifically on tests of executive function and working memory, compared to patients homozygous for the G and A alleles. RS138110 was unrelated to psychopathology and cognition. These results provide the first evidence of how genetic variation in *Sult4A1* may be related to clinical symptoms and cognitive function in schizophrenia, and permit future studies to attempt to replicate these potentially important findings.

1. Introduction

A number of genes located on chromosome 22q11–13 have been identified as increasing the risk for schizophrenia through association studies. Microdeletions of the 22q11 locus are associated with a greatly increased risk of developing schizophrenia (Karayiorgou and Gogos, 2004). Among the genes in the 1.5 Mb critical region of 22q11 which are candidates for increasing the risk are PRODH, ZDHHC8, TBX1 and COMT. There is; however, considerable evidence for one or more susceptibility genes distal to 22q11, e.g. in 22q12 or 22q13 (DeLisi et al., 2002; Mowry et al., 2004; Takahashi et al., 2003, 2005). One such gene may be the sultotransferase-4A1 (*Sult4A1*) gene, located in 22q13, which was first reported to be a candidate gene for schizophrenia on the basis of a microsatellite marker study targeting a polymorphism in its 5′ nontranslated region in 27 families having at least two siblings with schizophrenia or schizophrenia spectrum disorder (Brennan and Condra, 2005). Three single nucleotide polymorphism (SNPs) spanning a 37 kb segment which contained the Sult4A gene were evaluated. D22s1749e was found to be associated with risk for schizophrenia or

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schizophrenia spectrum disorder. Global chi-square analysis of haplotypes involving the single nucleotide polymorphisms (SNPs) (rs138060, rs138097 and rs138110) as well as D22s1749E showed significant transmission disequilibrium test (TDT) values. Subsequently, a more distal locus, centered at 61 cM, was also found to be associated with a broader disease definition which included schizotypal personality disorder (Condra et al., 2007). It was concluded that at least two separable, but closely linked, loci within 22q13 may influence susceptibility to schizophrenia spectrum disorders.

Phenolsulfotransferases, which catalyze the formation of dopamine sulfate (Goldstein et al., 2003), have been implicated in schizophrenia, based upon two studies which found elevated levels of dopamine sulfate in the cerebrospinal fluid of patients with schizophrenia (van Kammen et al., 1986; Risby et al., 1993), and a correlation between dopamine sulfate levels and negative symptoms (Risby et al., 1993). There is, as yet, no clarification of the specific importance, if any, of *Sult4A1* for specific symptoms of schizophrenia. Demonstrating a relationship between *Sult4A1* SNPs and psychopathology or cognitive disturbance in patients with schizophrenia would add to the evidence of the importance of *Sult4A1* for schizophrenia and suggest possible avenues by which its effects, if any, on brain monoamines and neurosteroids could be further investigated.

The *Sult4A1* gene is highly conserved between species and shows no variation in the coding sequence in a sample of 118 ethnically diverse humans (Hildebrandt et al., 2007). *Sult4A1* encodes a cytoplasmic sulfotransferase which is specific to the brain. It, and other members of the sulfotransferase superfamily, are involved in the sulfation and inactivation of neurotransmitters, including dopamine (DA) and norepineprhine, steroids (including neurosteroids), drugs and xenobiotics (Yu et al., 1985; Falany et al., 2000; Sakakibara et al., 2002; Liyou et al., 2003). Sulfotransferases have been implicated in the etiology of Alzheimer's disease (Miyata et al., 2007; Kimoto et al., 2001).

This study retrospectively examined associations between three *Sult4A1* SNPs and clinical symptoms, cognitive function, and quality of life in a sample of 86 Caucasian patients with schizophrenia.

2. Methods

2.1. Subjects

The subjects in this study included all Caucasian subjects for whom DNA was available from a larger group of patients with schizophrenia or schizoaffective disorder who participated in a comprehensive study of the biology of schizophrenia between 1986 and 1995 that was directed by the senior author (H.Y.M). All subjects provided written informed consent. The assessment of patients who participated in these studies has been described in detail elsewhere (Lee et al., 1999; Hagger et al., 1993; Kenny and Meltzer, 1991; Woodward et al., 2007). Briefly, diagnoses were established on the basis of structured interviews of the patient, examination of all available medical records, and confirmatory information from family members whenever possible. Diagnoses were a mixture of DSM-III-R or ICD-9 criteria but all have been updated to DSM-IV criteria by a research psychiatrist (H.Y.M). Exclusion criteria included history of learning disabilities, head trauma, stroke, or neurological illness, and active alcohol or drug abuse at the time they were assessed. Patients were assessed during inpatient admission and the majority of patients (67.4%) were medication free at the time, with 64% of subjects having undergone a minimum one day medication washout. With the exception of five patients, for whom medication status was unknown, the remaining subjects were receiving a typical APD, usually haloperidol. Clinical symptoms were rated on the Brief Psychiatric Rating Scale (BPRS: Overall and Gorham, 1962), Scale for Assessing Positive Symptoms (SAPS: Andreasen, 1984), Global Assessment of Function (GAF) from the DSM-IIIR, and Global Assessment Scale (GAS: Endicott et al., 1976). Social, occupational, and overall quality of life was assessed with the Heinrichs Quality of Life Scale (HQLS: Heinrichs et al., 1984). GAS and GAF data were unavailable for 6 subjects, 11 subjects were not rated on the SAPS, and the HQLS was completed on a subset of 66 patients. In addition, 49 subjects completed all or part of a neuropsychological assessment that included tests of attention, learning and memory, including working memory, and executive functions. The number of patients completing neuropsychological testing was lower because such testing was not initially part of the investigational protocol. A complete description of the tests included in the neuropsychological battery is included in a prior report (Woodward et al., 2007). Factor analysis of the neuropsychological battery revealed three factors that collectively accounted for 69% of the total variance and the minimum loading of any test on its respective factor was .58. The three factors were denoted: 1) Memory Function; 2) Attention and Verbal Fluency; and 3) Executive Function (Woodward et al., 2007). For the neuropsychological test data, the factor scores are reported as *Z*-scores which were created by standardizing each test variable (mean=0, SD=1) to a control sample that consisted of 26 subjects and averaging the standardized scores included in each factor (Woodward et al., 2007). A global cognitive score was created by averaging the mean *Z*-scores of all neuropsychological variables and this served as the primary outcome measure for the neurocognitive analysis.

2.2. Genotyping

Forty-seven of the blood samples were collected and sent to the Clarke Institute of Psychiatry in Toronto, Ontario, Canada. Genomic DNA was extracted from white blood cells using the high-salt method (Lahiri and Nurnberger, 1991). The DNA from the rest of the blood samples was extracted directly from fresh blood samples. Each subject's *Sult4A1* genotype status was determined by the Vanderbilt University Human Genetics Core Laboratory using the TaqMan assay developed by Applied Biosystems. A large proportion of the samples were also genotyped at the University of Louisville as described in Brennan and Condra (2005) with complete agreement between the two methods. For the TaqMan assay, the genomic sequence flanking the SNP was submitted to Applied Biosystems for development of an assay-by-design. Each unique TaqMan minor-grove-binding (MGB) allele-specific probe was labeled with either a 5′-FAM or a 5′VIC reporter dye. PCR amplifications of genomic DNA was performed in a 384-well plate in an ABI PRISM 7900. Allele

discrimination of the PCR products was performed on an ABI Prism 7900HT Sequence Detections System by use of Sequence Detector Software (SDS), version 2.2. Standard genotype calling was converted by a customized spreadsheet. In cases where a reaction failed (<2% of total), a second reaction was carried out to resolve discrepancies.

2.3. Statistical analysis

The primary dependent variables were the BPRS Total score, HQLS Total score, and Global Cognitive Summary measure. Each of these variables was examined with univariate ANOVAs to determine if genotype was a predictor. In cases when the assumption of equality of variances was violated, based on a significant Levene's test at alpha<.05, the groups were compared using the Welch test. Significant main effects of group at alpha=.05 were followed up with post-hoc pairwise contrasts to determine the source of the genotype differences. When necessary, significant group differences were confirmed after controlling for potential demographic differences in age, gender, education, age at illness onset, medication status, and treatment resistance status between genotype groups. Secondary dependent variables for psychopathology and cognition included the BPRS sub-scales, SAPS total score, GAS, and GAF, and individual cognitive domains and tests, respectively. Group differences on secondary dependent variables for psychopathology and cognition were only examined in cases where a main effect of genotype was observed on the respective primary dependent variable (BPRS Total or Global Cognitive Summary score).

3. Results

Demographic data for the whole sample and genotype sub-groups are presented in Table 1. A trend towards a significant relationship between the Global Cognitive Summary and HQLS was noted (r=.30, p<.09); although this was not the focus of the study and global cognition and HQLS scores were available for only 35 subjects. Each SNP did not differ from Hardy–Weinberg equilibrium (rs138060: Chi-squared p<.99; rs138097: Chi-squared p<0.84; rs138110: Chi-squared p<.94). The three SNPs were chosen because they define the four common haplotypes in the gene for Caucasians. The frequencies observed the rs138060–rs138097–rs138110 haplotypes in this sample were: TAT 0.33; GGT 0.27; GGC 0.20; TGC 0.20 as determined by the program Haploview. The frequencies of the genotypes and haplotypes for the three SNPs for this sample of Caucasian patients were not significantly different from that of the Caucasian probands with schizophrenia spectrum disorder studied by Brennan and Condra (2005). There were few significant demographic differences between genotype groups for each SNP. Patients homozygous for the T allele of rs138060 were significantly younger than G homozygous and heterozygous patients (F(2,83)=3.08, p<.05); and patients homozygous for the G allele of rs138060 had a significantly later onset age than both T homozygous and heterozygous patients (F(2,83)=3.95, p<.03). There were no significant differences between genotypes for rs138097 and rs138110.

3.1. Sult4A1 SNP associations with psychopathology and quality of life

Mean values stratified by genotype for the BPRS total, positive, withdrawal–retardation (negative symptoms), and anxiety/depression sub-scales, SAPS total, GAS, GAF, and HQLS are presented in Table 2.

3.1.1. RS138060

One-way ANOVAs revealed a main effect of genotype on the BPRS Total score (F(2,83)=3.10, p<0.05). In addition, group effects were also observed on the secondary psychopathology variables BPRS Anxiety/Depression sub-scale (F(2,81)=3.89, p<0.03) and SAPS Total score (F(2,72)=4.80, p<0.01). Post-hoc pairwise contrasts revealed that T/G patients demonstrated higher BPRS Total scores (p<0.02), BPRS Anxiety/Depression sub-scale (p<.007) and higher SAPS Total scores (p<0.01) than G/G patients. Adding age and age of onset as covariates had minimal effect on the results. The main effect of genotype remained significant at the trend level for BPRS Total

Table 1
Patient demographics*

	Sult4A1 genotype									
	Total sample	rs138060[C]			rs138097			rs138110[D]		
		T/T	T/G	G/G	A/A	A/G	G/G	T/T	T/C	C/C
No. of subjects	86	24	42	18	10	35	41	28	43	14
Age	32.9 (8.9)	29.6 (8.4)[E]	34.1 (7.6)	35.6 (10.5)	30.1 (9.3)	31.9 (8.4)	34.5 (9.0)	31.2 (7.8)	33.8 (10.0)	32.6 (7.7)
Education[A]	12.3 (1.8)	12.5 (1.6)	12.4 (1.8)	11.7 (1.9)	12.6 (1.4)	12.3 (1.8)	12.3 (2.0)	12.2 (1.5)	12.4 (1.9)	12.2 (2.3)
Onset age	20.4 (5.6)	19.2 (3.2)	19.9 (4.1)	23.6 (9.2)[F]	18.8 (3.2)	20.2 (4.1)	21.0 (7.0)	20.4 (5.4)	21.0 (6.3)	18.6 (3.1)
Duration of illness	12.5 (7.6)	10.4 (8.0)	14.3 (7.8)	12.0 (6.2)	11.3 (9.3)	11.8 (7.6)	13.5 (7.3)	11.4 (7.2)	12.9 (8.1)	13.9 (7.6)
No. of hospitalizations[B]	6.9 (6.4)	5.8 (6.1)	8.2 (7.3)	5.2 (3.2)	7.8 (7.2)	6.0 (6.5)	7.3 (6.1)	6.3 (5.6)	7.0 (7.2)	7.4 (5.8)
Treatment resistant (%)	79.1	75.0	81.0	83.3	80.0	80.0	78.0	78.6	81.4	71.4
Gender (% male)	70.9	66.7	73.8	72.2	70.0	65.7	75.6	71.4	67.4	85.7
Unmedicated (%)	67.4	66.7	66.7	72.2	60.0	80.0	63.4	67.9	67.4	71.4

* Mean (SD).
[A]Unavailable for 10 subjects.
[B]Unavailable for 5 subjects.
[C]Genotype not available for 2 subjects.
[D]Genotype unavailable for 1 subject.
[E]Main effect of age (F(2,83)=3.08, p<.053: T/T<T/G (p<.05); T/T<G/G (p<.03).
[F]Main effect of onset age (F(2,83)=3.95, p<.03: G/G>T/T (p<.02; G/G>T/G (p<.02).

Table 2
Sult4A1 SNPs, psychopathology and quality of life in schizophrenia[a]

	Sult4A1 genotype									
	Total sample	rs138060			rs138097			rs138110		
		T/T	T/G	G/G	A/A	A/G	G/G	T/T	T/C	C/C
No. of subjects	86	24	42	18	10	35	41	28	43	14
BPRS total[b]	31.0 (14.0)	30.3 (15.4)	34.2 (13.1)	24.6 (12.8)	30.1 (11.5)	34.0 (14.0)	28.6 (14.5)	31.4 (11.9)	31.6 (15.0)	27.8 (15.8)
BPRS Positive Sub-scale	11.1 (5.8)	10.0 (6.8)	12.2 (5.4)	10.4 (5.1)	10.9 (7.0)	12.3 (5.1)	10.2 (6.0)	11.7 (5.6)	11.7 (5.6)	8.0 (6.2)
BPRS Negative Sub-scale	4.4 (3.2)	5.1 (3.7)	4.4 (3.0)	3.7 (3.0)	4.8 (2.1)	4.4 (2.6)	4.3 (3.9)	4.5 (2.3)	4.4 (3.1)	4.1 (5.0)
BPRS Anxiety/depression[b]	5.6 (3.9)	5.6 (3.6)	6.5 (4.1)	3.5 (2.9)	6.0 (2.0)	6.2 (4.0)	5.0 (4.1)	5.3 (3.2)	5.9 (4.1)	5.2 (4.3)
SAPS—total[b]	7.9 (4.4)	6.8 (5.1)	9.5 (3.9)	6.1 (3.6)	6.7 (5.6)	9.5 (3.9)	6.7 (4.2)	8.3 (4.5)	8.5 (4.2)	4.8 (4.0)
GAS	33.6 (10.8)	33.4 (11.0)	32.6 (9.3)	34.7 (12.9)	33.3 (10.4)	33.5 (10.0)	33.8 (11.9)	33.9 (10.2)	32.5 (11.0)	36.2 (11.8)
GAF	33.6 (10.8)	33.4 (11.5)	32.6 (9.3)	34.7 (12.9)	33.3 (10.4)	33.5 (10.0)	33.8 (11.9)	33.9 (10.2)	32.5 (11.0)	36.2 (11.8)
HQLS—total	41.4 (20.4)	41.2 (17.8)	36.8 (18.1)	52.6 (26.2)	43.8 (18.3)	36.9 (15.1)	45.2 (25.1)	38.9 (20.4)	42.4 (15.6)	44.0 (31.2)

[a] Mean (SD).
[b] Main effect of rs138060 genotype ($p<.05$). See text for additional details.

score ($F(2,79)=2.88$, $p<0.06$) and the pairwise contrast between G allele homozygous and T/G heterozygous patients remained significant ($p<0.02$). The results for the BPRS Anxiety/Depression sub-scale were unchanged. Similarly, the main effect of genotype on SAPS total scores remained significant ($F(2,68)=4.15$, $p<0.02$) as did the pairwise contrast between G/G and T/G patients ($p<0.008$).

3.1.2. RS138097

No effect of genotype was observed in the primary psychopathology measures (BPRS Total score) or HQLS.

3.1.3. RS138110

No effect of genotype was observed in the primary psychopathology measures (BPRS Total score) or HQLS.

3.2. Sult4A1 SNP associations with cognition

As summarized in Table 3, rs138097 genotype showed extensive significant associations with cognition. A main effect of rs138097 genotype was observed on the Global Cognitive Summary score ($F(2,42)=3.74$, $p<.01$) and the executive function domain ($F(2,42)=5.71$, $p<.01$). For the Global Cognitive Summary score, A allele and G allele homozygous patients demonstrated superior scores compared to A/G heterozygous patients ($p<.02$ and $p<.01$, respectively). Additionally, A and G allele homozygous patients performed better on the executive function domain compared to heterozygous patients ($p<.01$ and $p<.01$). The genotype effects observed on the Global Cognitive Summary score and executive function domain remained significant after adding age, age at illness onset, sex, education, and medication status as covariates to the model ($F(2,27)=4.77$, $p<.02$; and $F(2,27)=3.87$, $p<.04$; respectively). Similarly, the post-hoc contrasts confirmed that G homozygous patients demonstrated superior Global Cognitive Summary and executive function domain scores than A/G heterozygous patients ($p<.005$ and $p<.02$, respectively). The contrasts comparing A allele homozygous patients to A/G heterozygous patients did not remain significant; however, this was due to the fact that years of education were unavailable for two patients in the A allele homozygous group leaving only four subjects in this

Table 3
rs138097 genotype and cognitive function in schizophrenia*

Cognitive domain	Total sample	rs138097			Summary statistics		
		A/A	A/G	G/G	*F*	*p*	Contrasts[A]
No. of subjects	49	6	21	22			
Cognitive domain Z-scores							
Global Cognitive Summary score	−1.35 (.96)	−0.74 (.34)	−1.84 (.98)	−1.10 (.86)	$F(2,40)=4.85$	.01	A/A and G/G>A/G
Memory Function	−1.48 (1.18)	−.93 (.78)	−1.97 (1.01)	−1.22 (1.27)	$F(2,40)=2.72$	.08	G/G>A/G
Attention and Verbal Fluency	−1.24 (.88)	−.85 (.78)	−1.54 (1.00)	−1.08 (.74)	$F(2,40)=1.96$	.15	–
Executive Function	−1.26 (1.48)	−.16 (1.10)	−2.07 (1.41)	−.87 (1.31)	$F(2,40)=5.71$	.007	A/A and G/G>A/G
Neuropsychological test raw scores							
ACTT	27.3 (8.6)	34.0 (4.5)	22.3 (7.1)	30.3 (8.3)	$F(2,46)=8.79$	.001	A/A and G/G>A/G
BSRT immediate recall	7.9 (2.3)	9.3 (1.4)	7.1 (2.1)	8.3 (2.4)	$F(2,46)=3.14$	.05	A/A>A/G
BSRT delayed recall	6.7 (3.1)	6.5 (2.8)	6.1 (3.1)	7.3 (3.2)	$F(2,46)=0.71$	.50	–
COWAT	32.7 (13.1)	37.2 (11.5)	28.3 (13.7)	35.6 (12.3)	$F(2,45)=2.11$	.13	–
CIGT	43.4 (14.3)	45.2 (11.5)	38.9 (14.3)	47.0 (14.4)	$F(2,45)=1.78$	.18	–
WAIS-R DSST	6.5 (2.2)	8.5 (2.6)	6.1 (2.1)	6.6 (2.0)	$F(2,46)=3.05$	.06	A/A>A/G
WCST categories completed	3.2 (2.5)	4.8 (2.4)	2.3 (2.3)	3.7 (2.3)	$F(2,46)=3.56$	.04	A/A>A/G
WCST perseverative errors	20.8 (14.0)	11.5 (5.2)	26.4 (14.8)	18.1 (13.0)	$F(2,46)=3.81$	.03	A/A and G/G>A/G
WISC-R mazes subtest	7.2 (3.6)	8.2 (1.3)	6.5 (3.7)	7.7 (4.0)	$F(2,45)=0.76$	.47	–

*Mean (SD).
[A] $p<0.5$.

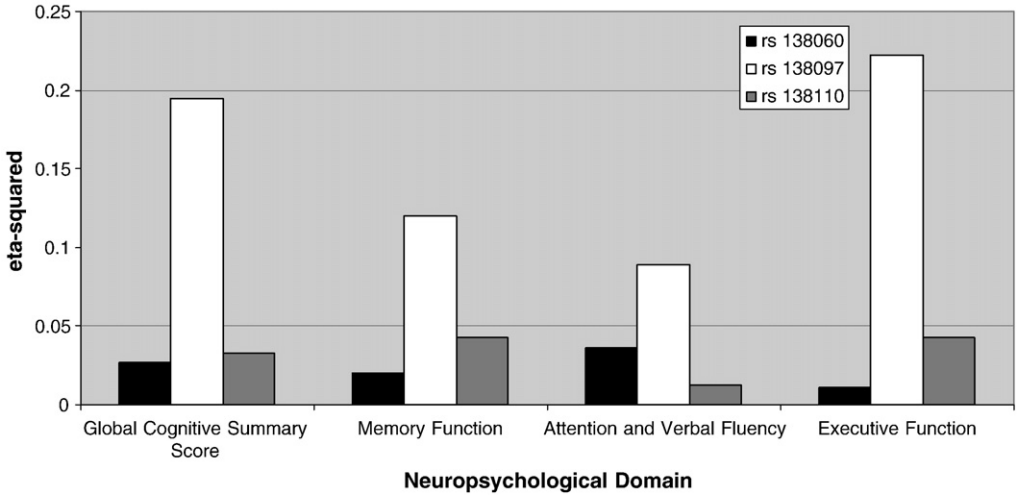

Fig. 1. Proportion of variance (eta-squared) in cognitive function explained by Sult4A1 genotype in patients with schizophrenia.

group for the ANCOVA analysis. The proportion of variance explained by each SNP (i.e. eta-squared) for the Global Cognitive Summary scores and individual domains is presented in Fig. 1. In general, rs138097 genotype accounted for 9–22% of the variance in cognitive function, whereas rs138060 and rs138110 accounted for less than 5% for any given cognitive domain. With respect to specific neuropsychological measures, significant genotype effects were observed on ACTT, WCST categories, and WCST perseverative errors at the $p<.05$ significance level. In each case, post-hoc pairwise contrasts revealed poorer performance in the rs138097 A/G genotype group compared to A and G allele homozygous groups (see Table 3 for the Global Cognitive Summary, domain, and individual test scores along with summary statistics for rs138097). No effect of rs138060 or rs138110 genotype was observed on the Global Cognitive Summary measure.

4. Discussion

The major findings of this study were that: 1) patients heterozygous (TG) for rs138060 had significantly worse clinical symptoms of schizophrenia, including overall symptomatology, positive symptoms, and anxiety and depression; and 2) patients heterozygous (AG) for rs138097 demonstrated significantly worse performance on neuropsychological testing, particularly on tests of executive function and working memory. Thus, the primary outcome measures for psychopathology, BPRS Total, and neurocognitive testing, the Global Cognitive Summary score, were found to be associated with specific *Sult4A1* SNPs. There were no relationships between rs138110 genotypes and psychopathology, and no indication that rs138060 and rs138110 were related to cognition in this sample of patients. The amount of variance explained by rs138097 genotype ranged from 9–22%, placing it among the largest SNP effects identified to date.

These three SNPs were chosen because they capture most of the common allelic variation in this gene (Brennan and Condra, 2005; Condra et al., 2007; M. Brennan, unpublished data). There are no data yet as to whether rs138060 and rs138097 are functional SNPs, but as they are intronic, it is unlikely that they are. Hildebrandt et al. (2007) found no variants in the coding region of this gene, not even synonymous variants; further, the intronic SNP variation in *Sult4A1* was significantly lower than that of most human genes, including other sulfotransferase genes. These authors suggested that decreased formation of protein or variations in how sulfotransferase interact with the cellular environment is the most likely basis for any phenotypic variation.

The finding that one SNP, rs138060, was related mainly to a spectrum of psychopathology and a second SNP, rs138097, was related to cognition, suggests that these intronic SNPS successfully tag different allelic variants of *Sult4A1*. Interestingly, the rs133097 heterozygotes, who have the poorest cognition, are highly enriched for the 213 nt allele of D22s1749E, which is preferentially transmitted to schizophrenic offspring by TDT (Brennan and Condra, 2005; M. Brennan unpublished).

Any number of mechanisms may underlie the observed findings. Variation in expression levels might affect dopamine or norepinephrine disposition, or components of the mechanisms relating to the magnitude and termination of dopamine and norepineprhine receptor stimulation, perhaps relating to the reported cell-type specific expression of the *Sult4A1* mRNA in CNS (Falany et al., 2000; Liyou et al., 2003). Both dopamine and norepinephrine are strongly implicated in psychosis, mood, and anxiety regulation, and cognition in schizophrenia as well as other psychiatric conditions. The *Sult4A1* protein presumably plays a major role in modulating catecholamine levels, either through sulfation or via novel pathways (Allali-Hassani et al., 2007). As noted in the Introduction, dopamine sulfate has been studied in the CSF of patients with schizophrenia and has been related to negative symptoms (Risby et al., 1993) and brain atrophy (van Kammen et al., 1986). However, current evidence suggests that another form of sulfotransferase, rather than *Sult4A1*, is involved in the sulfation reaction (Onasch et al., 2000). Rather, *Sult4A1* has been suggested to be related to intracellular trafficking of DA receptors.

A significant feature of the results reported here is that the heterozygotes for rs138060 (T/G) were found to have greater levels of psychopathology (higher BPRS Total Anxiety/

Depression scores, and SAPS Total scores than either T/T or G/G patients). Similarly, patients who were heterozygotous (A/G) for rs138097 were more impaired on the Global Cognitive Summary score and executive function domain than homozygotes. Specifically, the rs138097 heterozygotes were more impaired on the ACTT, WCST categories, and WCST perseverative errors. Heterozygotes showing worse function than homozygotes for quantitative traits is usually referred to as negative heterosis (Comings and MacMurray, 2000). However, in the absence of evidence that sulfotransferase enhances cognitive function, it is also possible that the results reported here could reflect positive heterosis (Barbato and Kruzelock, 1992). Heterosis is common in humans and has been reported for a variety of genes that have been implicated in schizophrenia, including DRD1, DRD2, DRD3, DRD4, and HTR2A.

The genetic and molecular basis of heterosis is poorly understood. It may be due to differences between heterozygotes and homozygotes in genome organization, e.g. loss of colinearity at various loci, differences in gene expression, including complex transcriptional networks which may vary at different developmental stages, altered dominance, complementation, repetitive DNA, transposons, and epistasis (Lippman and Zamir, 2007; Hochholdinger and Hoecker, 2007). This allelic variation provides a more comprehensive and unique array of alleles in the heterozygote that may lead to positive or negative heterotic phenotypes (Springer and Stupar, 2007). Further study is indicated to determine whether the heterozygotes for SNPs rs138997 or rs138110, or both, are associated with other allelic variations and distinctive levels of expression of *Sult4A1* product.

The main limitations of this study are the relatively small sample size and that the findings with regard to psychopathology and cognition were exploratory, post-hoc in nature and not predicted on the basis of *Sult4A1* physiology or previous findings, aside from the prior evidence that allelic variation in the gene is associated with schizophrenia susceptibility. Consequently, the results reported herein should be considered preliminary until replicated in larger sample sizes. We note that the power of the current study to detect a small effect size, as is likely to be the case given that the variables examined here are probably influenced by many genes, is approximately 8–12%. This is particularly relevant as the small sample size and relatively large number of uncorrected statistical tests performed exposes the results to greater Type I error rate. However, concern that the results relate entirely to Type I errors is mitigated to a certain extent by the fact that multiple measures of psychopathology and cognition demonstrated consistent relationships with the same SNPs. For example, both the BPRS and SAPS scales were associated with rs138060 and the pattern of findings across the measures was identical.

In conclusion, these results extend the previous findings that SNPs of *Sult4A1* are linked to schizophrenia by providing the first preliminary evidence that two of these SNPs are related to clinical symptoms and cognitive function in schizophrenia. It is extremely unlikely that these SNPs are themselves the basis for function change in sulfotransferase activity; however, they apparently tag allelic variation in the gene that is related to psychopathology.

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Contributors

Authors HYM and MDB conceptualized the purpose of the study, performed the literature search, and wrote the first and revised draft of the manuscript. Author NDW performed the statistical analysis and contributed to the writing of the first and revised drafts of the manuscript. Author KJ was responsible for database management and provided statistical expertise. Author HYM designed and implemented the original studies from which the current sample was acquired from.

Conflict of interest

Although no pharmaceutical products are discussed in this paper, author HYM has served as a consultant, board member, and/or speaker for Janssen, Novartis, and Pfizer, and has received grant/research support from AstraZeneca, Eli Lilly and Co., Janssen, Novartis, and Pfizer. For reasons of full disclosure, it is hereby noted that M.D.B. is a Major Shareholder in SureGene, LLC. Authors NDW and KJ have no conflicts of interest to report.

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