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Transmission Disequilibrium Suggests a Role for the Sulfotransferase-4A1 Gene in Schizophrenia

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Previous studies suggest a role for chromosome 22q13 in schizophrenia. This segment of chromosome 22 contains the sulfotransferase-4A1 (*Sult4A1*) gene, which encodes an enzyme thought to be involved in neurotransmitter metabolism in the central nervous system. To evaluate this candidate, we developed a microsatellite marker targeting a polymorphism in its 5′ nontranslated region (D22s1749E). Using samples obtained from the National Institutes of Mental Health Schizophrenia Genetics Initiative, we evaluated 27 families having multiple siblings with schizophrenia and schizophrenia-spectrum disorders for transmission disequilibrium (TDT) of this marker along with three single nucleotide polymorphisms (SNPs) spanning a 37 kb segment containing the *Sult4A1* gene. TDT for D22s1749E was significant ($P < 0.05$), with a tendency for the 213 nt allele to be preferentially transferred to affected children ($P = 0.0079$). Global chi-square values for haplotypes involving the SNPs (ss146366, ss146407, and ss146420) and D22s1749E, also showed significant TDT values ($P = 0.0006–0.0016$). Consequently, we proposed that *Sult4A* merited more careful scrutiny as a candidate gene for schizophrenia susceptibility.

KEY WORDS: candidate gene; catecholamine metabolism; chromosome 22q13; dopamine; neuropharmacogenomics

INTRODUCTION

Though numerous linkage and association studies have implicated chromosome 22q in the etiology of schizophrenia, the role of a particular gene or genes has yet to be established conclusively [Vallada et al., 1995; DeLisi et al., 2002; Jorgensen et al., 2002; Lewis et al., 2003; Takahashi et al., 2003]. Perhaps because distinct genes on this chromosome are involved in different lineages, analyses of candidate genes on 22q have resulted in conflicting results. For example, 22q11.2 contains the catechol-O-methyltransferase (*COMT*) gene, an attractive candidate whose significance is still under investigation, and the *proline dehydrogenase* gene, a gene whose role, if any, may be limited to Chinese lineages [Liu et al., 2002; Shifman et al., 2002; Williams et al., 2003; Li et al., 2004]. Likewise, for more distally located genes and polymorphisms, family-based transmission studies have given somewhat modest or even contradictory results [Vallada et al., 1995; Stober et al., 2000; Meyer et al., 2001; Georgieva et al., 2003; Takahashi et al., 2003; Kaganovich et al., 2004].

The sulfotransferase-4A1 gene (*Sult4A1*) is located in 22q13 and encodes a brain-specific sulfotransferase believed to be involved in metabolism of neurotransmitters [Falany et al., 2000; Sakakibara et al., 2002; Liyou et al., 2003]. Here we described a new microsatellite polymorphism in the 5′ end of this gene and evaluated this gene as a candidate for susceptibility to schizophrenia by family-based transmission disequilibrium (TDT) analysis of 27 families from the NIMH Schizophrenia Genetics Initiative.

MATERIALS AND METHODS

Samples

Twenty-seven nuclear and extended families, comprising 212 individuals, each having multiple affected siblings, were provided by the National Institutes of Mental Health (NIMH) Schizophrenia Genetics Initiative. Self-description of heritage was as follows: African-American, 12 families; European/Mediterranean, 11 families; Hispanic, 2 families; other, 2 families. DSM-IIIR criteria were compiled for all subjects by researchers at Columbia University, Harvard University, and Washington University. Detailed information on ascertainment, diagnosis, and informed consent for these families has been described previously [Cloninger et al., 1998]. Using the DSM-IIIR criteria for schizophrenia, the sample contained 51 affected sibling pairs, and using a broader disease definition that included schizotypal personality disorder and schizoaffective disorder, the sample contained 91 affected sibling pairs.

PCR Amplification and Genotyping

The upstream primer sequence for the microsatellite marker, D22s1749E was 5′-CAGCCGCACGCCATGGAACTCGAAG-3′ and the downstream primer sequence was 5′-GGCGCCATGACGTCACGCCTGC-3′. The upstream primer was labeled with fluorescent dye D2 at its 5′ end (Proligo Primer and Probes, Boulder, CO). Each 10 μl reaction contained 5 ng of genomic DNA, 1× PCR reaction buffer (Roche Molecular Diagnostics, Alameda, CA), 0.16 U of AmpliTaq Gold (Roche), 2.0 mM $MgCl_2$, 1 mM of each dNTP, 0.33 μM of forward and reverse primers, and 10% dimethyl sulfoxide (molecular biology grade, Fisher Scientific, Hampton, NH). PCR cycling conditions consisted of an initial enzyme activation at 95°C for 5 min, followed by 35 cycles of 93°C for 2 min, 92°C for 1 min, 71°C for 30 sec, and 72°C for 2 min, and a final incubation at 72°C for 5 min. PCR products were analyzed and fragment size was determined using the Beckman CEQ 8000 Analysis System.

Marker D22s683 was genotyped following standard procedures, using PCR reagents obtained from Applied Biosystems

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(ABI, Foster City, CA). Fragments were analyzed using an ABI PRISM 377 DNA sequencer, with GeneScan and Genotyper software packages.

Single nucleotide polymorphisms (SNPs) were genotyped by ABI Assays-on-Demand™ SNP kits using the conditions suggested by the supplier (5 μl reactions in 384 well-plates, containing 4.5 ng genomic DNA). PCR products were analyzed using the ABI Prism 7900HT Sequence Detection System. In cases where a reaction failed (<3% of total) or the results were not consistent with Mendelian inheritance (<0.5% of total), a second reaction was carried out to resolve discrepancies.

Genetic Analysis

Initial mapping of D22s1749E was performed using the MultiMap program (version 2.40), as described previously [Cox-Matise et al., 1996; Brennan et al., 2000]. TDT analysis was performed using TRANSMIT (version 2.5.2) [Clayton, 1999], with rare haplotypes aggregated so as to prevent elevation of χ^2 values that can arise due to expectations for rare haplotypes. The resulting global *P*-values for the χ^2 analyses estimate the significance of the transmission distribution for all haplotypes combined, with rare haplotypes being treated as a single haplotype. Similarly, χ^2 values for transmission of individual alleles and haplotypes, with one degree of freedom, were determined by TRANSMIT.

RESULTS

Using public database resources, we searched for candidate genes in 22q13. The *Sult4A1* gene is located in this region and encodes a brain-specific sulfotransferase believed to be involved in neurotransmitter catabolism in the central nervous system [Falany et al., 2000; Sakakibara et al., 2002; Liyou et al., 2003].

Alignment of the genomic sequences with several corresponding cDNA sequences indicated that the chromosomal segment encoding the 5′ nontranslated leader region of the *Sult4A1* mRNA might be polymorphic, with different alleles having varying numbers of imperfect GCC repeats (primary accession numbers Z97055, AF176342, AF188698, AF251263, AK091700, Al832543). To evaluate this directly, we developed a PCR procedure to amplify the genomic region corresponding to the 5′ nontranslated leader region of the encoded mRNA (Materials and Methods). Although the region is very G-C rich and refractory to amplification, we were able to get reproducible amplification for all families and confirm Mendelian inheritance in all cases. Using the MultiMap program, we verified that D22s1749E mapped approximately 10 cM distal to D22s683 (data not shown).

In this sample of families, we observed seven alleles of D22s1749E ranging in size from 198 to 216 nt (Table I). There was no statistically significant difference in allelic frequencies for families of different self-described heritage (data not shown).

Table I also summarizes the allele-specific TDT results for D22s1749E. Allele transmission frequencies did not differ significantly from expectations, with the exception of that for the 213 nt allele. The latter was transmitted more often than expected to affected individuals ($\chi^2 = 7.05$, 1df; $P = 0.0079$). After Bonferroni correction (five allele classifications) the result remained significant ($P < 0.04$).

We further evaluated the *Sult4A1* candidate gene by TDT analysis employing our new microsatellite marker along with three SNPs in the gene. Table II summarizes TDT analysis for these polymorphisms and haplotypes involving them. Significant global χ^2 values were seen for D22s1749E and various haplotypes involving D22s1749E and the three SNPs. A disease definition that included schizotypal personality disorder resulted in more significant results for several haplotypes. In contrast, a broader disease definition that included both schizotypal personality disorder and schizoaffective disorder resulted in lower global χ^2 values in most cases.

TABLE I. TDT Analysis for Alleles of D22s1749E for DSM-III Schizophrenia

Size (nt)	Frequency[a]	*P* (1df)[b]
198	0.0022	ND
202	0.0088	ND
207	0.385	0.83
209	0.033	0.15
212	0.286	0.39
213	0.165	0.0079[c]
216	0.022	ND

[a]Frequency for entire sample using only those parental genotypes that were observed directly or that could be unambiguously inferred.
[b]*P*-value, one degree of freedom, with rare alleles pooled (ND, not determined).
[c]Significant after Bonferroni correction (5 alleles or allele classes evaluated) $P < 0.04$.

Several studies have provided evidence that chromosome 22 loci lying proximal to *Sult4A1* may be involved in schizophrenia [reviewed in Mowry et al., 2004]. Accordingly, we wanted to address the possibility that a more proximal locus might account for the TDT results we observed for the *Sult4A1* region in these particular families. Microsatellite marker, D22s683, seemed a good choice to test this because others have seen evidence for transmission disequilibrium for this marker in a larger set of families, comprising in part, the samples we used here [Takahashi et al., 2003]. Despite the fact that D22s683 lies approximately 8 mb (about 10 cM) closer to candidate genes in 22q11 than does *Sult4A1*, we did not observed significant transmission disequilibrium for D22s683, regardless of the disease-definition, in this subset of NIMH families ($P > 0.19$ in all cases). Thus, it seems unlikely that a more proximal locus accounts for the TDT results we observed.

DISCUSSION

We provided evidence that *Sult4A1*, or perhaps some unknown polymorphism(s) in linkage disequilibrium with D22s1749E, may contribute to genetic predisposition to schizophrenia. As such, these findings add to a body of results pointing to a role for chromosome 22q in genetic predisposition to schizophrenia [Vallada et al., 1995; DeLisi et al., 2002; Jorgensen et al., 2002; Lewis et al., 2003; Takahashi et al., 2003; Mowry et al., 2004].

Given this relatively small sample of families, the suggestive evidence for a role of *Sult4A1* is intriguing. Interestingly, the 213 nt allele of D22s1749E is transmitted more often than expected to affected children. The 216 nt allele occurred too rarely in this small sample for the TDT analysis to be statistically valid, but tentatively, it too appears to be preferentially transmitted to affected offspring. These alleles are predicted to encode mRNAs with a longer 5′ nontranslated leader sequences than the shorter alleles. Conceivably, the longer nontranslated leader sequences might lower translatability of the mRNAs and result in lower final levels of the Sult4A1 enzyme. However, there are other possibilities, including a possible role for one or both of the SNPs in *Sult4A1* that result in nonsynonymous codon changes (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=25830). The latter are flanked by the two intronic SNPs (ss146366 and ss146407) used in the present study.

Two independent lines of evidence are consistent with the hypothesis that *Sult4A1* could play a role in schizophrenia.

TABLE II. TDT Analysis of *Sult4A1* Markers

Marker(s)	df	*P*-value for disease definition[a]		
		SZ	SZ + SPD	SZ + SPD + SD
D22s1749E	4[b]	0.047	0.054	0.046
ss146366-ss146407	3[b]	0.042	0.126	0.129
ss146366- D22Ss1749E	7[b]	0.008	0.004	0.017
ss146366-ss146407- D22s1749E	9[c]	0.0016[d]	0.0006[d]	0.005
ss146366- D22s1749E-ss146420	10[c]	0.010	0.0016[d]	0.018
ss146407- D22s1749E-ss146420	7[c]	0.042	0.034	0.050
ss146366-ss146407- D22s1749E-ss146420	11[c]	0.014	0.0064	0.040

[a]SZ, schizophrenia; SPD, schizotypal personality disorder; SD, schizoaffective disorder. Probabilities are given for Global χ^2 values, as determined by TRANSMIT.
[b]Haplotypes having frequencies of 3% or less aggregated to provide expectations of ≥3 for all transmission categories.
[c]Haplotypes having frequencies of 14% or less aggregated to provide expectations of ≥3 for all transmission categories.
[d]$P < 0.05$ after Bonferroni correction (15 marker combinations tested).

First, while the major physiological substrates of the Sult4A1 isozyme are yet to be identified, compounds present in brain extracts serve as substrates as do a variety of phenolic compounds structurally resembling dopamine [Sakakibara et al., 2002]. Furthermore, the enzyme is expressed, at various levels, in many regions of the brain including different segments of the cerebral cortex [Liyou et al., 2003]. If the longer alleles of D22s1749E result in lower protein levels and subsequent regional elevation of dopamine in the CNS, this would be consistent with the "dopamine hypothesis" of schizophrenia [Meltzer and Stahl, 1976; Seeman, 1987]. Clearly, more study will be necessary to determine if dopamine catabolism is affected by allelic variation in *Sult4A1*.

Of course, as is always the case with a novel candidate gene, larger sample sizes will be needed to critically address whether or not specific alleles of the *Sult4A1* locus confer susceptibility to schizophrenia. Using the narrow disease definition, our sample contained 70 affected individuals with informative transmissions of D22s1749E alleles. Assuming the observed frequency of the 213 nt allele is representative of the whole population, we estimated that this sample had a power of approximately 60% to detect TDT at an alpha level of 0.05 for a marker locus in complete linkage disequilibrium with the disease risk allele. For rarer alleles and haplotypes, correspondingly larger sample sizes will be necessary to detect significant TDT with 80% power at an alpha level of 0.05. Clearly then, specific haplotypes of the *Sult4A1* region will have to be analyzed in a larger number of families to provide the needed statistical power.

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