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Mutation analysis of the C1QTNF3 gene in patients with schizophrenia

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Abstract

We previously identified a small region on chromosome 5p13 related to schizophrenia in a Puerto Rican pedigree. We screened one of the positional candidate genes, C1QTNF3, for mutations in affected family members. The direct sequencing identified 10 sequence variants, including five shared by all affected family members. Genotyping of the shared variants in a Puerto Rican sample of 118 cases and 136 controls did not reveal either allelic or genotype association with schizophrenia.

Keywords

5p13; schizophrenia; C1QTNF3

1. Introduction

The C1q and tumor necrosis factor related protein gene (C1QTNF3) is one of the positional candidate genes for schizophrenia in the disease susceptibility locus on chromosome 5p13. The locus was identified by linkage analysis followed by fine mapping and haplotype analysis in the large Puerto Rican pedigree (Family 17) of Spanish descent (Silverman et al., 1996, Beshpalova et al., 2005).

The mechanisms of C1QTNF3 gene expression and regulation, as well as a specific function of the encoded protein, are mainly unknown. C1QTNF3 encodes the protein that shares 33% sequence identity with one of the members of the family of adipocytokines, adiponectin (Schäffler et al., 2007), and the extent of this homology suggests possible shared functional features as well (Brenner et al., 1998). Adiponectin (ADIPOQ) is a tissue-specific plasma protein with anti-inflammatory properties that is abundantly synthesized and secreted by differentiating adipose tissue, and plays a critical role in whole-body metabolism by increasing oxidation of fatty acids in muscle and increasing insulin sensitivity in both muscle and liver (Dyck, 2009; Schäffler et al., 2009). Animal models demonstrated a suppression of the *adipoq* gene expression in parallel with reduced insulin sensitivity during the development of obesity and diabetes mellitus (Hotta et al., 2001).

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Metabolic disorders, including insulin resistance, lipid abnormalities, and obesity, appear to be more common in patients with schizophrenia than among the general population, and taking antipsychotic medication may further increase the chance of developing the disorders (Lindenmayer et al., 2001; Henderson, 2005). Inflammation has also been connected to schizophrenia, as well as to metabolic disturbances in patients with schizophrenia (Fan et al., 2007). The fact that the paralog of C1QTNF3 has profound effects on both central metabolic functions and inflammation, and the location of C1QTNF3 at the locus involved in schizophrenia, suggest that C1QTNF3 could be not only a positional, but also a functional candidate gene for schizophrenia.

2. Methods

2.1. Subjects

All members of Family 17 were examined using the Schedule for Affective Disorders and Schizophrenia (Endicott and Spitzer, 1978). Family members, meeting DSM-III-R criteria for schizophrenia, schizoaffective disorder, or schizotypal personality disorder (SPD) or an a priori defined slight modification of DSM-III-R SPD were considered affected. The Institutional Internal Review Board of The Mount Sinai Medical Center and The San Juan VAMC approved the study, and all participants provided a written informed consent.

For the case-control study, 118 schizophrenia-affected and 136 healthy non-related Puerto Rican individuals of Spanish origin were collected. The two groups had the same male:female ratio. Unfortunately, metabolic information was not available in this sample. Putatively affected individuals were assessed with the Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994), and the final diagnosis was made based on DSM-IV criteria for schizophrenia. The controls were assessed with screening questions from the DIGS and FIGS, and were excluded if either they themselves or any first-degree relative screened positive for any symptoms of psychosis, major affective disorder, or suicidal ideation.

2.2. Sequence analysis

Genomic DNA was extracted from venous blood samples using the Gentra Systems PureGene DNA Extraction KIT (Puregene, Minneapolis, MN). The reference sequence of C1QTNF3 (NM_030945) was obtained from the University of California in Santa Cruz Genome Bioinformatics Site, <http://genome.ucsc.edu/>. Primers for polymerase chain reaction (PCR) were designed with the Primer-3 prediction program at http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi, synthesized by Applied Biosystems (Foster City, CA), and used for both amplification and sequencing (available upon request).

DNA amplification was performed using the programmable GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA), as described earlier (Bespalova et al., 2005). PCR products were sequenced using the ABI 3730XL DNA sequencer (Applied Biosystems). The data were analyzed using Basic Local Alignment and Search Tool (BLAST) at <http://www.ncbi.nlm.nih.gov>, and Clustal X multiple alignment software at <http://www.uib.no/mbi/seqlab/seq-analysis.html>.

2.3. Genotyping and statistical analysis

The genotyping was performed using the Applied Biosystems 7300 Real-Time PCR System and TaqMan 5'-exonuclease assays (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol.

Detected sequence variants were tested for Hardy-Weinberg equilibrium (HWE), and the genotype and haplotype association were investigated using Chi-Square Test (Preacher, 2001) and Haploview (Barrett et al., 2005), <http://www.broadinstitute.org/haploview/haploview>.

3. Results

We performed mutational analysis of coding regions, exon-flanking intronic regions, 3'- and 5'-non-translated areas, and about 2000 base pairs immediately 5' from the start codon of C1QTNF3, by direct sequencing of the longest transcript (NM_181435) of C1QTNF3.

DNA samples of two schizophrenia-affected siblings, 17-203 and 17-210, were sequenced. These siblings shared the maternal "at-risk" haplotype over the whole minimal critical interval (MCI) at 5p13 (Bespalova et al., 2005). Therefore, sequence variants identified in the maternal 'at-risk' allele were considered possibly disease related.

A total of 10 previously known sequence variants have been identified, including the heterozygous trinucleotide repeat polymorphism -/ACA in intron 1 (rs57826552), and nine single nucleotide polymorphisms (SNPs) in non-coding regions of C1QTNF3. Siblings 17-203 and 17-210 shared five of the nine SNPs, three in the promoter (rs299616, rs890952, rs89095) and two in the 3'-untranslated region (rs840386, and rs840385). The five SNPs were genotyped in the whole family, their sharing by only affected family members was confirmed, and then they were genotyped in 118 schizophrenia-affected individuals and 136 controls. To assess genotyping reproducibility, every 20th individual was duplicated. The mean discordance per sequence variant between pairs of samples was less than 0.0001%. All five variants did not depart from HWE ($p_{\min}=0.25$), and their genotype and allele frequencies did not differ significantly in cases and controls (Table 1). Haplotype analysis did not identify a haplotype associated with schizophrenia (data not presented).

4. Discussion

Several studies detected either positive LOD scores or structural changes at 5p13, suggesting that this region harbors schizophrenia-susceptibility genes (Crowe and Vieland, 1999; Ekelund et al., 2000; Garver et al., 2001; Paunio et al., 2001; Cooper-Casey et al., 2005; Suarez et al., 2006; Holliday et al., 2008; Walsh et al., 2008). None of the genes at 5p13 have been previously implicated in schizophrenia or other neurobehavioral disorders. The aim of the study was to screen C1QTNF3 gene for mutations in schizophrenia-affected members of Puerto Rican family, and to assess a frequency of detected mutations in ethnically matched case-control sample.

The direct sequencing of C1QTNF3 gene identified five sequence variants, which could potentially be involved in schizophrenia, but their genotyping in the case-control sample of the same ethnic origin did not confirm the predicted association. However, we cannot completely rule out a true association since the variants could have a small effect and be in linkage disequilibrium with strong schizophrenia susceptibility alleles. We also could have missed large rearrangements as well as large heterozygous deletions and insertions in the gene, since these structural variants cannot be detected by comparative sequence analysis. Subsequent follow-up studies to explore complex variations, and using a larger case-control sample would be desirable.

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Table 1

Genotype and allele frequencies of five SNPs in 118 cases and 136 controls

SNP	Group	Genotype	χ^2	p_2	Allele	χ^2	p_1
rs840386		AA	AG	GG	A	G	
	Case	26	56	36	1.97	0.37	1.07 0.30
	Control	32	73	31		137 135	
rs840385		AA	AG	GG	A	G	
	Case	21	58	39	0.70	0.71	100 136 0.37 0.54
	Control	19	70	47		108 164	
rs890950		TT	TC	CC	T	C	
	Case	13	43	62	2.48	0.29	69 167 2.11 0.15
	Control	18	60	58		96 176	
rs890952		TT	TC	CC	T	C	
	Case	48	49	21	1.08	0.58	145 91 0.59 0.44
	Control	47	64	25		158 114	
rs299616		CC	CG	GG	C	G	
	Case	92	23	03	2.50	0.29	207 29 2.47 0.12
	Control	115	20	01		250 22	

 p_1 and p_2 : p -value calculated at degree of freedom (df) 1 and 2, respectively.