Absence of Linkage for Schizophrenia on the Short Arm of Chromosome 5 in Multiplex Canadian Families

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Abstract

A VNTR for the human dopamine transporter gene (DAT-1) has been localized to chromosome 5p15.3. Silverman et al. [1996] found evidence for genetic linkage of the D5S111 locus, located just centromeric to DAT-1, to schizophrenia and related disorders in a large Hispanic family. We evaluated five markers on 5p, including D5S111 and the DAT-1 VNTR, in five multiplex schizophrenic families, assuming autosomal dominant transmission (subjects assessed $n = 122$, DNAs available $n = 96$, individuals with schizophrenia and schizoaffective disorder $n = 36$, broader spectrum disorders $n = 14$). LOD scores were negative across all families for all markers tested, and overall LOD scores were strongly negative (< -2.0 , $\theta = 0$) across all five families for each of the markers typed. Thus, there is no evidence to support the linkage of markers in this region of chromosome 5 to schizophrenia in this sample of families.

Keywords

schizophrenia; schizotypal personality disorder; linkage; dopamine transporter; chromosome 5p

INTRODUCTION

The cause of schizophrenia is currently unknown; however, family, twin, and adoption studies carried out over the past half century consistently indicate that genetic factors are involved in the etiology of this disease [Gottesman and Shields, 1982]. There is a well-documented role for dopamine in schizophrenia [Losonczy et al., 1987]. One model that could contribute to functional dopamine abnormalities is the dopamine transporter, which is responsible for termination of the activity of synaptic dopamine through reuptake [Hitri et al., 1994]. A genetic association study indicated a potential role for the dopamine transporter gene (DAT-1) in the etiology of psychosis in cocaine abusers [Gelernter et al., 1994]. Silverman et al. [1996] have recently reported evidence for genetic linkage of a locus near DAT-1 (D5S111, 5p14.1-13.1) to schizophrenia in one large pedigree. A maximum LOD score of 3.72 ($\theta = 0.01$) was found assuming autosomal dominant inheritance. Based on the

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above rationale, we were prompted to examine markers on the short arm of chromosome 5, including a VNTR for the dopamine transporter gene.

MATERIALS AND METHODS

Blood samples from five eastern Canadian families were collected by one of us (A.S.B.). Diagnosis was established (including schizophrenia, schizoaffective disorder, psychosis NOS, schizotypal personality disorder, or paranoid personality disorder) by using the Structured Clinical Interview for DSM-III-R (SCID-I, SCID-II) and available medical records [Bassett et al., 1993; Bassett and Honer, 1994]. Following from Silverman et al. [1996], we examined the broad spectrum of schizophrenia as the principal phenotype. Genomic DNA was extracted by using the high-salt method. We genotyped four highly informative microsatellite markers (D5S117, D5S108, D5S111, D5S411) and the dopamine transporter gene (DAT-1) VNTR, all of which are distributed across the short arm of chromosome 5. The DAT-1 VNTR was typed according to the method of Vandenberg et al. [1992]. For the microsatellite markers, the left primer was end labelled with γ -³²P ATP and T4 polynucleotide kinase. Polymerase chain reaction (PCR) products were separated by electrophoresis on 6% polyacrylamide gels. Autoradiography was performed overnight, and genotypings were analyzed by laboratory staff members who were blind to diagnoses. Allele frequencies were derived from unrelated individuals among the families. LOD scores for pairwise analyses were generated by using the MLINK program of the LINKAGE package, version 5.2. LOD scores were evaluated under the assumption of an autosomal dominant mode of inheritance (gene frequency = 0.85%, phenocopies = 0.001, penetrance = 70%).

RESULTS

The results of our LOD score analyses are shown in Figure 1. We were unable to replicate the positive LOD score of Silverman's group or to generate any suggestive positive LOD scores in any of the five families analyzed for D5S111 or any of the other flanking markers. Overall LOD scores were strongly negative across all five families for each of the microsatellite markers genotyped, resulting in an exclusion region (LOD score < -2.0) extending at least 10 centimorgans on either side of each marker. For the candidate gene DAT-1, linkage was excluded out to a 2-cM region flanking the marker. A recessive model was tested and also yielded strongly negative results for all markers.

DISCUSSION

We obtained negative results for each of the five markers that we tested on chromosome 5p. The discrepancy between our findings and those of Silverman et al. [1996] may be due to a chance result in their study, because they were not able to replicate this positive LOD score in any of their other 22 families, or our result could be a false negative. Another possible explanation is the problem of locus heterogeneity, which is probably present in most complex genetic disorders. It appears highly likely that there is more than one susceptibility locus for schizophrenia in the human genome. The family studied by Silverman et al., is unique, in that it has its origins in a relatively isolated rural area of Puerto Rico; thus, the genetic variant causing schizophrenia within this population would be expected to

demonstrate a higher degree of genetic homogeneity [Vazquez Calzada, 1981]. The limitations of our study are as follows: Only five families were analyzed, and we did not study an Hispanic racial subgroup. Therefore, our study cannot be considered to be a closely related replication attempt. Alternatively, the original findings may have been dependent upon the use of different diagnostic methods. Given the numerous arenas in which genetic studies can differ and given continued interest in DAT-1 as a candidate gene, other investigations of 5p, particularly in homogeneous populations, may be of interest.

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Fig. 1.

Pairwise analysis between schizophrenia and chromosome 5 markers: Map distances were obtained from the database at the website of the University of Southampton Genome Center (www.cedar.genetics.soton.ac.uk).