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Brief Research Communication

Linkage Disequilibrium for Schizophrenia at the Chromosome 15q13-14 Locus of the α7-Nicotinic Acetylcholine Receptor Subunit Gene (CHRNA7)

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The transmission/disequilibrium test was used for fine mapping of the linkage of schizophrenia to the chromosome 15q13-14 region, the site of a candidate gene, the α7 nicotinic acetylcholine receptor subunit gene (CHRNA7), in parent–child triads from the NIMH Schizophrenia Genetics Initiative families. This candidate gene was identified from neurobiological studies of deficits in schizophrenics of the inhibitory gating of the P50 auditory evoked potential. The neurobiological deficit was also used as a phenotype for subsequent linkage analysis. In the present study, significant genotype-wise disequilibrium ($P < 0.007$) was found at D15S165, a polymorphic simple sequence marker physically located within 1 megabase of both CHRNA7 and a partially duplicated, expressed sequence that includes exons 5–10 of CHRNA7. Replication of this result was found in an additional set of families. The results support this region as a chromosomal location involved in the genetic transmission of schizophrenia. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 105: 20–22, 2001. © 2001 Wiley-Liss, Inc.

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We reported an initial linkage for a neurophysiological phenotype associated with schizophrenia to genetic markers at chromosome 15q13-14 in nine multiply affected, European American families. The lod score for the neurophysiological phenotype at D15S1360 was $Z = 5.3$, $\theta = 0.0$. $P < 0.001$; the lod score for schizophrenia was lower, $Z = 1.3$, $\theta = 0.07$, $P < 0.007$, in an affecteds-only model [Freedman et al., 1997]. The lod score for schizophrenia was less than the lod score for the neurophysiological phenotype, principally because the neurophysiological phenotype diminished inhibition of the P50 auditory evoked response to repeated stimuli, has a highly penetrant dominant inheritance, whereas schizophrenia is a less penetrant and more heterogeneous phenotype [Freedman et al., 1999]. Although biological phenotypes have been successfully used as surrogates for the genetic analyses of other illnesses, including colon cancer (polyp formation), sickle-cell anemia (laboratory demonstration of red cell sickling), and hemochromatosis (serum iron levels), this approach has not been widely employed for psychiatric illnesses. The test of the value of this approach is whether it leads to replicable linkage findings and, ultimately, to the discovery of pathogenic molecular changes. Because P50 inhibition is not generally measured in genetic studies, replication of the finding in other samples has been performed using schizophrenia as the phenotype. A similar low positive score was reported in the NIMH Genetics Initiative Schizophrenia families by us at D15S1360, $Z = 1.5$, $\theta = 0.0$. $P < 0.002$ [Leonard et al., 1998]. The NIMH Genetics Initiative investigators independently published a replication, with a multipoint linkage score derived from markers in the region; the Z score was higher for African American than for European American families in the sample [Kaufmann et al., 1998]. A subsequent study of Bantu families with D15S1360 revealed a positive nonparametric linkage score and significant transmission disequilibrium [Riley et al., 2000], and two other groups

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reported negative findings [Neves-Pereira et al., 1998; Curtis et al., 1999]. Interestingly, one of the groups reporting negative findings has positive findings of transmission disequilibrium with D15S1360 in their bipolar families [Neves-Pereira et al., 1999]. The flanking marker ACTC has recently been reported to show a lod score of 3.46 in a selected population of lithium-responsive bipolar families [Turecki et al., 2000].

D15S1360 is a simple sequence repeat that is within 120 kb of the coding region of the α7 nicotinic acetylcholine receptor subunit gene (CHRNA 7) [Gault et al., 1998]. This marker was chosen because preliminary analysis had shown evidence for linkage of the P50 inhibitory abnormality at chromosome 15q14, which was subsequently shown to be the locus of CHRNA7 [Coon et al., 1993; Chini et al., 1994]. CHRNA7 is a viable candidate gene because of neurobiological evidence indicating the involvement in P50 inhibition of the nicotinic receptor that is coded for by this gene [Freedman et al., 1994]. CHRNA7 itself has a complicated genomic structure. The gene has 10 exons that are conserved across chick and other mammalian species. However, the human gene is part of a duplicated cassette of expressed sequences; the duplicons are located nearly adjacent to each other within approximately 1 mb of DNA at chromosome 15q14. The duplicon that contains CHRNA7, and is adjacent to D15S1360, is telomeric of a duplicon that contains an expressed sequence which has four novel exons followed by six exons which duplicate exons 5–10 of the full-length CHRNA7. The sequence homology between these last six exons and CHRNA7 is over 99% [Gault et al., 1998]. The functional significance of this duplication is unknown. Mutation screening of the coding region of the full-length CHRNA7 reveals no polymorphism that is uniquely found in a high proportion of people with schizophrenia [Gault et al., 1999]. Therefore, additional mapping and sequencing outside the coding region of CHRNA7 is required to find the molecular alterations that give rise to the linkage signal and to the dysfunction of the α7 nicotinic receptor in schizophrenia.

The fine mapping of complex traits to genetic loci is frequently necessary in the discovery of the molecular DNA polymorphisms that are responsible for pathogenic alterations in gene function. For rare genetic illnesses, single recombinations are often informative because the rareness of the illness makes it unlikely that an allelic or genetic variant or even a phenocopy is responsible for the apparent recombination. However, in a common, complex trait like schizophrenia, these alternative explanations are as likely, if not more likely, than a recombination, particularly in families ascertained by an affected sibpair. Therefore, linkage disequilibrium was selected as a strategy for fine mapping. All probability inferences are from χ^2 tests, unless otherwise noted.

The extended transmission disequilibrium test (ETDT) [Sham and Curtis, 1995] was used for fine mapping of the region indicated to be involved in linkage. D15S1360 was localized between two highly polymorphic markers, D15S165 and D15S144, with an odds ratio greater than 10^3, using CEPH pedigrees. The physical map confirms this location [Gault et al., 1998]. Heterozygosity of the markers was also estimated from CEPH families (D15S165 0.86; D15S1360 0.57; D15S144 0.69). The genetic distance between D15S165 and D15S144 is 6.4 cm; the physical distance is approximately 2.7 mb, contained within overlapping YAC's 940c5 and 810f11. The families of the NIMH Genetics Initiative, whose ascertainment has previously been described, were studied [Kaufmann et al., 1998]. Sixty-two parent–child triads in 50 families were examined. Genotyping with these markers has been previously described [Leonard et al., 1998].

Table I demonstrates significant genotype-wise transmission disequilibrium for D15S165 (genotype-wise $\chi^2 = 44.5$, 24 df, $P < 0.007$). The empirical P-value from 1,000 Monte Carlo simulations was $P < 0.0310 \pm 0.0055$ (SE). Because there is preexisting information about linkage in this region, the analysis was repeated using only the oldest affected individual in each sibling pair. The allele-wise significance was $P < 0.044$ and the genotype-wise significance was $P < 0.040$, demonstrating a positive test of association with D15S165. Replication of the D15S165 transmission disequilibrium was assessed in an independent sample of 47 triads from 47 families, 45 of which were European American [Cao et al., 1997]; a genotype-wise significance of $P < 0.019$ was found.

Stratification of the NIMH sample into African Americans and European Americans revealed similar genotype-wise disequilibrium in both groups at this marker. Transmission disequilibrium of D15S144, which was not significant in the entire sample, was significantly responsible for transmission disequilibrium in the European American sample. Transmission disequilibrium of D15S1360 was not significant in either the entire sample or in the two subsets. Examination of the transmission of specific alleles of each marker showed that a rare allele of D15S165 (206 bp, CEPH frequency 0.01) was transmitted to 14 of 15 schizophrenics in three African American and four European American pedigrees ($P < 0.000006$). In addition, the 190 bp allele of D15S165 (CEPH frequency 0.03) was significantly transmitted in African Americans

TABLE I. Linkage Disequilibrium Mapping of Schizophrenia at Chromosome 15q14

	Significance (P) of Transmission Disequilibrium:	
Marker	Allele-wise	Genotype-wise
Total sample		
D15S165	0.071	0.0069*
D15S1360	0.18	0.26
D15S144	0.16	0.17
African Americans		
D15S165	0.25	0.066
D15S1360	0.32	0.23
D15S144	0.94	0.87
European Americans		
D15S165	0.058	0.059
D15S1360	0.24	0.33
D15S144	0.036	0.012*

*Significant at $P < 0.05$ level after Bonferroni correction for testing at multiple markers; see text for transmission of specific alleles of these markers.

($P < 0.0077$), but not in European Americans. The European Americans' significant transmission disequilibrium at D15S144 is accounted for by near significant transmission ($P < 0.06$) of the 158 bp allele of D15S144 (CEPH frequency 0.03) and significant failure to transmit ($P < 0.008$) the 160 bp allele of the same marker (CEPH frequency 0.09). However, this transmission disequilibrium is dependent on the haplotype of D15S165 and D15S1360. For the haplotype D15S165, 182 bp (frequency 0.33), D15S1360, 112 bp (frequency 0.42), the transmission of the 158 bp vs. the 160 bp allele D15S144 is significantly different ($P < 0.019$), whereas on all other D15S165/D15S1360 backgrounds there is no difference in transmission for these two alleles of D15S144 ($P < 0.65$). Thus, the transmission disequilibrium at D15S144 is actually reflective of transmission of a haplotype in European Americans that includes the entire region between D15S165 and D15S144. However, D15S165 and D15S144 are not generally in disequilibrium in either the European American or the African American parental groups.

The goal of this study was to narrow the area of linkage support by using measures of linkage disequilibrium, because of the suggestion that areas as small as 0.5 cm can be identified with disequilibrium techniques. Empirical tests of this assumption on several chromosomes in schizophrenic pedigrees show a 96% likelihood of finding a 5% level of significance for markers within 0.5 cm and only an 8% likelihood within the 5–10 cm range accounted for by our 15q14 linkage markers [Kendler et al., 1999]. D15S165 is found in a 1.03 Mb YAC (969b11) that contains both CHRNA7 and its duplication, but excludes D15S144. D15S144 is found in a 1.170 mb YAC that includes CHRNA7, but excludes its duplication and D15S165 (791e6). D15S1360 is included in both YACs. The failure to find disequilibrium with D15S1360 is probably attributable to its low heterozygosity; it has only seven alleles, with two alleles having a combined frequency of 90% [Leonard et al. 1998]. Riley et al. [2000] used a haplotype of D15S1360 and D15S144 in Bantus, finding an allelewise $P < 0.073$. Their findings are similar to our findings in European Americans, which show disequilibrium for a D15S165-D15S1360-D15S144 haplotype.

The results do not distinguish between CHRNA7 and its duplication as a more likely site for mutations involved in the generation of the linkage signal from 15q14. However, the results add further support that a small region containing the CHRNA7 gene cluster is the likely site of a mutation, even in the absence of abnormalities in the coding region of the gene. Furthermore, the fine mapping performed in the NIMH Genetics Initiative families supports the validity of the initial discovery strategy, which used genetic linkage with a neurophysiological phenotype in schizophrenia as an alternative to the study of the disease itself.

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