

# Alleles at the Dopamine D<sub>4</sub> Receptor Locus Do Not Contribute to the Genetic Susceptibility to Schizophrenia in a Large Swedish Kindred

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The discovery of a functional polymorphism within the dopamine D<sub>4</sub> receptor gene (DRD4) has not only strengthened the hypotheses implicating DRD4 in the etiology of neuropsychiatric disorders, but also provided a genetic marker for testing these hypotheses. The possibility of the dopamine D<sub>4</sub> receptor as a candidate gene for schizophrenia was investigated in a large Swedish kindred segregating for schizophrenia. Linkage to schizophrenia was tested by linkage analyses of 6 polymorphic markers (at 4 loci) in chromosome 11p15.5 including the dopamine D<sub>4</sub> receptor (DRD4) and the tyrosine hydroxylase (TH) loci. Schizophrenia was excluded from close linkage to the DRD4 locus using two of the polymorphisms located within the dopamine D<sub>4</sub> receptor gene. The first DRD4 polymorphism consists of variation in the number of a 48 bp imperfect direct repeat in the third exon; the second consists of a variable number of repeated G nucleotides in the first intron. In addition, some of the individuals homozygous for four or seven copies of 48 bp repeat alleles were tested for previously reported sequence variation among repeats. No single haplotype of the DRD4 alleles or haplotype of other markers in chromosome 11p15.5 was found to be common to the schizophrenic individuals in this family. Therefore, we find no evidence for linkage of the D<sub>4</sub> receptor, or this region of 11p15.5, with genetic

susceptibility to schizophrenia in this kindred. © 1993 Wiley-Liss, Inc.

**KEY WORDS:** schizophrenia, genetics, dopamine D<sub>4</sub> receptor

## INTRODUCTION

For over 20 years, the dopamine system has remained a major candidate for the primary defect in schizophrenia. The disease is thought to be the result of hyperactivity of the dopaminergic system. This "dopamine hypothesis" developed from the role of dopamine in the action of amphetamine-induced paranoid schizophreniform psychosis [reviewed in Snyder, 1976]. Further support arose from the highly successful use of dopamine antagonists (neuroleptics) in the treatment of schizophrenia [see Seeman, 1981 for review]. The dopamine "system," however, is a complex set of interacting neurons, neurotransmitters, receptors, and all of the related functions. Attention has recently been focused on the roles of the dopamine receptors in schizophrenia, especially on the dopamine D<sub>2</sub> receptor [Seeman, 1981]. Although the majority of neuroleptics are known to bind or influence the serotonin, cholinergic, and dopamine systems, the correlation between their clinical potency and receptor affinity is greatest for the D<sub>2</sub> receptor.

Additional recently discovered dopamine receptors have properties similar to the D<sub>2</sub> receptor, raising the possibility that characteristics originally attributed to D<sub>2</sub> may have been an amalgam of characteristics of D<sub>2</sub> and those of the more recently found dopamine receptors, D<sub>3</sub> and D<sub>4</sub>. In contrast to D<sub>2</sub>, which is expressed in both limbic and caudate areas, the distribution of the D<sub>3</sub> receptor is more limited to the limbic area, the region involved in emotional processes; this suggests the possibility of a role for the D<sub>3</sub> receptor in psychosis.

The cloning of the fourth dopamine receptor generated considerable interest because the atypical neuroleptic clozapine (Sandoz) binds with ten times greater

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affinity to the D<sub>4</sub> receptor than to the D<sub>2</sub> or D<sub>3</sub> receptors [Van Tol et al., 1991]. Clozapine has been found to be extremely effective in reducing psychotic symptoms in many patients while being relatively free of drug-induced parkinsonism and tardive dyskinesia [Kane et al., 1988; Casey, 1989]. These observations could explain the apparent paradox that clozapine seems to be more effective in treatment of schizophrenics than predicted by its affinity for the D<sub>2</sub> receptor. Furthermore, the limbic localization of the D<sub>4</sub> receptor and the unique clinical profile of clozapine, including its high efficacy in treatment of patients who are nonresponders to classical neuroleptics, makes the D<sub>4</sub> receptor gene an interesting candidate for causation of schizophrenia.

Recently, a polymorphism, consisting of variation in the number of tandem repeats of a 48 bp unit, was reported in the putative third cytoplasmic loop of the D<sub>4</sub> receptor [Van Tol et al., 1992]. Interestingly, this polymorphism was shown to alter the biochemical properties of the receptor. The number of repeats of the 48 bp unit was shown to influence the binding of clozapine: variants with two or four repeats showed lower dissociation constants in the absence of sodium chloride than variants with seven repeat units [Van Tol et al., 1992]. Further, variation has been found to exist within the 48 bp repeat unit [Lichter et al., 1993]. Single base pair differences were identified within the repeat unit and 25 DNA sequence variants have been identified thus far [Lichter et al., 1993 and unpublished data]. The combination of the number of repeats and the sequence differences within the repeats results in substantial variation in the predicted amino acid sequence in this region. The effect, if any, of these various base pair changes on D<sub>4</sub> receptor function is as yet unknown. It seems relevant that the third cytoplasmic loop has been implicated in the specificity of G protein coupling.

## METHODS

The kindred used for this study is a large Swedish kindred originally described by J.A. Böök [1953]. The pedigree has been previously described in Wetterberg and Modrezewska [1989] and Kennedy et al. [1988] with some additional individuals added since that 1988 report. For details on the diagnostic criteria and methods used in the Swedish kindred, see Moises et al. [1991] and Wetterberg and Farmer [1991]. For parametric linkage analysis, schizophrenia was treated as an autosomal dominant trait with equal penetrances of 72% for homozygotes and heterozygotes. The gene frequency was set at 0.02 and phenocopies set at 0.001. For a detailed description of the genetic models see Barr et al. [1993].

The restriction length fragment polymorphisms (RFLPs) and simple tandem repeats (STRs) were typed by standard methods [Kidd et al., 1991; Weber and May, 1989]. Typing of the PCR-based RFLPs at H19 were as described in Zhang and Tycko [1992]. Description of the mononucleotide (G)<sub>n</sub> repeat can be found in Petronis et al. (submitted). Sequencing of the 48 bp repeats in the third exon was as described in Lichter et al. [1993].

Pairwise linkage analyses were performed using the LIPED program [Ott, 1974] and multipoint analyses

were performed using the LINKMAP program of the LINKAGE package [Lathrop et al., 1985].

## RESULTS

Pairwise lod scores for four loci in chromosome 11p15.5 are shown in Table I. Exclusion of close linkage by pairwise analyses was observed out to 2.04 cM at the TH locus, 2.04 cM at the H19 locus, and 0.20 cM at the DRD4 locus. Lod scores at the Harvey-ras (HRAS) locus were indeterminate because the low level of heterozygosity in this kindred allows only limited inference of segregation patterns.

At the DRD4 locus, the level of heterozygosity observed was lower than seen in other samples. Within this kindred the allele with four repeats of the 48 bp unit was the most common (allele frequency = .74) and 54% of the individuals were homozygous for this allele. When looking only at schizophrenics, 54% were homozygotes. Almost half of the individuals in this pedigree (47%) were also homozygous for the most common (G)<sub>n</sub> repeat allele (9 repeats of the G nucleotide). Haplotypes for the two DRD4 polymorphisms could not be explicitly constructed in all individuals because phase could not always be unambiguously determined. In individuals where phase could be determined, the most common DRD4 haplotype consisted of 4 repeats of the 48 bp unit in the third exon and 9 repeats of the G nucleotide in first intron (haplotype frequency = .48) with 28 of the individuals in the pedigree homozygous for this haplotype. This haplotype was common in both schizophrenic and nonschizophrenic individuals.

In individuals homozygous for the number of 48 bp repeats, we determined the sequence of the repeats in an attempt to uncover heterozygosity for base pair changes within the repeats. DNA from these "homozygous" individuals was amplified by polymerase chain reaction (PCR) and the sequence determined to see if base pair changes could be found within the repeat units in these individuals, as described in Lichter et al. [1993]. Unfortunately, we found no sequence heterozygotes among the individuals homozygous for 4 repeats. All individuals homozygous for 4 repeats were homozygous for the most common repeat/sequence combination, alpha/beta/theta/zeta [for information on nomenclature and sequence of repeats see Lichter et al., 1993]. This particular haplotype (4 repeats in the alpha/beta/theta/zeta configuration) was found in schizophrenics, unaffecteds, and individuals married into the pedigree. This is not particularly surprising considering that the frequency of this haplotype is .48 in a survey of 60 chromosomes in mixed European Caucasians [Lichter et al., 1993].

Genotypes of all five systems typed were placed on the pedigrees and the pattern of transmission of the entire region was determined. Two crossovers were observed in this kindred in the 11p15.5 region. The first crossover was observed between TH/H19 and DRD4. HRAS was uninformative in this branch of the family. The second crossover was observed between H19 and DRD4. Again, HRAS was uninformative, preventing placement of DRD4 either distal or proximal to HRAS.

Multipoint analyses were performed using two loci,

TABLE I. Pairwise Lod Scores for Chromosome 11p15.5 Loci\*

Locus symbol	Polymorphism	0.000	0.001	0.010	0.050	0.100	0.200	0.300	0.400	cM excluded
HRAS	MspI	1.56	1.56	1.52	1.33	1.10	0.69	0.35	0.12	
TH	STR	-4.10	-3.66	-2.71	-1.67	-1.07	-0.43	-0.16	0.03	2.04
H19	RsaI	-3.58	-3.32	-2.27	-1.02	-0.48	-0.08	0.01	0.01	2.04
DRD4	PvuII	0.39	0.38	0.37	0.33	0.28	0.20	0.11	0.05	
DRD4	STR	0.71	0.72	0.82	0.99	0.98	0.73	0.41	0.15	
DRD4	Haplotype	-2.17	-2.09	-1.56	0.04	0.49	0.57	0.35	0.14	0.20

\* Five polymorphic markers (4 loci) were tested for linkage to schizophrenia. Pairwise lod scores are shown for 6 recombination frequencies under the assumption that male and female recombination is equal. The DRD4 haplotype lod scores were calculated using the program LINKAGE by setting the distance between the two DRD4 polymorphisms to zero. Exclusion is taken as a lod score below -2 and the region excluded is the cM length calculated using Haldane's mapping function corresponding to twice the largest recombination fraction at which a lod score of -2 was observed.

TH and DRD4. The distance between TH and DRD4 was set to 6.3 cM [Gelernter et al., 1992]. Because the exact map location of DRD4 is not known with regard to HRAS, a multipoint analysis could not be performed over the entire marker set. The results from the multipoint are shown in Figure 2. Exclusion was observed across 24 cM of chromosome 11p15.5, spanning the region 9 cM proximal to the TH locus to 8.7 cM distal to DRD4. These results exclude the possibility of DRD4 as a single major locus contributing to schizophrenia under the described genetic model.

### DISCUSSION

Arguing against involvement of this region of chromosome 11 in the genetic susceptibility to schizophrenia is the finding of different 11p15.5 chromosomes in the affected members of the pedigree. If a gene in this region contributed to susceptibility to schizophrenia, then all schizophrenics within a family should share at least one ancestral chromosomal region containing the disease gene. As shown in Figure 2, they obviously do not. For individuals in which chromosomal phase could be determined, no single 11p15.5 chromosome was found to be common to all schizophrenic individuals within the entire kindred. More conclusively, looking at the smaller branches within the kindred, six affected relative pairs (uncle, aunt/niece, nephew or first cousins) were found that did not share 11p15.5 chromosomes that appeared to be identical by descent. In one nuclear branch, three affected individuals have 6 completely different chromosomes (Fig. 1).

Because of this finding, the involvement of an allele at the dopamine D<sub>4</sub> receptor locus as required but not sufficient for the development of schizophrenia (two locus or oligogenic model) can also be ruled out. This model would be supported only if schizophrenic individuals within a family share a haplotype identical by descent in addition to one or more unidentified disease alleles.

We can therefore rule out the possibility that the dopamine D<sub>4</sub> receptor has a role in the genetic predisposition to schizophrenia in this kindred, although not the possibility that variants at the DRD4 locus may in some way influence the response to neuroleptics. The role of the 48 bp variants and base pair mutations in the

third exon are unknown. The effect of these different alleles at the DRD4 locus on a patient's response to neuroleptics is of high interest and will be investigated in future studies.

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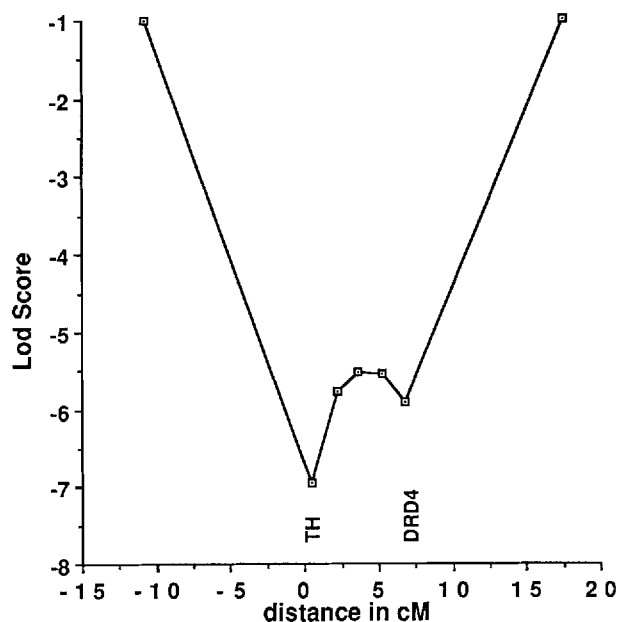


Fig. 1. Graphical representation of the multipoint analyses showing exclusion of schizophrenia 9 cM proximal to TH and 8.7 cM distal to DRD4.

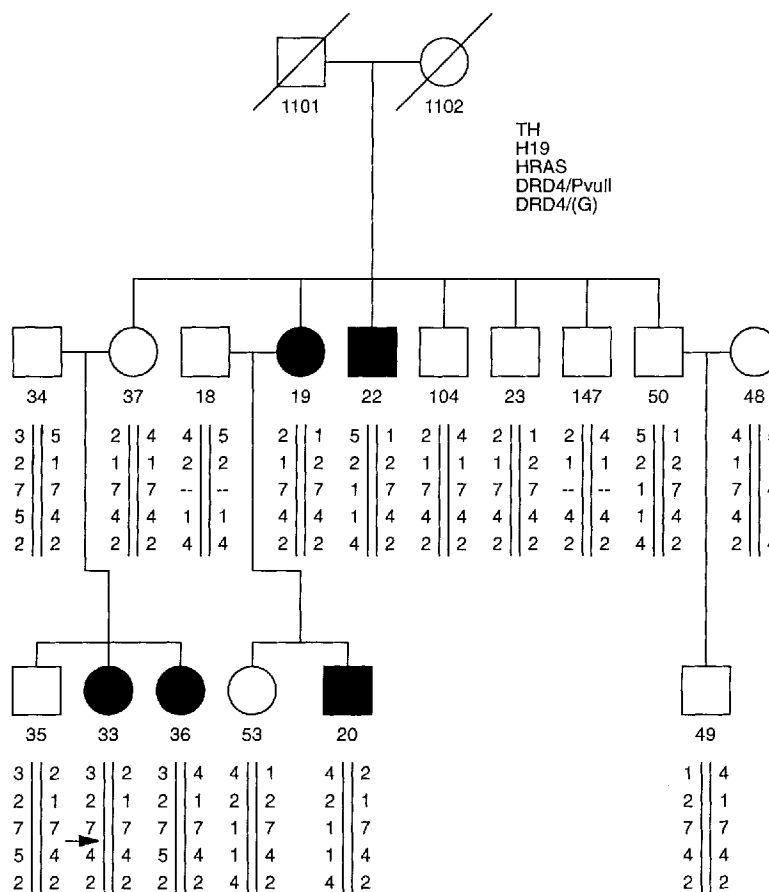


Fig. 2. Phase diagram of the 11p15.5 markers of one branch of the Swedish kindred. Three of the five affected individuals in this nuclear pedigree have 6 completely different chromosomes (individuals 22, 20, and 36). Of the other two, individuals 33 and 36 do not share the same maternal chromosome. Individual 19 shares one chromosome identical by descent with an affected sibling (22) but passes the other chromosome to the affected child. An arrow points to a meiotic crossover between TH/H19 and DRD4 on the paternal chromosome. The map position of DRD4 with regards to HRAS is unknown but was drawn distal to HRAS for the purpose of this figure. Because the segregation of HRAS was uninformative in the individual with the crossover, the placement of DRD4 proximal or distal to HRAS does not alter the interpretation of the inheritance of the chromosomes in this kindred.

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