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No Evidence for a Major Gene Effect of the Dopamine D_4 Receptor Gene in the Susceptibility to Gilles de la Tourette Syndrome in Five Canadian Families

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Gilles de la Tourette Syndrome (TS) is a neuropsychiatric disorder characterized by both motor and vocal tics affecting approximately 1/10,000 females and 1/2000 males. Because of the success of neuroleptics and other agents interacting with the dopaminergic system in the suppression of tics, a defect in the dopamine system has been hypothesized in the etiology of TS. In this paper we test the hypothesis that the dopamine D_4 receptor (DRD4) is linked to the genetic susceptibility to TS in five families. We tested three polymorphisms in the DRD4 gene and a polymorphism in the closely linked locus, tyrosine hydroxylase (TH). We found no evidence for linkage of DRD4 or TH to TS using an autosomal dominant model with reduced penetrance or using non-parametric methods. The presence of a mutation that results in a truncated non-functional D_4 receptor protein was also tested for, but was not observed in these families. © 1996 Wiley-Liss, Inc.

KEY WORDS: Gilles de la Tourette Syndrome, genetics, dopamine D_4 receptor

INTRODUCTION

A defect in the dopamine system has been hypothesized in the pathogenesis of a number of psychiatric diseases including schizophrenia [reviewed in Seeman, 1981], Parkinson's disease [reviewed in Nanko et al., 1994], and in TS [reviewed in Leckman et al., 1988]. A number of observations point to a defect in the dopaminergic system in TS. The most substantial observation being that tics in TS patients are successfully controlled with dopaminergic blocking agents (neuroleptics). Tic suppression has also been reported with alpha methylparatyrosine, an agent that blocks dopamine synthesis [Sweet et al., 1974] and tetrabenazine, an agent that blocks the accumulation of dopamine in presynaptic storage vesicles [Jankovic et al., 1984]. Agents that increase the levels of dopamine such as central nervous system stimulants (e.g., amphetamine, methyphenidate, and pemoline) often aggravate tics [reviewed in Golden, 1988]. These findings have led to the idea that TS is the consequence of hypersensitive postsynaptic dopaminergic receptors, either an increased number of dopamine receptors or increased receptor affinity for dopamine [Cohen et al., 1978; Butler et al., 1979; Singer et al., 1982]. Treatment with neuroleptics reduces the symptoms of TS in most individuals; however the symptoms are suppressed but not eliminated and not all TS patients respond to neuroleptics.

The cloning of the dopamine D_4 receptor generated a great deal of interest because of the binding properties of the receptor: the D_4 receptor binds the atypical neuroleptic, clozapine (Clozaril, Sandoz, Basel, Switzerland) with higher affinity than the D_2 and D_3 dopamine receptors. [Van Tol et al., 1991]. Clozapine has been successful in treating a high percentage of schizophrenia patients who were refractory to treatment with clinical neuroleptics, but preliminary evidence suggest that the frequency of tics in TS patients are not reduced by clozapine [Pfeiffer and Wagner, 1994]. It remains possible that a change in the D_4 receptor function may lead to tics, or modify susceptibility.

Because of the possible relevance of the D_4 receptor to neuropsychiatric diseases, the DRD4 locus has been the focus of intense investigation. Several polymorphisms have been identified for this locus including two polymorphisms in the coding region of the gene. The first polymorphism identified in the D_4 receptor is the result of differences in the number of a 48 base pair (bp) repeat in the third exon [Van Tol et al., 1992]. In addition, there is variation in the sequence within the repeats [Lichter et al., 1993]. The second polymorphism

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in the coding region results from a 12 bp insertion/deletion in the first exon [Catalano et al., 1993]. The effects on receptor function of these sequence variations are unclear.

A 13 bp deletion in the first exon resulting in a truncated protein has also been reported [Nöthen et al., 1994]. This mutation is present in approximately 2% of the German population. Preliminary results suggest that TS or other major psychiatric diseases do not result from the loss of one or both alleles due to this mutation [Nöthen et al., 1994].

Our study of the D_4 receptor and TS was prompted by the finding of transmission distortion of a DRD4 allele (seven repeats of the 48 bp repeat in exon 3) in several branches of a large Mennonite TS pedigree [Gelernter, 1995]. The DRD4 region had previously been excluded assuming an autosomal dominant model and locus homogeneity in a summary of several families segregating TS including the Mennonite TS pedigree [Pakstis et al., 1991].

Because of the transmission distortion finding and because of defect of the D_4 receptor is consistent with the hypothesized dopaminergic defect in the etiology of TS, we investigated the three variations in the coding region of DRD4 and an additional polymorphism in the first intron [Petronis et al., 1994] for linkage to TS. The gene for the rate-limiting enzyme in dopamine synthesis, tyrosine hydroxylase (TH), was also tested for linkage to TS.

METHODS

Diagnosis

Each subject was assessed in a direct interview by a research assistant trained to observe and recognize manifestations of TS and Obsessive Compulsive Disorder (OCD) in the context of a semistructured interview adapted from the Diagnostic Interview Schedule by Dr. David Pauls and colleagues at Yale [Pauls and Hurst 1987]. This interview schedule also includes an extensive section on OCD derived from the Yale-Brown Obsessive Compulsive Scale [Goodman et al., 1989] and provides diagnostic information about affective disorder, psychosis, anxiety disorder, attention deficit hyperactivity disorder (ADHD), substance abuse, and antisocial behavior. The diagnostic checklist for ADHD provides additional information regarding concentration and hyperactivity in childhood and at the time of assessment.

The above information is augmented by three self-administered instruments: i) SCL 90, a general mental health questionnaire well described in the literature [Derogatis, 1977], ii) OCD inventory derived from Leyton Obsessive-Compulsive inventory [Frankel et al., 1986], and iii) Conner's Parent Rating Scale [Goyette et al., 1978] for children under age 16. All of the above information was reviewed and summarized by an experienced neuropsychiatrist who then derived a diagnostic classification according to the criteria outline below.

Tourette Syndrome is diagnosed according to DSM-III [American Psychiatric Association, 1987] criteria and diagnosis is refined as suggested by Kurlan [1989], to indicate the quality of the information. This gives subjects a possible or definite diagnosis of Tourette Syndrome, chronic multiple tic disorder (motor or vocal), or other tic disorder. Similarly OCD is diagnosed according to DSM-IIIR [American Psychiatric Association, 1987] criteria and classified by the neuropsychiatrist as possible, probable or definite. For the affected status we used definite and probable TS and definite CMT. In these five families chosen for linkage analysis only five individuals, other than obligate carriers, change from unaffected to affected status with the inclusion of CMT as affected (two of these individuals also have OCD) and only two individuals, who are not obligate carriers, have OCD without CMT or TS. Our simulation studies show that the choice of clinical phenotype for analysis will have very little impact on the lod score using these families.

Isolation of DNA and Marker Typing

DNA was extracted directly from blood lymphocytes or from Epstein-Barr virus established cell lines using the high salt extraction method of Miller et al. [1988]. The $(G)_n$ mononucleotide repeat polymorphism located in the first intron of DRD4 was typed according to Petronis et al. [1994]. The 48 bp repeat polymorphism was typed according to Lichter et al. [1993] and the 12 bp insertion/deletion polymorphism in the first exon was typed by the method described in Catalano et al. [1993]. The presence of the null mutation in the first exon of DRD4 was assessed using the method described by Nöthen et al. [1994]. The tetranucleotide repeat polymorphism at TH was typed as described in Edwards et al. [1992].

Linkage Analysis

Pairwise linkage analyses were performed using the LIPED program [Ott, 1974]. Haldane's mapping function [Haldane, 1919] was used to convert recombination fractions to map distances. The genetic model for two-point analyses were as follows: a single autosomal dominant gene with gene frequency .003 [Pauls et al., 1990]. An age of onset correction was included using a linear function increasing from age 2 years to 21 years with a minimum penetrance of .048 and a maximum of .999 for males and a minimum penetrance of .027 and maximum penetrance of .561 for females. A small rate of phenocopies was also incorporated into the model: minimum .0002 to maximum .005 for males and minimum 0 to maximum .0001 for females. The small rate of phenocopies (penetrance value for homozygotes for the non-TS allele) is included in the model to compensate for a small rate of false positive diagnoses resulting from environmentally caused symptoms, or the possibility that a second TS susceptibility locus is brought into the pedigree.

Although the exact mode of inheritance for TS is not known, two-point analysis is relatively robust (i.e., insensitive) to most model misspecification [Clerget-Darpoux et al. 1986]. If the model is misspecified and there is truly linkage, a high lod score will still be seen but the recombination fraction will be overestimated. The only specification for which this does not hold true is the degree of dominance (i.e., if the disease is reces-

sive and it is analyzed as dominant, and vice versa). In this case, the lod score suffers considerably. Other misspecification such as wrong values for disease allele frequency and for the penetances inflate the recombination fraction without much damage to the lod scores.

The data were also analysed non-parametrically using a modified version [Ward, 1993] of the Affected-Pedigree-Member method of Weeks and Lange [1988]. In this method, the test statistic is extended to include contrast between affected and unaffected pedigree members [Ward, 1993]. For this analysis unaffected family members under 19 years of age were classified as unknown to allow for the possibility of the development of the disorder during the age of risk. Multipoint analyses were performed using the Linkmap option of the LINKAGE program package [Lathrop et al., 1985].

RESULTS

We tested three different polymorphisms at the DRD4 locus and a polymorphism at the closely linked locus, TH for genetic linkage to TS. DRD4 is located 6.3 cM distal to TH on chromosome 11p15.5 [Gelernter et al., 1992; Petronis et al., 1993]. Pairwise lod scores for the four polymorphic systems are shown in Table I for the autosomal dominant model. Lod scores for each family are shown individual and as a sum of all the families tested for each polymorphism. No evidence was seen for linkage using the genetic model specified. Genotypes from the two most informative polymorphisms at DRD4, the mononucleotide repeat in the first intron and the 48 bp repeat in the third exon, were also analysed using the non-parametric methods described in Ward [1993]. No significant results were observed.

We also tested for the presence of the 13 bp deletion mutation in the first exon resulting in a truncated non-functional protein [Nöthen et al., 1994]. We checked only the founders of each pedigree for the mutation or if the founders were not available we checked the founders of each of the branches of the pedigree. We did not observe the null allele in any of the 18 family members tested.

Also within the first exon, variation in the number of a 12 base pair repeat has been reported [Catalano et al., 1993]. The 12 bp repeat codes for four amino acids in the extracellular N-terminus of the receptor. The most common form of the gene has two copies of the repeat and in the rare variant there is only one copy of the repeat. We observed only the most common allele (two repeats) in three of the five families tested. In the other two pedigrees we observed 4 out of 23 individuals with

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

Locus symbol	Polymorphism	Family	0.000	0.010	0.050	0.100	0.200	0.300	Region excluded
TH	STR	T001	−2.44	−2.02	−1.30	−0.88	−0.44	−0.21	Locus excluded
		T004	0.69	0.68	0.61	0.52	0.35	0.18	
		T005	−4.28	−3.01	−1.96	−1.30	−0.64	−0.33	Locus excluded
		T006	−3.48	−2.82	−2.14	−1.65	−0.89	−0.42	11 cM
		T008	−0.69	−0.68	−0.64	−0.52	−0.40	−0.23	
		Sum	−10.19	−7.86	−5.44	−3.82	−2.02	−1.02	50 cM
DRD4	exon 1—ins/del	T001	N.I.						
		T004	N.I.						
		T005	0.58	0.56	0.51	0.44	0.29	0.15	
		T006	N.I.						
		T008	−2.70	−1.80	−1.13	−0.82	−0.40	−0.28	Locus excluded
		Sum	−2.12	−1.24	−0.62	−0.38	−0.11	−0.13	Locus excluded
DRD4	exon 3—48 bp rep	T001	−2.30	−1.84	−1.09	−0.66	−0.26	−0.09	Locus excluded
		T004	−2.06	−1.96	−1.35	−0.91	−0.45	−0.22	Locus excluded
		T005	−0.01	−0.01	−0.01	−0.01	0.00	0.00	
		T006	−3.61	−2.60	−1.35	−0.67	−0.08	0.12	Locus excluded
		T008	−0.13	−0.13	−0.13	−0.12	−0.10	−0.04	
		Sum	−8.10	−6.54	−3.93	−2.37	−0.80	−0.23	22 cM excluded
DRD4	intron 1—(G)n	T001	−2.51	−1.92	−1.05	−0.60	−0.21	−0.06	Locus excluded
		T004	−0.43	−0.39	−0.27	−0.17	−0.07	−0.02	
		T005	−3.08	−1.21	−0.56	−0.30	−0.10	−0.03	Locus excluded
		T006	−1.80	−1.14	−0.36	0.01	0.27	0.30	
		T008	−5.86	−4.31	−2.68	−1.76	−0.85	−0.38	Locus excluded
		Sum	−13.68	−8.97	−4.92	−2.82	−1.26	−0.19	22 cM excluded
DRD4	Haplotype	T001	−3.06	−2.09	−1.06	−0.57	−0.16	−0.02	11 cM excluded
		T004	−1.94	−1.88	−1.34	−0.90	−0.44	−0.23	
		T005	−3.09	−1.18	−0.53	−0.28	−0.09	−0.02	Locus excluded
		T006	−3.96	−2.96	−1.67	−0.96	−0.31	−0.04	Locus excluded
		T008	−5.35	−4.00	−2.58	−1.70	−0.83	−0.37	11 cM excluded
		Sum	−17.39	−12.11	−7.18	−4.41	−1.82	−0.67	22 cM excluded

*Linkage results for TH and DRD4 polymorphisms. Four polymorphic markers (two loci) were tested for linkage to TS using an autosomal dominant model. Pairwise lod scores are shown for six 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 most informative 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 without interpolation. The designation "locus excluded" indicates that exclusion is only at the locus and N.I. indicates the marker was not informative in that family.

one copy of the 12 bp repeat in pedigree T005 and 6 out of 32 individuals with one copy of the 12 bp repeat in pedigree T008. This variation did not segregate with the disorder in either of the two pedigrees.

Linkage analysis of the haplotypes of the two most informative DRD4 polymorphisms was performed using multipoint analyses over the two DRD4 polymorphisms setting the distance to zero between the markers. The resulting lod scores are shown in Table I. Also, haplotypes were determined by hand for the DRD4 polymorphisms on all families. No single haplotype of markers was found to be shared in the affected individuals within a single pedigree.

DISCUSSION

We have presented data to exclude the involvement of DRD4 as a major genetic susceptibility locus for TS in the five families examined as a group and also individually. All the families examined were excluded at the DRD4 locus with at least one of the polymorphisms. The combined exclusion area extended to 11 cM on either side of the DRD4 locus and 25 cM either side of the TH under an autosomal dominant hypothesis. Under the autosomal dominant model we could also exclude the gene for TH, a critical enzyme because of the role of this enzyme as the rate limiting step in the synthesis of the catecholamines dopamine and nonadrenaline.

We were able to exclude the DRD4 locus under an autosomal dominant model as well as non-parametrically. The strongest evidence against the D4 gene as a susceptibility locus for TS is that we found no sharing of DRD4 haplotypes in affected individuals in these families. If a defect in the DRD4 gene arose on an ancestral chromosome then affected members within a family should share the same D4 haplotype inherited identical by descent. We did not find this in any of the five families studied. These findings also exclude DRD4 in an oligogenic or two locus model because affected members would share a D4 haplotype in addition to sharing at the unidentified second locus.

Three of the polymorphisms in the DRD4 locus result in a change in the coding sequence of the gene. The change in the number of the 48 bp repeat in the third exon, a 4 amino acid deletion/insertion in the first exon and a 13 bp deletion mutation. We did not find evidence for any of these polymorphism in the genetic susceptibility to TS in the five families studied. Because locus heterogeneity has not been ruled out, the possibility remains that DRD4 may be responsible for TS in a subset of families which have not been reported.

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