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Linkage Study of the Dopamine D_5 Receptor Gene and Gilles de la Tourette Syndrome

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A defect in the dopamine system has been hypothesized as the etiological defect in Gilles de la Tourette syndrome (TS). In this report, we test the hypothesis that the dopamine D_5 receptor locus (DRD5) is linked to the genetic susceptibility to TS in five families studied in Canada. We tested for linkage to the dopamine D_5 receptor gene using a microsatellite polymorphism located in the same cosmid clone. Using an autosomal dominant model with reduced penetrance, we were able to exclude linkage in four of the five families for the TS and chronic multiple tics (CMT) phenotype. Also, no evidence for linkage was found using nonparametric methods in all five families. ***Am. J. Med. Genet. 74:58–61, 1997.***

KEY WORDS: Gilles de la Tourette syndrome; genetics; dopamine D_5 receptor

INTRODUCTION

Gilles de la Tourette syndrome (TS) is a neuropsychiatric disorder characterized by both motor and vocal tics. In addition, affected individuals frequently display symptoms such as attention deficit hyperactivity disorder and/or obsessive compulsive disorder.

Current evidence suggests that TS may result from a defect in the dopamine system [reviewed in Leckman et al., 1988]. This hypothesis is supported by the successful reduction of tics in the majority of patients using neuroleptics (dopaminergic blocking agents) [Shapiro et al., 1989]. Tic suppression also has been reported with an agent that blocks the accumulation of dopamine in presynaptic storage vesicles, tetrabenazine [Jankovic et al., 1984], and an agent that blocks dopamine synthesis, alpha methylparatyrosine [Sweet et al., 1974]. In addition, tics are often aggravated by agents that increase the levels of dopamine such as central nervous system stimulants [reviewed in Golden, 1988].

Of the dopamine receptors characterized, the D_5 receptor most closely resembles the D_1 subtype; both receptors stimulate adenylate cyclase and are intronless. The dopamine D_5 receptor was identified by homology screening with the D_1 receptor gene as a probe [Grandy et al., 1991; Sunahara et al., 1991] and was found to display a higher affinity for dopamine than the D_1 receptor [Grandy et al., 1991; Sunahara et al., 1991]. The D_5 receptor is expressed specifically in the neurons and primarily localized in the limbic region of the brain (Sunahara et al., 1991). The gene for the D_5 receptor was originally localized to chromosome 4p16.1 by *in situ* hybridization [Grandy et al., 1992] and later mapped to 4p15.1–p15.3 by a panel of somatic cell hybrids [Eubanks et al., 1992].

A highly polymorphic microsatellite has been identified within the cosmid containing the D_5 receptor gene [Sherrington et al., 1993]. This polymorphism, $(CT/GT/GA)_n$, was previously used to test for linkage to TS using a single large pedigree with multiple affected members [Brett, 1995]. In this family, no evidence was found for linkage using an autosomal dominant model with reduced penetrance. We tested this same polymorphism for linkage to TS in five families collected in Canada.

MATERIALS AND METHODS

Diagnosis

A detailed description of the assessment procedure and diagnostic criteria for the families used in this study can be found in Barr et al., [1996]. 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]. In addition, the diagnostic interview was augmented by three self-administered instruments [see Barr et al., 1996, for a detailed description]. All information was reviewed and

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summarized by an experienced neuropsychiatrist who then derived a diagnostic classification according to the criteria outlined below. Tourette syndrome was diagnosed according to DSM-IIIR [APA, 1987] criteria, and diagnosis was refined as suggested by Kurlan [1989] to indicate the quality of the information. This gave subjects a possible, probable, or definite diagnosis of TS, chronic multiple tic disorder (motor or vocal), or other tic disorder. Similarly, OCD was diagnosed according to DSM-IIIR [APA, 1987] criteria and classified by the neuropsychiatrist as possible, probable, or definite.

Several lines of evidence support a genetic relationship between TS and some forms of OCD, including a high incidence of obsessive compulsive symptoms (OCS) in TS patients and a higher incidence of OCD in relatives of TS probands than in the general population [Pauls et al., 1986, 1991]. It is not uncommon to find obligate carriers of TS with OCD or OCS, and within the same family males are more likely to have TS or tics, whereas females are more likely to have OCS [Pauls et al., 1991].

For the affected status we used two classifications of phenotype for the analysis: (1) definite and probable TS as affected, and (2) definite and probable TS, or definite CMT as affected. 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 (2 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 $(CT/GT/GA)_n$ repeat polymorphism located in the cosmid clone containing the D5 receptor gene was typed according to Sherrington et al. [1993]. Allele frequencies observed in our sample of unrelated individuals were comparable to the previous reported allele frequencies [Sherrington et al., 1993]. Two alleles previously reported, 156 and 154 bp, with allele frequencies of .008 and .038, respectively, were not seen in our smaller sample of chromosomes.

Description of Pedigrees

Five extended families (3–4 generations) were used for linkage analysis for this study. For the smallest pedigree, DNA was available on 15 family members (4 individuals with CMT or TS), and for the largest pedigree, DNA was available on 29 family members (10 individuals with CMT or TS). In total, 106 individuals (37 with CMT or TS) were typed for the marker at DRD5.

Linkage Analysis

The LIPED program [Ott, 1974], was used to perform pairwise linkage analyses. Recombination fractions were converted to map distances using Haldane's [1919] mapping function. For the TS phenotype, the following genetic model was used: a single autosomal dominant gene with gene frequency .0004 [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 .0429 and a maximum of .45 for males and a minimum penetrance of .016 and maximum penetrance of .171 for females [Pauls et al., 1990]. We included a small rate of phenocopies into the model: minimum 0 to maximum .0001 for males and females 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.

For the TS/CMT phenotype, the following genetic model was used: 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 [Pauls et al., 1990]. We included a small rate of phenocopies into the model: minimum .0002 to maximum .005 for males and minimum 0 to maximum .0001 for females.

We have used for the parametric analysis the genetic model supported by segregation analysis. Two-point analysis is relatively robust (i.e., insensitive) to most model misspecification except for misspecifications with regard to degree of dominance (i.e., if the disease is recessive and it is analyzed as dominant, and vice versa). If the degree of dominance is misspecified and there is truly linkage, the lod score suffers considerably [Clerget-Darpoux et al. 1986]. 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 [Clerget-Darpoux et al., 1986].

As the exact mode of inheritance for TS is at this point unknown, we also analyzed the data nonparametrically using the Affected-Pedigree-Member Method (APM) statistic of Weeks and Lange [1988] and a modified version of this statistic [Ward, 1993]. In the modified 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.

RESULTS

We tested a highly informative microsatellite polymorphism at the DRD5 locus [Sherrington et al., 1993] for linkage to TS in five families. Pairwise lodscores for DRD5 using an autosomal dominant model are shown in Table I for the TS/CMT phenotype and Table II for the TS only phenotype. Lod scores for each family are shown individually and as a sum of all the families tested. No evidence was detected for linkage using the autosomal dominant model and phenotype classification specified above. We were able to exclude four of the five families tested for using the TS/CMT phenotype classification and three of the five pedigrees with the

TABLE I. Pairwise Lod Scores for DRD5: TS/CMT Phenotype*

Family	0.000	0.050	0.100	0.150	0.200	0.250	Region excluded
T001	0.526	0.562	0.547	0.503	0.442	0.370	
T004	−2.977	−1.550	−1.031	−0.733	−0.531	−0.382	Locus excluded
T005	−4.615	−3.739	−3.112	−2.410	−1.816	1.333	36 cM excluded
T006	−3.329	−2.143	−1.770	−1.367	−0.989	−0.681	10 cM excluded
T008	−2.104	−0.411	−0.097	0.037	0.090	0.100	Locus excluded
SUM	−12.499	−7.281	−5.463	−3.970	−2.804	−1.926	50 cM excluded

* Linkage results for DRD5 microsatellite polymorphism tested for linkage to TS/CMT using an autosomal dominant model. Pairwise lod scores are shown for six recombination frequencies under the assumption that male and female recombination is equal. 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.

TS only phenotype. One family was not sufficiently informative to exclude or prove linkage using an autosomal dominant model with either phenotype classification (Tables I and II) and a second family was not sufficiently informative to exclude or prove linkage using the TS only classification (Table II). Summed over all five families, 25 cM on either side of the DRD5 locus could be excluded assuming an autosomal dominant model and locus homogeneity.

Genotypes from these families were also analyzed using the APM statistic of Weeks and Lange [1988] and a modification of this statistic [Ward, 1993]. No significant results were observed for any of the five families.

DISCUSSION

We were able to exclude the involvement of DRD5 as a major genetic susceptibility locus for TS in the five families examined as a group and also individually. Four of the five families were excluded using an autosomal dominant model and the TS/CMT classification and no evidence was seen for linkage using nonparametric methods in any of the five families. We also were able to exclude an area 25 cM on either side of the DRD5 locus under an autosomal dominant hypothesis assuming locus homogeneity. By visual inspection we observed no sharing of DRD5 alleles in affected individuals in these families—further evidence against the D_5 receptor gene as a susceptibility locus for TS.

Because locus heterogeneity has not been ruled out, the possibility remains that DRD5 may be responsible for TS in a subset of families that have not been studied. With 98% of the genome excluded assuming autosomal dominant inheritance and locus homogeneity among the families studied [Barr et al., unpub.], locus heterogeneity should be considered a possibility for this disorder.

Only one genome scan report with markers for this region has been published for TS at this point [Pakstis et al., 1991]. It noted a 15 cM exclusion on either side of the marker D4S10, which is estimated to be located at 25 cM from DRD5 [Sherrington et al., 1993]. DRD5 has not been mapped in relation to the distal marker, D4S123 used in the Pakstis et al. [1991] study, and therefore an exact position of DRD5 in the exclusion maps cannot be determined. As there is a gap between the exclusion zones of the two markers D4S10 and D4S123, DRD5 may be located in a position that has not previously been excluded. In addition, only the summed exclusion for all families studied for this region were published, and therefore the number of families contributing to the exclusion is not specified at each locus. If the markers D4S10 and D4S123 were uninformative or slightly positive in a subset of families, then linkage may not have been detected.

Our study extends the findings of Brett et al. [1995] where a single large informative TS family was tested for linkage to DRD5 using an autosomal dominant model. With the combined results of both studies of the exclusion of a total of five families using an autosomal dominant model and no evidence for linkage with nonparametric analyses in our study, the possibility of the involvement of DRD5 in the genetic susceptibility to TS becomes less likely.

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TABLE II. Pairwise Lod Scores for DRD5: TS Phenotype*

Family	0.000	0.050	0.100	0.150	0.200	0.250	Region excluded
T001	−1.068	−0.066	0.119	0.191	0.216	0.213	
T004	−2.525	−0.694	−0.419	−0.270	−0.174	−0.109	Locus excluded
T005	−4.023	−2.356	−1.973	−1.546	−1.166	−0.857	10 cM excluded
T006	−4.036	−1.948	−1.568	−1.197	−0.879	−0.627	Locus excluded
T008	−1.668	−1.197	−0.729	−0.443	−0.263	−0.148	
SUM	−13.320	−6.261	−4.570	−3.265	−2.266	−1.528	50 cM excluded

* Linkage results for DRD5 microsatellite polymorphism tested for linkage to TS using an autosomal dominant model.

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