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Report

Significant Linkage for Tourette Syndrome in a Large French Canadian Family

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Family and twin studies provide strong evidence that genetic factors are involved in the transmission of Gilles de la Tourette syndrome (TS) and related psychiatric disorders. To detect the underlying susceptibility gene(s) for TS, we performed linkage analysis in one large French Canadian family (127 members) from the Charlevoix region, in which 20 family members were definitely affected by TS and 20 others showed related tic disorders. Using model-based linkage analysis, we observed a LOD score of 3.24 on chromosome 11 (11q23). This result was obtained in a multipoint approach involving marker D11S1377, the marker for which significant linkage disequilibrium with TS recently has been detected in an Afrikaner population. Altogether, 25 markers were studied, and, for level of significance, we derived a criterion that took into account the multiple testing arising from the use of three phenotype definitions and three modes of inheritance, a procedure that yielded a LOD score of 3.18. Hence, even after adjustment for multiple testing, the present study shows statistically significant evidence for genetic linkage with TS.

Gilles de la Tourette syndrome (TS [MIM 137580]) is a neuropsychiatric disorder with onset during childhood and is characterized by chronic intermittent motor and vocal tics (The Tourette Syndrome Classification Study Group 1993; American Psychiatric Association 1994). Despite an undeniable demonstration of a genetic component in TS, linkage studies have not yet been successful in determining the chromosomal location of a susceptibility gene for TS (for a review, see Barr and Sandor 1998). Recently, two genome screens were completed. In the first one, involving 386 markers (Barr et al. 1999), no LOD score >2 was found by a model-based linkage analysis, but the model-free analysis yielded eight low *P* values that the authors of the study interpreted with caution, given that the affected-pedigree method that they used is known to have a high false-positive rate. The Tourette Syndrome Association International Consortium for Genetics (1999) also performed a genome screen, which was based on 110 sib pairs. Although no results reached acceptable statistical significance, suggestive results were found in two regions (4q and 8p). The lack of definite evidence for linkage with TS, as well as the inconsistencies among linkage results obtained so far, may be due to either obstructing factors, such as the uncertainty regarding the mode of inheritance for TS and the exact phenotype definition, or complicating factors such, as locus heterogeneity.

Attempts to detect linkage disequilibrium between various candidate genes, mainly dopamine-receptor genes, and TS also have led to inconclusive results, mainly because of an inability to replicate original suggestive results (for a review, see Barr and Sandor 1998). However, more recently, a large-scale association study in an Afrikaner population, which compared DNA samples from patients affected by TS versus those of unaffected control subjects, yielded promising results (Simonic et al. 1998). Of the 1,167

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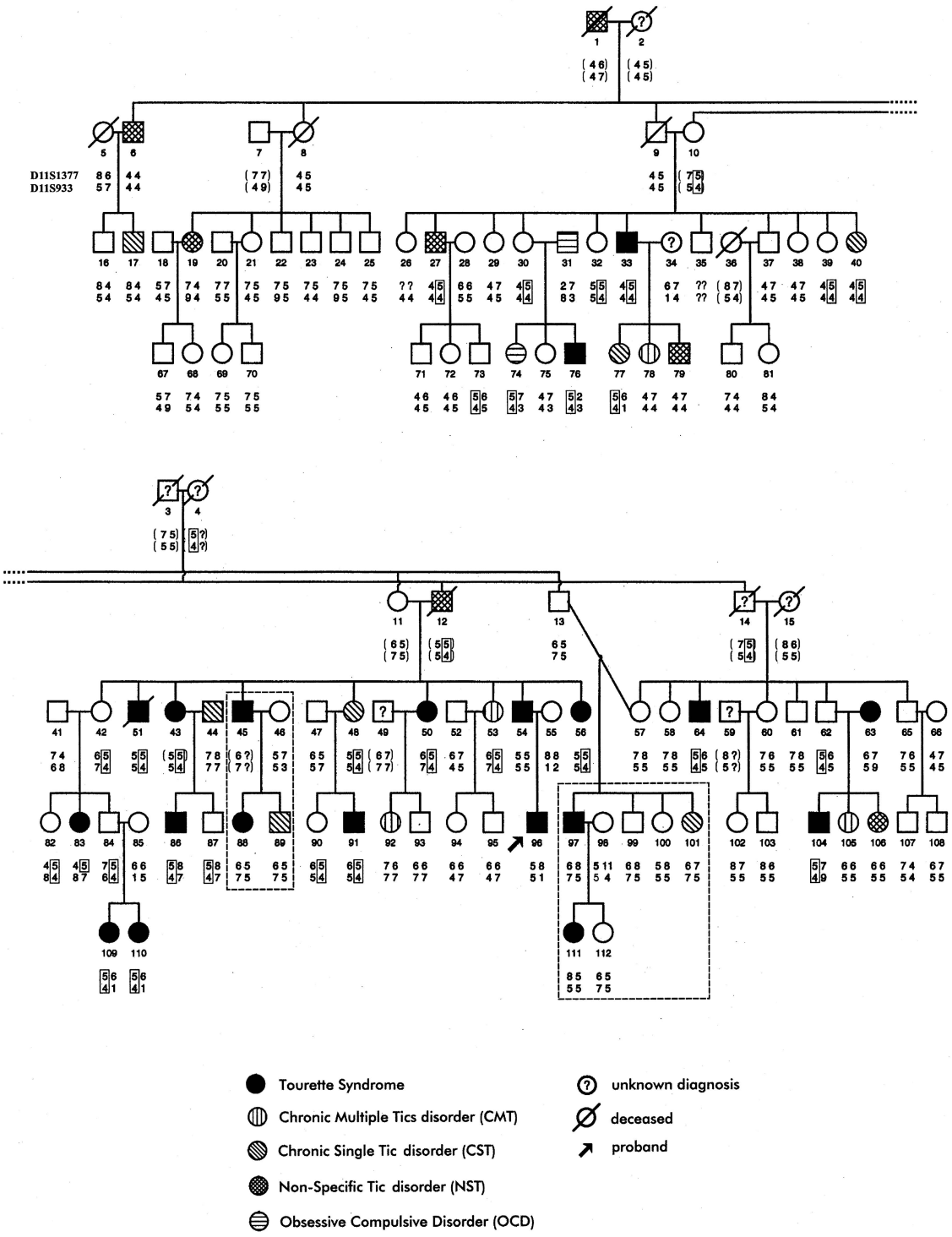

Figure 1 Large French Canadian family (127 members) from the Charlevoix region in the province of Québec, in which (i) 20 members were affected by definitive TS; (ii) 33 members entered the intermediate phenotype definition, which includes definite TS, definite CMT, definitive CST, and OCD; and (iii) 40 members were classified as belonging to the broad phenotype definition that added definite NST to the intermediate definition. To preserve the anonymity of the family, 15 members have been omitted from the pedigree shown in the figure, the sex of some individuals has been changed (although the family sex ratio remains the same), and a few deceased subjects are designated as being alive, and vice versa. One particularity of this pedigree is the presence of two marriage loops: three individuals from one family (subjects 9, 11, and 13) are married to three individuals belonging to another family (subjects 10, 12, and 57, respectively). For each member, haplotypes were constructed on chromosome 11, by use of markers D11S1377 and D11S933. The two boxes enclosed by broken lines contain the two family branches that did not contribute to the LOD-score result for chromosome 11.

Table 1

Descriptive Data on 127 Family Members, by Phenotype

Phenotype and Affection Status (*N*)	Proportion of Males (%)	Mean Age ± SD (years)	
		At Onset	When Studied
Narrow:			
Affected (20)	55.0	7.1 ± 2.1	30.7 ± 17.3
Unknown (49)	46.9	...	41.4 ± 21.8
Unaffected (58)	53.4	...	37.7 ± 17.2
Intermediate:			
Affected (33)	45.4	7.2 ± 2.2	29.7 ± 15.7
Unknown (36)	55.5	...	46.2 ± 22.4
Unaffected (58)	53.4	...	37.7 ± 17.2
Broad:			
Affected (40)	47.5	7.9 ± 3.8	34.3 ± 20.9
Unknown (29)	51.7	...	43.9 ± 20.3
Unaffected (58)	53.4	...	37.7 ± 17.2

Note.—Of the 127 family members, 101 were genotyped.

markers tested, 24 reached a significance level that suggested disequilibrium, according to a two-stage strategy that incorporated a confirmatory sample. We selected these 24 markers to investigate genetic linkage with TS in the large Charlevoix family (fig. 1) that we studied, in which 20 members were definitely affected by TS and in which 20 others showed related tic disorders. The 24 markers are listed in tables 2 and 3 of the article by Simonic et al. (1998) and cover 12 chromosomes (1, 2, 4–6, 8, 11–14, 20, and 21). We added marker D13S1325 to this list, to obtain two adjacent markers on chromosome 13. The Charlevoix region, an area of 100 × 10-15 km that is located in the eastern part of the province of Québec, is a particularly suitable region for genetic studies, because of its founder effect (Morissette 1991).

Family members were given a personal explanation of the study and signed a consent form. All subjects were evaluated at home, with the Schedule for Tourette and Other Behavioral Syndromes (Pauls and Hurst 1987). A review of the past and present medical history was also done. All adults and children >9 years of age were personally interviewed. For the children <18 years of age, an interview concerning the child was also conducted with one of the parents (usually the mother). Every interview was audiotaped. The interview lasted 1–3 h and was conducted by trained psychiatric nurses. Family history (FH) interviews using the Family History Method Using Diagnostic Criteria (Andreasen et al. 1977) and some sections of the Family Informant Criteria (Pauls and Hurst 1987) were conducted with close relatives, to collect data about each subject. Medical records were obtained with the written consent of the subject.

Consensus best-estimate diagnoses were made on the basis of the aforementioned sources of information; for each subject, a board of three of the four certified psychiatrists (C.C., H.B., A.P., and F.R.), who were blinded with regard to both the subject's position within the pedigree and the presence of any psychiatric disorder in close family members, was formed. Two members of the board reviewed the information independently of each other. Then the board met to discuss and decide on a diagnosis. The diagnostic criteria were based on those developed for TS genetic-linkage studies and were from either The Tourette Syndrome Classification Study Group (1993), in the case of TS, and the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association 1994), in the case of all other psychiatric disorders. A diagnosis was considered *definite* only if all diagnostic criteria were met.

Of the 127 family members, 103 were interviewed directly. Sixteen additional individuals were evaluated on the basis of FH interviews. A mean of 3.0 FH interviews was conducted on each subject. Eight subjects received a diagnosis of "unknown," because of a lack of information. Blood samples were collected from 101 family members, for immortalization of the cells and DNA extraction. Forty subjects were classified as *definitely* affected: 20 subjects had definite TS, 4 had definite chronic multiple motor- or phonic-tic disorder (referred to as "chronic multiple tics" [CMT]), 7 had a definite chronic single-tic disorder (CST), 7 had definite nonspecific-tic disorder (NST), and 2 had obsessive-compulsive disorder (OCD) without one of the other diagnoses.

Given the uncertainty regarding the exact phenotype definition to be used in a genetic study of TS, we defined three phenotypes: (1) a *narrow* definition, which included definite TS only ($N = 20$); (2) an *intermediate* definition, which included definite TS, definite CMT, definite CST, and definite OCD ($N = 33$); and (3) a *broad* definition, which added definite NST to the immediately preceding definition ($N = 40$). Subjects having one of the diagnoses included in the definition in use were classified as "affected." Subjects with either a nondefinite diagnosis or a diagnosis included within the broad phenotype definition but not included within the definition in use were classified as "unknown." The 58 subjects who did not show any symptom related to the diagnoses included within the broad phenotype definition were classified as "unaffected," regardless of the phenotype definition in use. Table 1 provides, for each phenotype definition, the number of subjects per affection status, the proportion of males, the mean age at onset, and the mean age at the time of the study.

Manual radioactive genotyping was performed essentially according to the method of Maziade et al. (1997), by means of MapPairs™ primers purchased from Re-

Table 2

Penetrance Parameter Values Used in the Present Linkage Study

	Penetrance for[a]								
	Model 1			Model 2			Model 3		
Sex and Age	tt	Tt	TT	tt	Tt	TT	tt	Tt	TT
Males:									
0–5 years	0	.126	.126	.0005	.196	.196	.0013	.126	.126
6–10 years	0	.219	.219	.0021	.425	.425	.0022	.219	.219
11–15 years	0	.311	.311	.0033	.654	.654	.0031	.311	.311
16–20 years	0	.404	.404	.0044	.884	.884	.0040	.404	.404
⩾21 years	0	.450	.450	.0050	.999	.999	.0045	.450	.450
Females:									
0–5 years	0	.048	.048	0	.098	.098	.0013	.126	.126
6–10 years	0	.083	.083	0	.233	.233	.0022	.219	.219
11–15 years	0	.118	.118	0	.367	.367	.0031	.311	.311
16–20 years	0	.153	.153	0	.502	.502	.0040	.404	.404
⩾21 years	0	.170	.170	0	.569	.569	.0045	.450	.450

[a] Data are the probabilities of having TS, for each of the three possible genotypes—tt, Tt, and TT (where the T allele identifies the susceptibility allele). Models 1 and 2 are from Gelernter et al. (1990); model 3 was derived to better suit our data from the family that we studied. The disease-gene frequency used for models 1, 2, and 3 was .0004, .003, and .0004, respectively.

search Genetics. The information pertinent to the informativity and the relative map positions of the genotyped microsatellite loci was extracted from either The Genome Database, The Cooperative Human Linkage Center, or the Center for Medical Genetics, Marshfield Medical Research Foundation. Allele frequencies were estimated on the basis of a sample of 168 unrelated subjects that included the 16 spouses in the Charlevoix family.

Although the mode of inheritance for TS is uncertain (Seuchter et al. 2000), model-based (or parametric) linkage analyses (Ott 1991) were performed, since there is growing evidence that, even when the mode of inheritance specified is only approximately correct, this method is more powerful than model-free (or nonparametric) analysis (Durner et al. 1999). Two-point LOD scores were computed by the FASTLINK version (Schäffer 1996) of the LINKAGE programs (Lathrop et al. 1984). When a two-point LOD score was >1.0, a three-point analysis was performed with both the marker involved in the two-point result and the most adjacent marker.

We used the two autosomal dominant inheritance models proposed by Gelernter et al. (1990). Table 2 shows the corresponding age-dependent penetrances. In both models, the penetrance was higher in males than in females, but model 2 assumed an almost completely penetrant gene and some phenocopy for males. Given that, in this family, males and females were equally likely to be affected, we derived a third model (model 3 in table 2), which better suited our data, via a few modifications of model 1: model 3 assumed reduced penetrances and no dependence on gender and allowed for phenocopy. Model 1 was used for the analysis with the narrow phenotype definition, whereas model 2 was used for the analysis with the intermediate and broad phenotype definitions. Model 3 was used with each of the three phenotype definitions. Hence, each marker was analyzed six times. For each marker, the best result was, then, a mod score obtained by maximization of the maximum LOD score (Z_{max}; maximized over the recombination fraction [θ]) for these six combinations. Although this mod-score approach has a greater power to detect linkage than does a single model (Hodge and Elston 1994), the major drawback is an inevitable inflation of the rate of type I error. To correct for such multiple testing, we raised the Z criterion for level of significance (Morton 1955) by .18, following the guidelines of Hodge et al. (1997). Hence, in our study, the hypothesis of no linkage was rejected if a mod score was >3.18. The LOD-score thresholds for significance that have been proposed by Lander and Kruglyak (1995) were not appropriate for the present study, because we did not perform a whole-genome scan.

According to the two-point linkage analysis, two markers yielded LOD scores >2. When model 3 and the narrow phenotype definition were used, $Z_{max} = 2.40$ ($\theta = .10$) was obtained at D11S1377, whereas $Z_{max} = 3.2(\theta = .0)$ was obtained at D13S788. Table 3 shows the two-point Z_{max} values obtained for chromosomes 11 and 13, for each of the six combinations of a phenotype definition (table 1) and a transmission model (table 2). All other markers tested in the present study yielded Z_{max}

Table 3

Two-Point Z_{max} Values for Linkage Analysis with TS in a Charlevoix Pedigree ($N = 127$), for Chromosomes 11 and 13

Marker	Map Position (cM)	Z_{max} (θ) for Narrow Phenotype ($N = 20$)		Intermediate Phenotype ($N = 33$)		Broad Phenotype ($N = 40$)	
		Model 1[a]	Model 3[a]	Model 2[a]	Model 3	Model 2	Model 3
D11S1377	120.9	1.60 (.1)	2.40 (.1)	0 (.5)	1.30 (.15)	.70 (.25)	.90 (.15)
D11S933	124.1	1.01 (.2)	1.27 (.2)	.13 (.4)	.67 (.2)	.43 (.3)	1.15 (.15)
D13S1325	45.6	.30 (.25)	.10 (.3)	0 (.45)	.10 (.1)	0 (.35)	1.30 (0)
D13S788	45.6	2.60 (0)	3.20 (0)	.10 (.45)	1.80 (.05)	0 (.5)	.70 (.1)

[a] Penetrance values characterizing the model are given in table 2.

values ≤ 1.0. Three-point linkage analyses that combined two adjacent markers with the disease locus were performed on chromosomes 11 and 13, for the narrow phenotype definition under model 3. On chromosome 11, the analysis involved markers D11S1377 and D11S933, assuming a distance of 3.2 cM between them. A three-point Z_{max} value of 3.24 was obtained that localized the TS locus 10 cM centromeric to D11S1377. On chromosome 13, the analysis involved markers D13S1325 and D13S788, assuming a θ value of .0. The corresponding three-point Z_{max} was only 0.0.

We assessed the potential impact that a misspecification in marker-allele frequencies would have on our results, by reanalyzing chromosomes 11 and 13 on the basis of two different sets of marker-allele frequencies, fixing the model-phenotype combination to model 3 with the narrow phenotype definition; we used both a set of published marker-allele frequencies (using the different sources of marker information provided above) and a set obtained by assigning the same frequency to all alleles. The three-point analysis involving markers D11S1377 and D11S933 and these two new sets of marker-allele frequencies led to three-point Z_{max} values of 3.35 and 3.38, respectively. With marker D13S788, the two sets yielded two-point Z_{max} values of 3.1 and 3.8, respectively, compared with the 3.2 originally obtained by use of our population-based estimates. Hence, globally, the impact that varying marker-allele frequencies had on our results was to slightly increase the linkage evidence obtained by use of our own population-based estimates.

Figure 1 shows the family with haplotypes constructed on the basis of markers D11S1377 and D11S933. Although haplotype 5-4 was often found among affected subjects, it was not seen systematically in all of them. Therefore, we performed a sensitivity analysis to study the impact that each individual branch of the family had on our results. We found that, when the branch starting with subjects 13 and 57 was ignored by giving an unknown phenotype to the seven implied subjects (encompassed by the larger broken-line box in fig. 1), the three-point Z_{max} on chromosome 11 rose to 4.53. When the branch including subjects 45 and 46 and their two children was ignored, the three-point Z_{max} rose to 3.92. Moreover, when both branches were ignored, the three-point Z_{max} reached 5.94. This sensitivity analysis reflected the absence of haplotype 5-4 in these two branches. The impact of ignoring any other branch was to decrease the LOD score on chromosome 11. The presence of haplotype 5-4 among unaffected subjects was compatible with the mode of inheritance (model 3) that assumed incomplete penetrance. The sensitivity analysis performed on chromosome 13 showed that all branches contributed to the two-point Z_{max} of 3.2 obtained at D13S788, because omitting any one of them decreased the LOD score.

The three-point Z_{max} of 3.24 obtained on chromosome 11 with markers D11S1377 and D11S933 reached our predetermined threshold for significance—that is, 3.18. It is interesting to note that D11S933 is only 7 cM from D11S912, a marker that, in the genome scan reported by The Tourette Syndrome Association International Consortium for Genetics (1999), yielded a multipoint maximum-likelihood score of 1–2. Moreover, D11S1377 is the marker that, in the Afrikaner population studied by Simonic et al. (1998), showed the most significant linkage disequilibrium with TS. Therefore, we concluded that, in this Charlevoix family, a gene for TS may be located on chromosome 11 (11q23). Although the evidence provided by the present study is statistically significant, one must have a replication or an extension study before reaching a final conclusion as to the existence of a TS gene on 11q23, as recommended for complex disorders (Lander and Kruglyak 1995). To accomplish this, we are increasing the sample by studying additional families.

With regard to chromosome 13, results remained ambiguous, because the Z_{max} of 3.2 that initially was found for marker D13S788 in the two-point analysis was not supported by the multipoint analysis involving both D13S788 and D13S1325. However, negative results in multipoint analysis must be interpreted with caution, because such an analysis is sensitive to power loss due to misspecification of intermarker distances (Halpern

and Whittemore 1999). In future work, we will attempt to clarify the situation, by genotyping additional markers in the vicinity of D13S788.

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Electronic-Database Information

The accession number and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics (for microsatellite marker information)

Cooperative Human Linkage Center, The, http://lpg.nci.nih.gov/CHLC/ (for microsatellite marker information)

Genome Database, The, http://www.gdb.org (for microsatellite marker information)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim (for TS [MIM 137580])

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