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Indications of Linkage and Association of Gilles de la Tourette Syndrome in Two Independent Family Samples: 17q25 Is a Putative Susceptibility Region

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Gilles de la Tourette syndrome (GTS) is characterized by multiple motor and phonic tics and high comorbidity rates with other neurobehavioral disorders. It is hypothesized that frontal-subcortical pathways and a complex genetic background are involved in the etiopathogenesis of the disorder. The genetic basis of GTS remains elusive. However, several genomic regions have been implicated. Among them, 17q25 appears to be of special interest, as suggested by various independent investigators. In the present study, we explored the possibility that 17q25 contributes to the genetic component of GTS. The initial scan of chromosome 17 performed on two large pedigrees provided a nonparametric LOD score of 2.41 near D17S928. Fine mapping with 17 additional microsatellite markers increased the peak to 2.61 ($P = .002$). The original families, as well as two additional pedigrees, were genotyped for 25 single-nucleotide polymorphisms (SNPs), with a focus on three genes in the indicated region that could play a role in the development of GTS, on the basis of their function and expression profile. Multiple three-marker haplotypes spanning all three genes studied provided highly significant association results ($P < .001$). An independent sample of 96 small families with one or two children affected with GTS was also studied. Of the 25 SNPs, 3 were associated with GTS at a statistically significant level. The transmission/disequilibrium test for a three-site haplotype moving window again provided multiple positive results. The background linkage disequilibrium (LD) of the region was studied in eight populations of European origin. A complicated pattern was revealed, with the pairwise tests producing unexpectedly high LD values at the telomeric *TBCD* gene. In conclusion, our findings warrant the further investigation of 17q25 as a candidate susceptibility region for GTS.

Introduction

Gilles de la Tourette syndrome (GTS [MIM %137580]) is a neurodevelopmental disorder with onset in childhood. The phenotype includes the presence of multiple motor and phonic tics that occur in bouts and that wax and wane in severity over a period of days, weeks, or months (Leckman 2002). Tics are sudden habitual movements or vocalizations that typically mimic some fragment of normal behavior and involve discrete muscle groups (Leckman and Riddle 2000). The mean age at onset of the disorder is 7 years (range 2–15 years), and, in uncomplicated cases, the severity of tics peaks early in the 2nd decade of life, with symptoms often showing a striking decline in frequency and severity by age 19 years (Leckman et al. 1998). This suggests that the substrate for GTS is not neurodegeneration; rather, the disorder may be due to features of the developing brain that are present to a lesser degree in the mature nervous system.

Once thought to be as rare as 1–10/10,000, GTS is now considered much more common, with estimated prevalence in the range of 1%–3.8% (Singer 2000; Robertson 2003). This variation among studies can probably be attributed to selection of the target population and ascertainment bias. Tics have the greatest effect on a patient's self-esteem and peer and family relationships during ages 7–12 years. The high comorbidity of GTS with other behavioral disorders detracts even more from the patient's quality of life (Spencer et al. 1998; Carter et al. 2000; Elstner et al. 2001; Peterson et al. 2001). Indeed, the behavioral spectrum of GTS and related tic disorders includes obsessive-compulsive (OC) symptoms or even formal obsessive-compulsive disorder (OCD

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Table 1

Frequencies of Rarer Alleles

Known Gene and SNP	Physical Position (bp)	Allele Frequency in[a]							
		Adygei (UID SA000017I; 2*N* = 108)	Chuvash (UID SA000491O; 2*N* = 84)	Russians (UID SA000019K; 2*N* = 96)	Ashkenazim (UID SA000490N; 2*N* = 166)	Finns (UID SA000018J; 2*N* = 72)	Danes (UID SA000007H; 2*N* = 102)	Irish (UID SA000057M; 2*N* = 226)	European Americans (UID SA000020C; 2*N* = 184)
EVER2[b]:									
1.C_11489838_10[c]	76,572,635	.396	.500	***.573***	.380	.410	.314	.405	***.511***
2.C_3068804_10[c]	76,595,312	.031	.025	NA	.020	.051	.015	.062	.021
3.C_11488062_10[c]	76,779,757	***.611***	***.586***	.451	.412	.382	.343	.424	.467
NPTX1:									
4.C_152603_10[c]	78,956,093	.422	.488	.478	.411	.353	.490	.395	.375
5.C_465993_10[c]	78,978,702	***.510***	.463	.380	.367	.279	.469	.335	.338
6.C_9176659_10[c]	79,009,826	.147	.232	.239	.179	.176	.160	.198	.213
7.C_106441_10[c]	79,091,403	.250	.317	.283	.272	.309	.280	.293	.283
IRSP53:									
8.C_216379_10[c]	79,583,267	.192	.220	.250	.281	.106	.200	.239	.276
9.C_150018_10[c]	79,589,081	.231	.341	.375	.453	.348	.382	.365	.365
10.C_7985808_10[c]	79,598,546	.173	.171	.156	.270	.088	.216	.159	.192
11.C_213917_10[c]	79,622,389	.275	.329	.326	.187	.324	.235	.243	.227
12.C_179850_10[c]	79,641,502	.327	.263	.344	.393	.265	.324	.252	.318
13.C_209341_10[c]	79,652,019	.216	.134	.177	.257	.162	.250	.110	.230
14.C_399266_10[c]	79,664,539	.067	.063	.052	.101	.030	.088	.041	.076
SECTM1:									
15.C_11600340_10[c]	80,819,047	.481	***.519***	***.571***	***.569***	.450	.386	.495	.490
TBCD:									
16. rs1056534[d]	81,238,364	.442	.341	.419	.462	.371	.373	.364	.397
17. rs635996[d]	81,284,335	.450	.313	.430	.468	.424	.430	.399	.410
18. rs662669[d]	81,305,806	.365	***.563***	.432	.354	.500	.461	.482	.457
19. C_1674439_10[d]	81,339,492	.410	.287	.337	***.533***	.353	.353	.349	.345
20. rs733342[d]	81,351,843	.202	.171	.239	.348	.286	.265	.234	.224
21. rs3744161[d]	81,357,820	.423	.305	.375	***.538***	.357	.410	.361	.393
22. C_1674406_10[c]	81,373,505	.202	.171	.227	.348	.271	.270	.231	.238
23. C_1674398_10[c]	81,380,863	.390	.333	.386	***.538***	.357	.412	.360	.400
24. rs3214032[d]	81,417,007	.202	.154	.239	.329	.271	.265	.216	.212
25. C_1674327_10[c]	81,437,491	.442	.488	***.512***	***.558***	***.586***	***.598***	.472	***.506***

Note.—Frequencies of alleles that are actually more frequent in that particular population are shown in bold italics.

[a] 2*N* = no. of studied chromosomes.

[b] *EVER2* = epidermodysplasia verruciformis gene 2.

[c] Applied Biosystems assay SNP ID.

[d] dbSNP ID.

Table 2

STRPs Used for Linkage Analysis in Large Families

Marker	Physical Position[a] (bp)	Genetic Position[b] (in cM), According to		
		DeCode	Généthon	Marshfield
D17S849[c]	386,285	.63	.6	.63
D17S938[c]	6,194,052	17.06	14.8	14.69
D17S945[c]	9,766,279	27.99	22	21.01
D17S799[c]	13,114,012	37	32.8	31.96
D17S925[c]	27,158,736	52.17	49.5	49.67
D17S798[c]	31,138,469	55.6	53.9	53.41
D17S933	33,045,783	62.09	58.3	57.71
D17S1814	38,029,065	68.61	62.2	61.48
D17S1299	38,903,071	NA	NA	62.01
D17S791[c]	45,098,507	NA	65	64.16
D17S1795	48,267,058	75.53	69.4	68.44
D17S787[c]	53,523,963	NA	75.7	74.99
D17S1799	53,782,650	81.61	75.7	74.99
D17S957	55,815,576	87.36	81.7	80.38
D17S808[c]	61,012,911	91.86	84.2	82.56
D17S1816	64,838,112	95.49	87.6	85.94
D17S949[c]	68,929,847	102.96	94.9	93.27
D17S1826	70,999,722	106.23	97.2	95.99
D17S1352	72,462,356	110.6	99.3	98.14
D17S1807	72,824,696	113.1	100.4	99.21
D17S785	74,895,412	115.34	104.7	103.53
D17S802[c]	76,695,601	120.84	108.2	106.8
D17S1847	77,485,779	123.75	112.3	111.22
D17S836	77,760,910	125.04	114	112.92
D17S1806	77,906,603	NA	115.4	114.41
D17S1822	78,344,934	129.24	117.7	116.86
D17S1830	78,360,040	NA	117.7	116.86
D17S784[c]	78,366,192	129.62	117.7	116.86
D17S928[c]	80,782,601	135.67	128.7	126.46
17qter STR	81,447,000	NA	NA	NA

[a] Physical position refers to the beginning of each STRP. Positions are based on National Center for Biotechnology Information build 33. Both physical and genetic positions for the STRPs used can be found at the University of California–Santa Cruz Genome Bioinformatics Web site, except for 17qter STR (the position of which is based on local contig assembly).

[b] Genetic positions are given relative to pter. NA = not applicable.

[c] ABI PRISM linkage mapping set.

[MIM %164230]), other anxiety disorders, mood disorders, and attention-deficit and disruptive behavior disorders (Kurlan et al. 2002). It seems likely that these disorders share a common or overlapping neurobiological basis.

The neuroanatomic localization of GTS is unknown, but it is currently thought that the pathophysiology of the disorder involves the cortico-striatal-thalamo-cortical circuits (Singer and Minzer 2003). A popular model of basal ganglia functional anatomy suggests that involuntary movements are associated with decreased inhibitory output from the basal ganglia resulting in excessive activity in fronto-cortical areas (Mink 2001). This model has been invoked to explain several "hyperkinetic" movement disorders (tics, chorea, and dystonia), as well as many psychiatric disorders (schizophrenia, OCD, and depression). It has been hypothesized that the same mechanisms that are involved in habit formation are also involved in tics (Leckman and Riddle 2000).

Several lines of evidence suggest that GTS is an inherited disorder. In twin studies, the concordance rate was 53%–56% in MZ twins versus 8% in DZ twins, indicating a genetic basis of the disorder (Price et al. 1985; Hyde et al. 1992). At the same time, the fact that the concordance rate between MZ twins is not 100% demonstrates the importance of environmental or other nongenetic factors in the pathogenesis of the disorder. First-degree relatives of individuals with GTS have a 10- to 100-fold increased risk of developing the disorder, compared with individuals in the general population (Pauls et al. 1991). Early segregation-analysis studies reported either a pattern consistent with autosomal dominant inheritance (Pauls and Leckman 1986; Eapen et al. 1993) or a model in which the penetrance of heterozygous individuals was intermediate between those of the homozygotes (Hasstedt et al. 1995). More recent studies, however, were interpreted as showing evidence for a significant multifactorial (polygenic) background (Walkup et al. 1996). On the other hand, Seuchter et al. (2000) could not support Mendelian transmission of GTS and related conditions. Nevertheless, the majority of studies suggest that the etiology of GTS has a strong and, most likely, complex genetic component.

Despite the numerous genetic studies undertaken by various groups (reviewed by Pauls [2003] and Singer [2000]) targeting a large number of both large pedigrees and small nuclear families, the genetic basis of GTS has so far remained elusive, accentuating the likelihood of the heterogeneity of the disorder. To date, neuroleptics are the main treatment for GTS, suggesting that a dysfunction in dopaminergic pathways might be implicated in the development of the disorder. Consequently, several genes involved in these pathways have been studied, but their role in the etiopathogenesis of GTS still remains unclear. A positive-association result between the dopamine receptor gene *DRD4* (MIM 126452) and GTS has been reported (Grice et al. 1996; Cruz et al. 1997; Díaz-Anzaldúa et al. 2004), but other studies have failed to replicate this result (Brett et al. 1995; Hebebrand et al. 1997; Comings et al. 1999). Positive association was also found between the monoamine oxidase A gene (*MAOA* [MIM 309850]) and GTS (Gade et al. 1998; Díaz-Anzaldúa et al. 2004). Chromosomal abnormalities in individuals and families with GTS have also been studied in the hope of identifying a gene or genes of major effect that would be disrupted by the rearrangement (Brett et al. 1996; Kroisel et al. 2001; Petek et al. 2001; Crawford et al. 2003; and State et

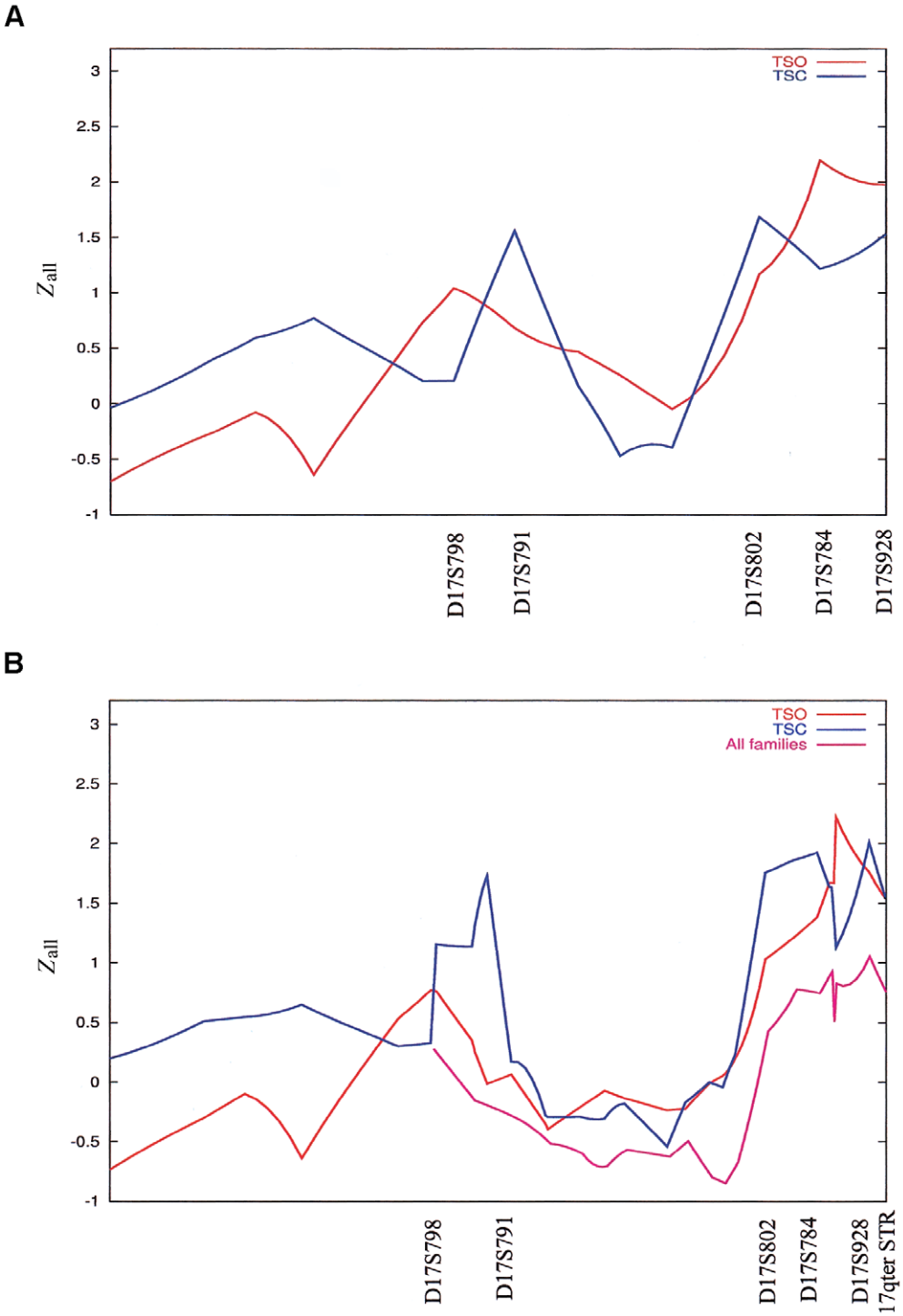

Figure 1 Multipoint linkage analysis (GENEHUNTER) of large pedigrees (Yale sample). *A*, Analysis of TSO and TSC with initial STRP panel. *B*, Analysis by use of a fine-mapping STRP panel.

al. 2003, among others). Using this approach, Verkerk et al. (2003) hypothesized that disruption of the contactin-associated protein 2 gene (*CNTNAP2* [MIM 604569]) could lead to the GTS phenotype. Simonic et al. (1998), in a genomewide search using a case-control strategy, reported positive associations, with markers in seven regions and, in a subsequent study, provided additional evidence for loci on chromosomes 2, 8, and 11 (Simonic et al. 2001). It is interesting that linkage was found with the same marker on chromosome 11 re-

Table 3

Nonparametric Single-Point Linkage Analysis (GENEHUNTER)

Pedigree and Marker	NPL Score	*P*
TSO:		
D17S784	1.97	.007
D17S928	1.54	.017
TSC:		
D17S802	1.70	.041
D17S928	1.82	.033
TSO and TSC:		
D17S802	1.67	.026
D17S784	2.19	.007
D17S928	2.39	.004

ported by Simonic et al. in a large French Canadian pedigree that was analyzed by use of a multipoint approach (Mérette et al. 2000).

Whole-genome scans performed either on large families or on families with two affected sibs have provided indications for linkage of GTS with several genomic regions. The results between studies are, however, quite inconsistent. This is believed to be due to uncertainties in the definition of the phenotype, diagnostic assessment, and family ascertainment schemes, as well as a misspecified genetic model used for the data analysis. A partial genome scan in 1991 excluded 50% of the genome, under the assumption of an autosomal dominant gene in all of the families studied (Pakstis et al. 1991). Barr et al. (1999) reported genomewide significant linkage with eight markers, using the affected-pedigree method, a nonparametric approach. In the same study, two of those markers on chromosomes 5 and 19 also gave weak indications of linkage by use of the parametric LOD score. A genome screen of 110 affected sib pairs performed by the Tourette Syndrome Association International Consortium for Genetics (TSAICG [1999]) provided suggestive positive-linkage results with markers on chromosomes 4 and 8. When affected individuals in this study were stratified according to OC symptoms, significant allele sharing was noted for hoarding phenotypes for markers at 4q34-35, 5q35, and 17q25.4 (Zhang et al. 2002).

Among the regions providing indications for linkage to GTS so far, we considered 17q25 to be of particular interest, as suggested by various independent studies and investigators. A genomewide linkage study performed on a large pedigree from Utah gave the highest LOD score (2.2) at marker D17S802 (106 cM from 17pter) (Leppert et al. 1996). The finding of evidence for linkage to marker D17S784 was supported both by the TSAICG (1999) and by Zhang et al. (2002). The TSAICG found a weak peak, with a maximum-likelihood score (MLS) of 0.6, at this marker. However, as mentioned above, Zhang et al. (2002) found a high nonparametric LOD (NPL) score at marker D17S784 ($P < .00002$) in a subset of affected sib pairs positive for the OC symptom of hoarding. These results indicated that 17q25 deserved further evaluation as a possible GTS-susceptibility locus.

Here, we report results of an initial screen of chromosome 17 and the follow-up fine mapping of the candidate region. Furthermore, the linkage disequilibrium (LD) pattern of the region was studied in samples from eight populations of European ancestry to facilitate the interpretation of our association results and to provide background information for subsequent studies. Linkage analysis was performed initially on two large families with multiple members affected with GTS. Next, a map of increased density of STRPs was typed on a total of four large families, including the two original families, as well as two additional pedigrees. For one of the additional families, additional individuals were added to extend the pedigree structure. To further reduce the large candidate interval, we typed 25 SNPs, focusing on three loci that, according to their expression patterns and function, could constitute putative susceptibility genes for GTS—neuronal pentraxin 1 (*NPTX1* [MIM 602367]), insulin receptor substrate p53 (*IRSP53* [MIM 605475]), and tubulin specific chaperone D (*TBCD* [MIM 604649]). Tests of both single-marker and haplotype association were undertaken, and multiple positive results were obtained. The same SNPs were then typed in an independent sample of small nuclear families participating in the study at a second site (Toronto), and the initial findings were replicated to some extent.

Samples and Methods

Samples

The study was approved by the appropriate institutional review boards and ethics committees at both sites (Yale and Toronto), and informed consent was obtained from the participating individuals.

Yale family sample.—At Yale, linkage and association analysis was performed on four large, multigenerational families with multiple members affected with GTS. The families investigated originated from Canada, Kansas (TSK), Michigan (TSM), and Oregon (TSO). The largest branch of the Canadian family has been analyzed separately and is designated hereafter as "TSC." The pedigrees are extended to four generations and consist of 462 individuals, with 105 individuals affected with GTS (68 male and 37 female). None of the married-in spouses presented with any GTS symptoms, according to our data. These kindreds have been described in detail elsewhere (Kurlan et al. 1986; Pauls et al. 1990; Pakstis et al. 1991). In all families, each individual was assessed in a direct interview by use of a structured questionnaire (Pauls and Hurst 1987). Diagnoses were based on criteria from the revised third edition of the *Diagnostic and*

Table 4

Haplotype Analysis of TSO and TSC

	Allele at Marker										% (no./total) of Chromosomes	
Pedigree	D17S785	D17S802	D17S1847	D17S836	D17S1806	D17S8122	D17S1830	D17S784	D17S928	17qter STR	Affected Individuals	Unaffected Individuals
TSO:												
	6	7	1	2	2	2	7	Allele 8 or 1	11	12	26.67 (8/30)	2.86 (2/70)
	6	7	3	2	11	1	5	3	2	1	16.67 (5/30)	2.86 (2/70)
TSC:												
			1	1	1	1	2	2	1	1	13.24 (9/68)	1.61 (1/62)
			1	2	2	3	1	3	Allele 2 or 3	6	19.12 (13/68)	6.45 (4/62)

Table 5

TDT for Single Markers (GAS)

Sample and Marker	Known Gene	Allele	No. of Alleles		*P*
			Transmitted	Not Transmitted	
Yale families:[a]					
C_11488062_10[b]	...	G	27	9	.002
rs3744161[c]	*TBCD*	G	29	17	.052
Canadian families:[d]					
C_11488062_10[b]	...	G	18	8	.038
rs662669[c]	*TBCD*	T	23	12	.045
rs3744161[c]	*TBCD*	G	23	11	.029
Toronto families:					
C_11600340_10[b]	*SECTM1*	G	76	49	.0098
rs1056534[c]	*TBCD*	C	65	43	.021
rs662669[c]	*TBCD*	T	65	44	.027

Note.—Transmission of all 25 SNPs genotyped was tested. Only statistically significant results are presented here.

[a] Analysis performed on the entire sample of large families available at the Yale site.

[b] Applied Biosystems assay SNP ID.

[c] dbSNP ID.

[d] Analysis performed on the large Canadian families only.

Statistical Manual (DSM-IIIR) (American Psychiatric Association 1987) and were refined, as suggested elsewhere (Kurlan 1989), to indicate the quality of the information. This gives subjects a possible, probable, or definite diagnosis of GTS or chronic multiple tics. We decided to take a more conservative approach and considered as affected in our analysis only those individuals who presented with definite or probable GTS, according to the diagnostic scheme. DNA was extracted, using standard procedures, from permanent lymphoblastoid cell lines that were established for all the families at the Yale site.

Toronto family sample.—In Toronto, 330 individuals from 96 small, nuclear families were available for analysis. DNA was extracted from whole blood according to standard procedures. The sample consisted of 41 families with one affected child and 55 families with two affected children. Of the 96 families studied, 90 are of European origin. Two families of southwestern Asian origin and one family of East Asian origin were also included in the sample. In addition, in two families, the father is of European origin, and the mother is East Asian; in one family, the mother is European and the father is African American. The diagnostic assessment of these families has been described elsewhere (TSAICG 1999). In brief, information about symptoms associated with GTS was collected, using a self- and family report, on the basis of the Yale Global Tic Severity Scale (Leckman et al. 1989). The information was checked by an experienced neuropsychiatrist or neurologist, who also performed a direct examination of each proband.

Yale population samples.—Background LD in the genomic region studied was estimated in unrelated samples from seven populations originating from Europe (Adygei, Chuvash, Russians, Ashkenazi Jews, Finns, Danes, and Irish). A more diverse sample of European Americans was also studied. The mean sample size was 65 individuals. The vast majority of families used for linkage and association studies here (all of the Yale families and 90 of 96 Toronto families) are of European descent, and their ethnic origin cannot be easily defined any further. The populations used to obtain background information are representative of European ancestry, especially given the fact that genomic variation among European populations is quite homogeneous (Kidd et al., in press). Descriptive information and literature citations for these population samples can be found in the Allele Frequency Database (ALFRED) under the unique identification numbers (UIDs) shown in table 1. DNA was extracted from lymphoblastoid cell lines available at the Yale site.

Genotyping

STRPs.—For the initial screen, the TSO and the TSC pedigrees were typed for 13 markers on chromosome 17 (ABI PRISM linkage mapping set, panels 24 and 25). The average intermarker distance was 10 cM. All of the large pedigrees at the Yale site were then typed for 17 additional STRPs, increasing the density of the map between marker D17S798 and 17qter (table 2). All STRPs were typed by use of fluorescently labeled primers for PCR amplification, and electrophoresis of the denatured products was performed on an acrylamide gel by use of the ABI 377 instrument. The fine-mapping markers were organized into two sets, so as not to overlap, according to their fluorescent label, and allele size and the PCR products of each set were pooled before electrophoresis.

Table 6

Results of TDT by Use of 3-Site–Haplotype Sliding Window in the Yale Families

SNPs[a] and Haplotype	Transmissions		Var(Observed − Expected)	χ^2 (P)	Global χ^2 (P)[b]
	Observed	Expected			
2-3-4:					
A-G-G	34.805	41.065	8.16	4.79 (.021[c])	6.68[d] (.271[c])
3-4-5:					
G-A-G	34.275	28.078	5.76	6.65 (<.001)	NC
G-A-A	16.313	21.648	3.73	7.62 (.01)	
4-5-6:					
G-A-G	12.771	9	1.93	7.31 (<.001)	18.07[e] (.022)
G-G-A	21.98	27.37	8.78	3.31 (.031)	
5-6-7:					
G-G-C	38.10	31.88	8.22	4.7 (.041)	17.08[e] (.019)
A-G-T	4.44	3.18	.89	1.79 (.02)	
6-7-8:					
A-C-G	44.05	52.8	9.75	7.86 (.001)	16.03[e] (.01)
G-C-T	43.33	35.96	10	5.40 (.005)	
7-8-9:					
C-G-A	49.83	55.85	10.281	3.519 (.043)	8.08[e] (.122)
8-9-10:					
T-A-G	8.53	5.58	2	4.28 (<.001)	36.4[f] (.041[c])
T-G-G	11.014	16.18	2.79	9.54 (.001)	
9-10-11:					
G-G-C	17.26	25.75	7.07	10.186 (<.001)	33.109[g] (.001)
11-12-13:					
C-A-A	63.915	59.26	5	4.31 (.032)	13.5[f] (.081)
T-A-A	7.71	5.63	1.65	2.62 (.002)	
12-13-14:					
A-A-C	71.414	65.465	5.24	6.74 (.016)	37.3[f] (.023)
13-14-15:					
G-C-A	29.92	40.624	14	8.14 (.019)	8.62[d] (.341[c])
17-18-19:					
T-T-G	14.811	21.017	6.36	6.05 (<.001)	20.82[e] (.007)
C-T-G	9.13	14	3.37	7.04 (.029)	
18-19-20:					
T-G-G	15.693	24.356	6.66	11.268 (<.001)	21.72[g] (.009)
T-T-A	12.823	9.91	2.79	3.025 (.002)	
19-20-21:					
T-A-G	13.83	10.464	2.66	4.25 (<.001)	NC
20-21-22:					
A-G-G	16.792	12.966	3.1	4.72 (<.001)	NC
21-22-23:					
G-G-T	24.2	19.047	4.52	5.86 (<.001[c])	NC
23-24-25:					
C-T-C	4.86	3.68	1.16	1.20 (.005)	12.669[e] (.165)

Note.—Results shown for joint analysis of all pedigrees.

[a] SNP numbers correspond to SNP order shown in table 1.

[b] NC = value could not be calculated.

[c] Used −c5 flag.

[d] 4 df.

[e] 7 df.

[f] 5 df.

[g] 6 df.

The primers used for PCR amplification can be found at the GenLink site for all markers, except for the 17qter STR. This is an STRP identified in our lab; the primers and the amplification conditions can be found on the ALFRED Web site. Size assignment and allele calling were performed using GeneScan and Genotyper software.

SNPs.—Twenty-five SNPs were typed in all families available for this study. The SNPs were chosen from the Applied Biosystems "assays on demand" catalogue and were typed as 5′ TaqMan assays (Livak 1999). Most of the SNPs are intronic, with no apparent functional significance. SNPs rs3214032 and rs1056534 are exonic

Table 7

Results of TDT by Use of 3-Site–Haplotype Sliding Window in the Toronto Families

SNPs[a] and Haplotype	Transmissions		Var(Observed − Expected)	χ^2	P	Global χ^2 (P)
	Observed	Expected				
2-3-4:						
G-G-G	2.37	5.95	2.16	5.91	.017[b]	17.294[c] (.027[b])
3-4-5:						
G-A-G	25.80	20.14	6.45	4.97	<.001	9.54[c] (.076)
4-5-6:						
G-G-G	13.04	17.27	5.58	3.19	.02	6.25[c] (.308)
7-8-9:						
C-T-A	14.90	11.44	3.10	3.85	.032	6.56[c] (.447)
11-12-13:						
T-C-G	.68	4.03	1.52	7.36	<.001	8.26[d] (.035)
12-13-14:						
C-G-C	.88	5.17	2.06	8.93	<.001	11.173[e] (.007)
C-A-T	1.06	2.28	.96	1.53	<.001	
14-15-16:						
C-G-C	95.10	81.59	26.52	6.88	.017	10.762[c] (.064)
15-16-17:						
G-C-T	101.10	85.40	27.16	8.97	.004[f]	10.557[d,f] (.037[f])
18-19-20:						
C-G-A	35.04	44.71	14.82	6.31	.002	7.98[d] (.012)

[a] SNP numbers correspond to SNP order shown in table 1.
[b] Used −agg3 flag.
[c] 7 df.
[d] 4 df.
[e] 5 df.
[f] Used −c5 flag.

but code synonymous changes. The total region screened spans 4.8 Mb, with greater marker density in three regions of 135 kb (*NPTX1* locus), 80 kb (*IRSP53* locus), and 199 kb (*TBCD* locus) (table 1). We studied the whole of the *IRSP53* and *TBCD* genes, with markers at an average spacing of ~10 kb and ~21 kb, respectively. In the *NPTX1* locus, we studied three SNPs at the 3′ end as well as one SNP in intron 2–3 of the gene. The SNPs were chosen on the basis of their heterozygosity and also their chromosomal position. An attempt was made to investigate in more detail the region that produced high NPL scores but had low STRP coverage. At the same time, the genes studied could constitute candidate genes for GTS. In this article, for reasons of simplicity, we refer to each of the SNPs by its order number, as shown in table 1.

Statistical Analysis

The STRP genotyping data were used to perform both single- and multipoint nonparametric linkage analysis, as implemented by GENEHUNTER (Kruglyak et al. 1996). STRP allele frequencies were calculated in the unrelated individuals in the sample by use of locally written software. Haplotypes shared identical by descent among affected individuals were examined in detail in the families yielding high NPL scores (TSC and TSO). Haplotypes were constructed using GENEHUNTER and were then compared among affected and unaffected individuals in each pedigree.

The transmission/disequilibrium test (TDT) for individual markers (Spielman et al. 1993) was performed using the software package GAS. The program TRANSMIT (Clayton 1999) was used for the inference of haplotypes in the family samples and for the implementation of a haplotype-based TDT. The bootstrap option of the program was used, and, for each haplotype, the test was replicated 1,000 times, thus deriving exact P values. For some of the intervals, in which rare haplotypes or alleles existed, the −c5 or −agg3 flags were used to disregard rare haplotypes (frequency <0.05) or aggregate rare alleles (frequency <0.03), respectively, before haplotype construction.

Allele frequencies of individual SNP sites were calculated by gene counting. The assumption of Hardy-Weinberg ratios was tested by means of an auxiliary program, FENGEN (Kidd et al. 1998). The multisite haplotype frequencies were calculated with HAPLO (Hawley and Kidd 1995), which implements the expectation-maximization algorithm. By use of the haplotype frequency estimates, pairwise LD coefficients were computed as Δ^2 (Pritchard and Przeworski 2001). The HAPLO/P program (Zhao et al. 1999) was used to perform permutation-based calculations of the pairwise LD values and to provide a test of statistical significance.

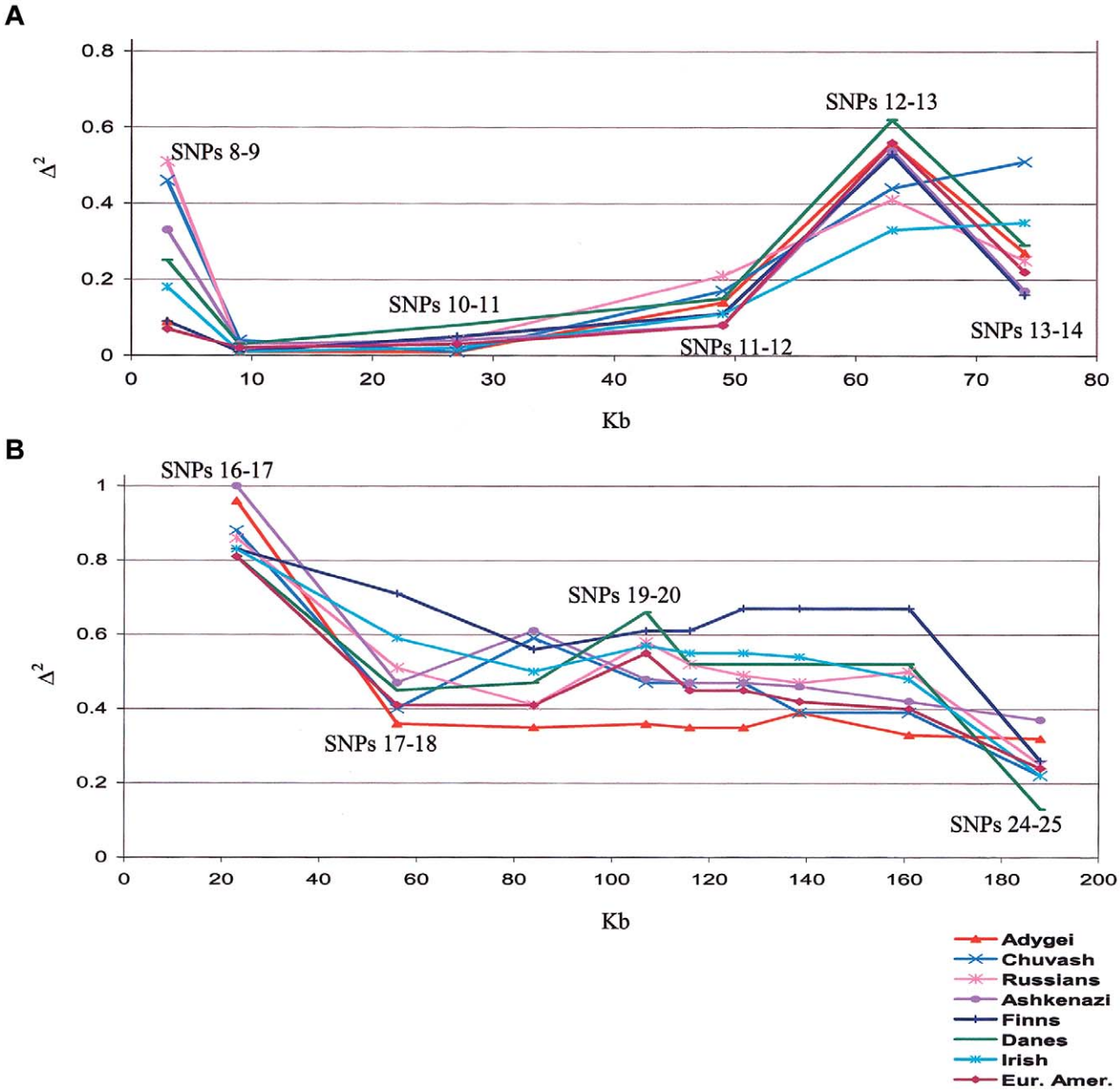

Figure 2 Pairwise LD tests at *IRSP53* and *TBCD* regions (*X*-axis corresponds to distance in kb; LD values are shown at the midpoint of each interval). *A,* Tests at *IRSP53* are significant for all the populations at $P < .006$ for the intervals between SNPs 11 and 12, 12 and 13, and 13 and 14. At the interval between SNPs 8 and 9, the test is significant at $P < .002$ for Chuvash, Russians, Danes, Irish, and European Americans. *B,* Analysis of the *TBCD* region. Each point corresponds to adjacent SNP intervals (SNPs 16–17, 17–18, 18–19, 19–20, 20–21, 21–22, 22–23, 23–24, and 24–25). All Δ^2 values are associated with $P < .001$, except for the test for the final interval (SNPs 24–25) for the Chuvash ($P = .014$).

Results

Linkage and Association Studies

The results of the initial scan of chromosome 17, by use of only the ABI panel STRPs and a multipoint linkage analysis approach on each pedigree, are shown in figure 1*A*. Analysis of the TSO genotyping data gave a peak between marker D17S784 and 17qter ($Z_{all} = 2.19$) and a smaller peak at marker D17S798 ($Z_{all} = 1.03$). For TSC, three peaks were detected, at markers D17S791 ($Z_{all} = 1.31$), D17S802 ($Z_{all} = 1.67$), and D17S928 ($Z_{all} = 1.51$). With both pedigrees analyzed together, the multipoint NPL score reached 1.61 at

marker D17S791, whereas, at 17q25, a broad peak between markers D17S802 and D17S928 was produced (highest $Z_{all} = 2.46$ at marker D17S928; $P = .0037$). Results of the single-point analysis are shown in table 3. For TSO, the highest NPL score was produced at marker D17S784 with an associated *P* value of .007, whereas TSC gave the highest NPL score at marker D17S928 ($P = .033$). Single-point nonparametric linkage analysis of the genotyping data of both pedigrees produced interesting NPL scores at markers D17S784 ($P = .007$) and D17S928 ($P = .004$).

We decided to pursue these findings further and to increase the density of markers between marker D17S798 and 17qter. To this end, we typed 17 additional STRPs in both of these pedigrees as well as in additional individuals from TSC, TSK, and TSM. For TSO, the peak sharpened and shifted to marker D17S928, reaching a *P* value of .003, whereas the peak at marker D17S798 was reduced (fig. 1*B*). For TSC, the peak at marker D17S791 became more distinct, whereas, at 17q25, there was a broad peak between markers D17S802 and D17S1822 and a sharper peak at marker D17S928 (fig. 1*B*). With both pedigrees analyzed together, the peak NPL score at marker D17S928 reached 2.61, with a *P* value of .002. Multipoint analysis of the TSK pedigree produced an NPL score that peaked close to marker D17S802 ($Z_{all} = 1.35$; $P = .07$), whereas TSM analyzed alone did not provide positive results in this region (data not shown). When all of the families were analyzed together, the NPL score only reached 1 ($P = .09$) close to marker D17S928 (fig. 1*B*).

We examined in detail the haplotypes that were shared identical by descent in the two pedigrees (TSC and TSO) and that yielded the highest NPL scores. The pattern of haplotypes inherited by the affected individuals is quite complex, with no single haplotype always segregating with the affection status in either of the families analyzed. However, excessive haplotype sharing among affected individuals could be seen (table 4). In TSO, two haplotypes account for 43.3% of the chromosomes in the affected individuals and only 5.7% of the chromosomes in the unaffected individuals. In TSC, two different haplotypes in the same region are inherited identical by descent in 32.35% of the chromosomes in individuals with GTS and are found in only 8% of the chromosomes of the unaffected individuals.

We continued our fine-mapping efforts, focusing on 17q25 and, particularly, the regions producing the highest NPL scores. We typed 3 SNPs around marker D17S802 and 22 SNPs between markers D17S784 and 17qter STR. Initially following a map-based approach, we chose to type SNPs in regions that gave strong indications of linkage but had low STRP coverage in our analysis. At the same time, 22 of the SNPs studied span three genes (*NPTX1, IRSP53,* and *TBCD*) that, according to their expression patterns and functions, could constitute susceptibility candidates for GTS (see the "Discussion" section). Finally, we decided to study *TBCD,* the last known gene on chromosome 17, as an anchoring point for our analysis.

The TDT revealed overtransmission of alleles and positive association with one SNP close to marker D17S802 (C_11488062_10) and two in the *TBCD* region (rs662669 and rs3744161) (table 5). To make the sample more informative, we continued our analysis, performing an association test with a three-site–haplotype moving window. Two-site–haplotype tests were also performed, but they did not add any information and pointed toward the same regions (data not shown). Multiple small haplotypes, spanning most of the region studied, were found to be overtransmitted or undertransmitted to affected offspring (table 6).

This intriguing result required replication in an independent sample. Our collaborators at the Toronto Western Hospital agreed to type their sample of small nuclear families with one or two members affected with GTS for the 25 SNPs that we had typed in the large pedigrees. The TDT revealed positive association, with three of the markers (C_11600340_10, rs1056534, and rs662669) having an allele overtransmitted to the affected offspring (table 5). Markers rs1056534 and rs662669 are situated at the beginning of the *TBCD* gene, whereas marker C_11600340_10 resides 400 kb upstream of *TBCD* at an intronic region of secreted and transmembrane protein 1 (SECTM1 [MIM 602602]). The same allele of rs662669 that was overtransmitted in the Yale sample was also transmitted in excess in the Toronto sample. The three-site–haplotype TDT produced positive findings in six small haplotype regions (table 7).

LD Patterns

We collected background information about the SNPs included in this study, to allow better interpretation of our association results and to provide a framework for subsequent studies. Since all the families analyzed at Yale and most of the families analyzed in Toronto are of European descent, we calculated the SNP frequencies and the pairwise LD values in unrelated samples from eight populations of European origin. The pairwise LD tests were performed between SNPs at the three loci (*NPTX1, IRSP53,* and *TBCD*) that were studied at a somewhat higher density.

The frequencies of all SNPs that were genotyped are shown in table 1. The table shows only the results of pairwise LD tests between consecutive SNPs. At *NPTX1,* we studied four SNPs spanning 135 kb at an average spacing of 45 kb. Moderately significant LD was found only between SNPs 4 and 5 (C_152603_10 and C_465993_10), with an associated *P* value of <.006 for

all the populations studied. At *IRSP53,* seven SNPs were studied spanning 80 kb at average intervals of 13 kb (fig. 2*A*). The highest LD was observed between SNPs 8 and 9 (C_216379_10–C_150018_10), SNPs 11 and 12 (C_213917_10–C_179850_10), and SNPs 12 and 13 (C_179850_10–C_209341_10). The LD test results are most interesting at the telomeric *TBCD* region (fig. 2*B*). One would expect little or no LD between markers, because of generally high recombination rates near telomeres. However, in a region spanning 199 kb with 10 SNPs at an average spacing of 22 kb, there is high LD between all the marker pairs studied. If selection has not operated, this finding indicates low recombination rates across this telomeric region. It should also be noted that, in all three regions, LD does not break down with distance in a simple manner (data not shown). The populations studied are historically close and genetically homogeneous, when compared with other regions of the world (Tishkoff et al. 1998; Kidd et al. 2000, in press). In the regions analyzed here, some variation among populations is seen, but it does not appear significant.

Discussion

We have presented several lines of evidence implicating 17q25 in the etiopathogenesis of GTS. Our study was initiated as a fine-mapping effort following up on previous indicative findings. Nonparametric linkage analysis in the multigenerational pedigrees included in this study provided indications of linkage with a region between markers D17S802 and D17S784 as well as between marker D17S928 and 17qter STR. A previous linkage analysis study, performed on a large pedigree from Utah, yielded a parametric LOD score of 2.2 at marker D17S802 (Leppert et al. 1996). On the other hand, marker D17S784 produced one of the highest NPL scores in the genome screen performed by Zhang et al. (2002) on families with two sib pairs affected with GTS who were concordant for the OC phenotype of hoarding. In view of these previous results, our findings on independent samples become more significant.

In addition to the positive linkage suggested in the large pedigrees studied, we obtained multiple positive association results after analyzing the genotyping data from the 25 SNPs that we typed in our region of interest. TDT results with single markers point in the direction of the *TBCD* gene as well as to the region close to marker D17S802. A TDT that we performed using a three-site–haplotype sliding window seems also to be implicating the other two genes investigated (*NPTX1* and *IRSP53*).

An independent sample of 96 small nuclear families with one or two children affected with GTS and their parents, was genotyped for the SNPs included in our initial study. The results make our case stronger by replicating a finding of positive association with two markers in the *TBCD* region as well as with over- or undertransmitted haplotypes in the entire region studied. The fact that the large-family sample produces many more positive results with the haplotype approach than do the Toronto small families can be explained by the nature of the sample. It is expected that fewer genetically independent haplotypes will exist in a sample of large families, thus reinforcing the positive results. It has also not escaped our attention that in each of the two family samples studied, different haplotypes are associated with GTS. This may be due to the LD pattern of the region. In genomic regions of low LD, the recombination rates are simply too high, increasing the number of observed haplotypes. Thus, mutations involved in the etiology of GTS may have occurred on multiple haplotype backgrounds. On the other hand, for intervals demonstrating strong LD (as is the case, e.g., in the *TBCD* region studied here), the existence of multiple haplotypes that are positively associated with the disorder implies the presence of more than one mutation allele. We have to note that, at this point, this is only speculation.

The two families that were used for the initial scan of chromosome 17 (TSO and TSC) have also been used in a whole-genome scan that did not produce any positive findings in the region investigated here (Barr et al. 1999). In that study, the diagnostic scheme used was much broader, including subjects who presented with chronic motor tics in the analysis of affected individuals. Furthermore, different statistics were estimated (the parametric LOD score and the nonparametric affected-pedigree method statistic).

We believe that, in the current study, the fact that not all large families show linkage to the markers suggested in the two largest pedigrees simply underlines the heterogeneity of the disorder. Bilineality of susceptibility transmission in large families is an issue that has long been proposed as the cause that hampers the linkage analysis studies on large pedigrees with GTS (Comings et al. 1989; Kurlan et al. 1994).

The comorbidity of GTS with other psychiatric disorders could be explained by a common neurological basis. OC symptoms among individual with GTS range from 11%–80% (King et al. 1998), whereas 30% of adults with GTS meet full criteria for OCD. Hanna et al. (2002), in a recent genomewide scan for OCD, obtained a suggestive NPL score of almost 1 at a marker close to 17qter. Mood disorders have also been associated with GTS, and some studies have suggested that bipolar disorder is overrepresented in patients with GTS (Kerbeshian et al. 1995; Berthier et al. 1998; Robertson 2000). A recent genomewide linkage analysis of bipolar disorder produced a LOD score of 2.4 at marker D17S928 (Dick et al. 2003). Such overlapping findings

could be of special interest for disentangling the association of different psychiatric-disease phenotypes.

The genes studied in greater detail here could constitute candidate susceptibility genes for GTS. All three are related to neuronal plasticity, maintenance, and development. *NPTX1* is a gene with a protein product that is expressed almost exclusively in the human brain and plays a role in excitatory synaptogenesis, most likely in the developing brain (Omeis et al. 1996; Xu et al. 2003). Insulin receptor substrate protein p53 (IRSP53) is also expressed primarily in the brain and functions as an insulin receptor tyrosine kinase substrate (Abbott et al. 1999). It is considered to play an important role in neurite outgrowth, influencing the shape and dynamic of cytoskeletal structures (Oda et al. 1999; Krugmann et al. 2001; Bockmann et al. 2002). It has also been identified as interacting with the dentatorubral-pallidoluysian atrophy (*DRPLA*) gene, which is associated with a neurodegenerative disease (Okamura-Oho et al. 1999). DRPLA symptoms are similar to those of Huntington disease and include chorea, ataxia, lack of coordination, and dementia (Ross et al. 1997). The third gene we chose to study encodes TBCD, a protein important for the correct folding of tubulin and the formation of the functional $\alpha\beta$-heterodimer (Fleming et al. 2000). When overexpressed in vitro, it acts as a microtubule-destabilizing protein (Martin et al. 2000). As part of the microtubule-assembly machinery, TBCD may play an important role in the establishment of neural networks as well as axonal transport and maintenance. It has been recently shown that mutations in the proteins responsible for the folding and assembly of tubulin subunits into functional heterodimers can cause neurological disease (Bommel et al. 2002; HRD/Autosomal Recessive Kenny-Caffey Syndrome Consortium 2002; Martin et al. 2002).

The etiopathogenesis of GTS seems to result from the interaction of genetic susceptibility, environmental factors, and neurobiological systems active in the developing brain. It is clear that the disorder is both genetically and phenotypically heterogeneous. Our study has identified three genes that could confer susceptibility or protection for GTS and that should be further investigated. The identification of genes that contribute to the genetic component of GTS will lead treatment of the disorder in new directions and will elucidate the complex brain procedures involved in habit formation and tics.

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Electronic-Database Information

The URLs for data presented herein are as follows:

ALFRED, http://alfred.med.yale.edu
Applied Biosystems, http://www.appliedbiosystems.com/
GenLink, http://www.genlink.wustl.edu/
National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for GTS, OCD, *DRD4, MAOA, CNTNAP2, NPTX1, IRSP53, TBCD,* and SECTM1)
University of California–Santa Cruz Genome Bioinformatics, http://www.genome.ucsc.edu/

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