Genome Scan for Tourette Disorder in Affected-Sibling-Pair and Multigenerational Families

The Tourette Syndrome Association International Consortium for Genetics*

Tourette disorder (TD, or Gilles de la Tourette syndrome [MIM #137580]) is a childhood-onset chronic neuropsychiatric disorder characterized by multiple motor and phonic tics that wax and wane over time. Once thought to be rare, with prevalence estimates ranging from 1:20,000 to 1:2,000, it has recently been observed that, in school-age populations, the prevalence may be as high as 1% among boys. Family studies demonstrate that TD is familial, and twin studies provide evidence that genetic factors are important in its transmission. Initial complex segregation analyses of nuclear family study data were consistent with autosomal dominant inheritance. However, subsequent studies suggest that the mode of inheritance is more complex. Evidence from several genetic epidemiologic studies has pointed to a common genetic basis for TD, chronic-tic (CT) disorder, and obsessive-compulsive disorder (OCD). When these diagnoses are considered together, bilineal transmission is common in families ascertained for probands with TD.

The likelihood that some forms of TD, CT disorder, and OCD appear to be variant expressions of the same genetic susceptibility factors complicates linkage studies of TD and may partially explain the equivocal results of such studies, particularly those performed using parametric analyses. It is also difficult to compare the results of several independent linkage studies that have been performed in extended pedigrees affected with TD, since these have often employed different parametric models and have used marker sets that were substantially nonoverlapping.

Given the complications in genetic analyses of TD in large pedigrees, the Tourette Syndrome Association International Consortium for Genetics (TSAICG) undertook a complementary approach and started a multisite collection of affected sib pairs (ASPs). An initial linkage analysis of 92 ASPs from 76 nuclear families provided moderate evidence (maximum LOD score [MLS] 2) of linkage in two regions: one on chromosome 4q with a peak MLS of 4.42 and two adjacent segments on chromosome 8 bounded by markers D8S1106, D8S1145, and D8S1136. The TSAICG has extended this prior work by collecting a larger sample of ASPs for genome screening.

We report here the results of a complete genome screen that used 390 highly polymorphic DNA markers in a sample that includes 304 independent ASPs (from 238 nuclear families) and 18 large multigenerational pedigrees. The major rationale for including both ASPs and large pedigrees is that it provides the first uniform analysis of the majority of large pedigrees reported so far for TD. Data from the large pedigrees, collected by members of the TSAICG with the use of similar assessment protocols, have now been evaluated using the same markers and by nonparametric analysis methods that were not available when the initial studies of these pedigrees were conducted. Furthermore, the ASP and large family samples can easily be combined for further exploration of any linkage findings in each of the samples separately.

Material and Methods

Sample

Data from 238 ASP families and 18 large multigenerational families (a total of 2,040 individuals: 1,052 in the ASP sample and 988 in the multigenerational family sample) were analyzed in the genome scan. Of 255 sib-pair families, 17 were excluded from the analyses either because both sibs did not meet the diagnostic criteria for TD after complete assessment and consensus diagnosis (n = 12) or because non-Mendelian inheritance was observed in the family (n = 5). Two multigenerational families were excluded from the large family sample because they were uninformative for linkage.
All 238 ASP families included at least two offspring affected with TD and/or CT disorder. All diagnoses were made using DSM-IV-TR criteria. The ASP families consisted of 182 with two affected offspring, 47 with three affected offspring, 8 with four affected offspring, and 1 with five affected offspring, yielding a sample equivalent to 304 pairwise “independent” sib pairs. An additional 34 unaffected siblings were included in the sample. These individuals were used to obtain allele-frequency estimates but were not included in the linkage analyses. As noted above, there were 1,052 individuals in the ASP sample. DNA samples were not obtained from 6 mothers and 27 fathers, which yielded a total of 1,019 individuals for whom genotypes were available for most loci. There were no families in which DNA was unavailable for both parents. Of the 33 parents with unavailable DNA, 20 were also missing phenotypic data. There were 647 individuals (including offspring and parents) with TD and 69 with CT disorder in these families. Of the 716 with a diagnosis of either TD or CT disorder, 311 also had a diagnosis of OCD. Only 31 individuals in these families. Of the 716 with a diagnosis of either TD or CT disorder, 311 also had a diagnosis of OCD. Only 31 individuals (all parents) received a diagnosis of OCD without TD or CT disorder. The 18 multigenerational families range in size from 14 individuals with 3 members affected with TD or CT disorder to 272 individuals with 91 members affected with TD or CT disorder. Altogether, there were 214 individuals with TD or CT disorder in these 18 multigenerational families.

This research was approved by the ethics committees of each participating site. All individuals 18 years and older signed informed consent forms. For those individuals younger than 18, parents signed consent forms, and the children signed assent forms before participating.

Phenotypic evaluation.—When a family entered the study, information concerning diagnosis was collected as follows. Initially, information regarding symptoms associated with TD, OCD, and attention-deficit hyperactivity disorder (ADHD) was obtained with an interview that included a tic inventory and ordinal severity scales modified from the Yale Global Tic Severity Scale, the Diagnostic Confidence Index, a Modified Yale-Brown Obsessive-Compulsive Scale, and the Conners Rating Scales (parent and adult versions). This interview was then followed by a comprehensive assessment of other psychopathology, with use of the Kiddie-SADS–Lifetime Version (K-SADS-PL) and the Structured Clinical Interview for DSM (SCID).20

Earlier versions of the TD and OCD instruments have been shown to have a high level of agreement with expert clinician ratings of tic and obsessive-compulsive symptom severity. These instruments, when compared with clinician diagnoses, have been shown to be both valid (the rate of agreement between interview-derived diagnoses and clinical diagnoses was 0.98 for TD and 0.97 for OCD) and reliable for the diagnoses of TD (κ = 1.00) and OCD (κ = 0.97).31 As noted above, for the assessment of other psychopathology, the K-SADS-PL was used for children younger than 18, and the SCID was used for adults. Both interviews have established reliability. For this report, only individuals with a diagnosis of TD or CT disorder were considered to be affected. Future analyses will include information about OCD, ADHD, and other comorbid conditions.

Best-estimate diagnoses.—All diagnoses were made using the best-estimate approach. Before the initial diagnostic estimate was made, separate files for each individual were prepared. These files contain all available information about the individual, including the completed interview packet, medical records (if available), and all reports from relatives. Web-based document access and data input facilitated the best-estimate consensus diagnostic process. De-identified records for all families were scanned into PDF files and made available via password-protected login to clinicians at each of the individual clinical sites on three continents. Assessment instruments include all materials in the assessment battery.

Diagnostics used DSM-IV-TR criteria for all disorders. For each subject in the sample, two diagnosticians—neither from the originating site of the subject—individually reviewed the assessment materials and recorded diagnoses. A software algorithm compared diagnoses and notified the clinicians if discrepancies were found. Clinicians then used e-mail to resolve differences or to schedule telephone calls for discussion of the differences. In the rare event of an unresolved disagreement, a third diagnostician was asked to review the case and bring resolution. For cases in which consensus was not possible because of missing or ambiguous data, further clinical data were requested from the site of origin. For the diagnosis of TD and CT disorder, the initial independent diagnoses of the two consensus diagnosticians were in agreement 94% of the time (i.e., only 6% of the time was there a disagreement between the two independent diagnosticians that required discussion). Failure to reach consensus occurred in only 21 cases, primarily because of the lack of adequate case data. No individual was included for the linkage study unless consensus diagnosis was achieved.

DNA Markers

Genotyping was completed at the Centre National de Génotypage in Evry, France. A total of 390 STR markers were genotyped (LMS2 Applied Biosystems). These markers had an average heterozygosity of 0.78. Standard DNA amplification protocols were used and were optimized to amplify an average of four markers in the same well.

Fine-mapping genotyping also used standard protocols. Amplified products were pooled, depending on the fluorescent dye label and the size of the products, and were combined with a size standard before being analyzed on an ABI3730. GeneMapper v3.5 was used to analyze the raw results from the ABI3730. However, a genotype was not considered final until two laboratory personnel had independently checked (and verified or corrected) the GeneMapper results and had agreed. The rate of missing genotypes was <4% in both the initial genome scan and the fine-mapping experiments.

Data Analysis

Automatic genotyping was performed on the basis of a series of software processes, trace processing, fragment sizing, allele calling, and the assignment of genotype quality scores (QS) that are implemented in Genetic Profiler software (version 1.1). In addition, an independent manual review of all samples was also completed. Two independent readings of all genotypes were completed. When differences in the genotypes scored in the two readings were observed, the data were reverified to resolve inconsistencies. After raw data genotyping analyses were completed and the results were exported, allele rebinning was performed for each marker on the basis of the distribution of allele peak locations over all samples in this study. Once final allele bins were defined, allele codes were assigned for each individual or microsatellite.

Consistency of the data with Mendelian inheritance, for both the genome scan and the fine-mapping genotypes, was examined
Figure 1. $Z_{pairs}$ scores for ASP families, with use of TD (broken line) and TD + CT (solid line) as diagnostic groupings (MERLIN)

using PedCheck.\textsuperscript{33} Genotypes were reverified when inconsistencies were detected. When the inconsistency could not be resolved, the family was removed from the study.

The data were also examined to determine whether the removal of unlikely genotypes would alter the results of linkage analysis. Unlikely genotypes were identified using the error-checking algorithm implemented in MERLIN,\textsuperscript{34} which is based on the detection of double-recombinant events. The default parameter, which identifies erroneous genotypes as those with a likelihood ratio of $P < .025$, was used. The same procedure was used both for the original markers and for those used in fine mapping. As expected, given the framework map used in the linkage studies, there was little difference in the results whether unlikely genotypes were kept or removed; thus, results are reported for all genotype data.

Analyses were completed using two diagnostic classifications. The first set of analyses included as affected only those individuals with a diagnosis of TD (TD). The second set of analyses included as affected all individuals with a diagnosis of either TD or CT (TD). The same procedure was used both for the original markers and for those used in fine mapping. As noted above, the families included in the final analyses yielded a total of 304 pairwise independent sib pairs. Analyses of the multigenerational families were conducted with the computer program SIMWALK2.\textsuperscript{35–38} Finally, the two samples were combined and were analyzed using MERLIN and SIMWALK2. MERLIN was used to calculate the $Z$ scores in all sib-pair families and in several multigenerational families, and those results were then imported, using MEGA2,\textsuperscript{39} into SIMWALK2, which was used to analyze the remaining multigenerational families. For the SIMWALK2 analyses, empirical $P$ values were estimated by randomly sampling from the very large inheritance-vector space, to obtain an estimate of pointwise significance. This is in contrast to other linkage software programs that are constrained to smaller family units and that calculate an exact $P$ value by calculating the value of the test statistic for all possible inheritance vectors. In much larger pedigrees, calculating exact $P$ values is impossible, given the large number of possible inheritance vectors. However, SIMWALK2 can calculate the values for a large number of the inheritance vectors, thus estimating the pointwise $P$ value. Analyses were completed using 10,000, 20,000, 40,000, and 100,000 simulations to estimate significance. The reason for conducting these analyses was that, for each of the first three sets of simulations, our most significant result was always equal to the minimum $P$ value possible for that set of simulations. That is, for 10,000 simulations, our most significant results corresponded to $P = 1 \times 10^{-4}$ ($-\log P = 4.00$); for 20,000 simulations, the most significant result observed corresponded to $P = 5 \times 10^{-5}$ ($-\log P = 4.30$); for 40,000 simulations, the most significant $P$ value observed was equal to $2.5 \times 10^{-5}$ ($-\log P = 4.60$); and for 100,000 simulations, the most significant $P$ values observed were not at the bound of 100,000 simulations, the most significant $P$ value observed was equal to $1 \times 10^{-4}$ ($-\log P = 4.00$).

In addition, the run time for the Markov chain–Monte Carlo analyses was extended more than three times the default value in SIMWALK2, to increase the likelihood of achieving the optimal solution in the sample of large families. Only the results of the analysis in which 100,000 simulations were completed are reported.

**Results**

For both diagnostic classifications, the genome scans of the ASP sample and the multigenerational family sample were analyzed separately and together. For the analyses in the ASP sample in which only individuals with TD were included as affected, no $Z_{pairs}$ scores $\geq 3$ were obtained, but scores $\geq 2$ ($P < 1 \times 10^{-2}$) were observed for markers on chromosomes 2p, 3p, 3q, 4p, 6p, 10p, 15p, 21p, and Xp (fig. 1). The analysis of the multigenerational family sample, which included only individuals with TD as affected, yielded the highest peak on 2p ($-\log P = 2.34$, $P =$}
were genotyped at an average spacing of 2 cM. Markers (fig. 3), compared with 3.99 ( ) when the anal-
ergative families that used the same diagnostic group-
ing (TDtigenerational families slightly increased the linkage signal on 2p (−logP = 3.99, P = 1 × 10−4) at marker D2S165. Additional scores >2 were observed on chromosomes 5p and 6p (fig. 2). It should be noted that the highest scores observed in the ASP sample on 2p for both sets of analyses (TD alone and TD + CT) were also observed for marker D2S165.

Analysis of the combined sample of ASP and multige-
erational families slightly increased the linkage signal on 2p for the analyses that included individuals with TD or CT disorder as affected. For the analyses including only individuals with TD, the peak at D2S165 was −logP = 3.23 (P = 5.8 × 10−4), compared with 2.34 (P = 4.6 × 10−5) when the data sets were not combined. When individuals with TD or CT disorder were included as affected, the peak at D2S165 was −logP = 4.40, P = 4.0 × 10−5) (fig. 3), compared with 3.99 (P = 1 × 10−4) when the analyses were completed with the large families alone.

Given these findings, fine mapping on 2p in both ASP and large families was completed; five additional STR markers (D2S2233, D2S220, D2S2221, D2S144, and D2S352) were genotyped at an average spacing of 2 cm. Markers D2S2233, D2S220, D2S2221, and D2S144 are between D2S305 and D2S165 (the two markers giving the highest −logP score in the original genome scan data). Marker D2S352 is between D2S165 and D2S367. The analyses in the combined sample of ASP and multigenerational families yielded a maximum Zparam − log(P) = 4.42 (P = 3.8 × 10−5) at marker D2S144 with the diagnostic classification of TD + CT, confirming the findings from the initial 10-cM genome scan (fig. 4). The 95% CI, calculated using BINOM,41 for this −logP score is 4.01–4.96. Fur-
thermore, support at nearly the same level was maintained over an ∼5-cM region (sex averaged, encompassing ∼8 Mb). A larger region of ∼16 cM encompasses all markers with a drop of −logP−1.

Discussion

The current study represents the largest series of families with TD to have been included in a genome scan. The strongest evidence of linkage is at marker D2S144 on chromosome 2p23.2. Moreover, the evidence is strengthened by the fact that (1) both the sib-pair and the multigener-
erational pedigree samples support linkage to this region and (2) a more clearly defined linkage peak was observed when additional markers were typed. An additional piece of evidence that may support the existence of a TD locus in this region comes from the observation of a complex chromosomal rearrangement in which two children with TD, OCD, and mental retardation inherited an insertion of 2p21-23 (without any apparent disruption of any genes) into a region of chromosome 7q35-36.41 This segment of chromosome 2, which is trisomic in the affected offspring, contains the 15-Mb interval that lies beneath the linkage peak (between D2S305 and D2S367). This region has the highest priority for follow-up studies.

Other regions may also be important to follow up. When examined separately, several regions emerged as potentially interesting in the sib-pair sample, with segments on 3p, 3q, and 14q all displaying NPL scores >2.5. However, none of these regions were supported substan-
tially by the total multigenerational sample. Similarly, two regions (5p and 6p) yielded −logP scores >2.0 in the pedi-
gree sample. Both regions had positive NPL scores in the ASP sample (5p, −logP = 1.11; 6p, −logP = 2.42). Of note is that, in the analyses of the combined samples,
$-\log P = 2.73$ in the 6p region. Although none of these regions achieve statistical significance, they all warrant additional follow-up, since they may represent the effect of gene variants that are contributing to TD susceptibility in only a subset of the families. Given the size of this sample, it was not computationally feasible to complete simulations necessary to provide $-\log P$ scores that define the thresholds for suggestive and significant linkage. For example, one complete genome analysis of the combined data set on a Linux cluster of 30 processors requires $>3$ mo to complete.

In a previous linkage study, using a subset of the sib pairs employed in the current genome scan, the TSAICG\textsuperscript{18} reported moderate evidence of linkage on chromosomes 4q and 8p (MLS $>2$). A third region on chromosome 4p showed weak evidence of linkage (MLS $>1$) in that initial study.\textsuperscript{18} Of note, in the current study, the evidence of linkage to 4p is strengthened in both the ASP ($Z_{pairs}$ score $>2$) and the large family ($-\log P > 2$) samples, as well as in the combined sample ($-\log P > 2$); however, neither the region on 4q nor the region on 8p are supported, even in the ASP sample alone. The diminution of evidence in successive linkage studies of complex traits has been observed for several other disorders.\textsuperscript{42,43} In addition, theoretical studies have predicted that this would be the case,\textsuperscript{44} on the basis of the inherent etiologic heterogeneity of such traits, especially where there is variability in either the ascertainment or the assessment of the subjects. In our study, we attempted to minimize such variability (e.g., through the use of our best-estimate assessment procedures); nevertheless, we ascertained the families for this study in multiple sites in Europe and North America. This study also does not provide support for other regions implicated in prior smaller-scale linkage studies of TD, some of which focused on families that were included in the current study. Mérette and colleagues\textsuperscript{45} reported moderate evidence of linkage to chromosome 11q23 in one large French Canadian kindred. In a genomewide linkage study of seven multigenerational families, Barr et al.\textsuperscript{46} observed a linkage signal for TD in two regions (19p13.3 and 5p13-q11.2). Whereas neither of these regions showed significant linkage in our combined sib-pair and pedigree analysis, the positive linkage results on chromosome 5 in the multigenerational families in the current sample are consistent with the Barr et al. findings.\textsuperscript{46} However, this may be due largely to the inclusion of four of the families from the Barr et al.\textsuperscript{46} study in the present study. Paschou and colleagues\textsuperscript{47} identified suggestive linkage results in 17q (spanning the interval from D17S784 to D17S928) in three multigenerational families. Although the families included in the Paschou et al. study\textsuperscript{47} are also included in the current sample, the findings of the combined sample of ASP and pedigree families and the total pedigree sample do not support the results of Paschou et al.\textsuperscript{47}

Other approaches have also been employed in the search for TD susceptibility loci. A recent finding reported a possible association between the gene “slit and Trk-like 1” (SLITRK1) and TD\textsuperscript{48}; SLITRK1 was examined as a candidate gene because of its proximity to a de novo chromosomal inversion on chromosome 13q31.1 in a child with TD and on the basis of the finding of a frameshift mutation and two independent occurrences of the identical variant in the binding site for microRNA hsa-miR-189 among 174 unrelated probands (but not in >3,600 control chromosomes). Unfortunately, there was no support for a locus on chromosome 13 in the current study, suggesting that, if SLITRK1 is a susceptibility gene for TD, it is not one with major effect in the population studied and reported in this article.

The fact that we obtained our strongest linkage finding when we included as affected those individuals with CT disorder, as well as those given diagnoses of TD, underscores the continuing uncertainty regarding the optimal phenotypic definition for linkage and association studies.
Figure 4. $Z_{\text{pair}} -\log P$ scores for fine mapping of chromosome 2p, with use of TD and TD + CT as diagnostic groupings (MERLIN and SIMWALK2). The dotted line represents TD-only genome-scan markers; the dotted and broken line represents TD-only fine-mapping markers; the broken line represents TD + CT genome-scan markers; and the solid line represents TD + CT fine-mapping markers.

of these disorders. Since we have obtained, in the families studied, information on other qualitative phenotypes (e.g., OCD and ADHD) that may be related to TD, as well as a wide range of quantitative phenotypic features, it will be possible to conduct further analyses of the genome-scan data. These analyses may suggest additional genome regions that warrant follow-up investigation in these families, as well as in the trios with TD that we have sampled.

In conclusion, this sample of 238 ASP families and 18 multigenerational families provides significant evidence of linkage to marker $D2S144$ on chromosome 2p32.2. Other chromosomal regions—including 3p, 3q, and 14q—had NPL scores >2.5 in the sib-pair sample but not in the multigenerational pedigrees. These results are consistent with a complex inheritance model that includes locus heterogeneity and a gene of major effect on 2p32.2. Since it appears likely that other regions may harbor additional genes that contribute to susceptibility for TD, it is important that analyses be undertaken to examine the extent of linkage heterogeneity and phenotypic variation in this sample and to attempt to determine if genes in these regions may be linked in a subset of individuals with TD.

Sib-pair and pedigree samples are most suitable for identifying relatively infrequent variants of moderate-to-large effect. Given that our collection of such samples has involved an exhaustive international effort, our efforts so far represent an effective screen for most such variants that contribute to TD. However, it remains important to employ different strategies, such as whole-genome association studies, that will facilitate the identification of variants that may have a lesser effect in any given individual with TD but that will make a relatively larger contribution to the manifestation of TD in the population as a whole.

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Web Resources

The URLs for data presented herein are as follows:

BINOM version 20, http://www.genemapping.cn/linkutil.htm

References


