Am. J. Hum. Genet. 68:848–858, 2001

Disruption of a Novel Gene (*IMMP2L*) by a Breakpoint in 7q31 Associated with Tourette Syndrome

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Gilles de la Tourette syndrome (GTS) is a complex neuropsychiatric disorder characterized by multiple motor and phonic tics. We identified a male patient with GTS and other anomalies. It was determined that he carried a de novo duplication of the long arm of chromosome 7 [46,XY,dup(7)(q22.1-q31.1)]. Further molecular analysis revealed that the duplication was inverted. The distal chromosomal breakpoint occurred between the two genetic markers D7S515 and D7S522, which define a region previously shown to be disrupted in a familiar case of GTS. Yeast and bacterial artificial chromosome clones spanning the breakpoints were identified by means of FISH analysis. To further characterize the distal breakpoint for a role in GTS, we performed Southern blot hybridization analysis and identified a 6.5-kb *Sac*I junction fragment in the patient's genomic DNA. The DNA sequence of this fragment revealed two different breaks in 7q31 within a region of ~500 kb. *IMMP2L,* a novel gene coding for the apparent human homologue of the yeast mitochondrial inner membrane peptidase subunit 2, was found to be disrupted by both the breakpoint in the duplicated fragment and the insertion site in 7q31. The cDNA of the human *IMMP2L* gene was cloned, and analysis of the complete 1,522-bp transcript revealed that it encompassed six exons spanning 860 kb. The possible role of *IMMP2L* and several other candidate genes within the region of chromosomal rearrangement, including *NRCAM, Leu-Rch Rep,* and *Reelin,* is discussed. The 7q31 breakpoint interval has also been implicated in other neuropsychiatric diseases that demonstrate some clinical overlap with GTS, including autism and speech-language disorder.

Introduction

Gilles de la Tourette syndrome (GTS [MIM 137580]) is a neuropsychiatric disorder characterized by multiple involuntary motor and vocal tics, with an estimated general population prevalence of ~2 per 10,000 (Robertson 1994). Freeman et al. (2000) studied 3,500 people who had GTS. The male:female ratio observed was 4.3:1, with onset of the tics at age <10 years in 93% of cases. Most patients were diagnosed with other conditions in addition to GTS, including attention deficit–hyperactivity disorder, obsessive-compulsive disorder, obsessive-compulsive behavior, learning disorders, and conduct disorder–oppositional defiant disorder (Freeman et al. 2000).

Although GTS appears to be genetically transmitted (Curtis et al. 1992), several linkage studies so far have failed to determine a specific chromosomal locus for a susceptibility gene (Barr and Sandor 1998). Merette et al. (2000), by investigating a single large French Canadian family, described the most significant GTS linkage (LOD score 3.24) to the long arm of chromosome 11. In other cases in which structural chromosomal anomalies associated with GTS are reported, no specific common locus could be identified.

In 1996, Boghosian-Sell et al. reported a familial form of GTS with partial or full expression of the disease, which was shown to segregate with an apparently balanced t(7;18)(q22-q31;q22.3) translocation. Because a patient with GTS with a deletion of 18q had been described (Donnai et al. 1987) and because of apparent behavioral problems in other 18q cases, a putative GTS locus was suggested on the long arm of chromosome 18. However, no gene(s) in this region have yet been demonstrated in the etiology of GTS. By means of somatic cell-hybrid analysis, the 7q breakpoint could be positioned between D7S515 and D7S522 (Boghosian-Sell et al. 1996).

In the present article, we describe a detailed molecular genetic analysis of a 13-year-old boy, described elsewhere, with a de novo duplication dup(7)(q22.1-q31.1), who developed vocal and motor tics at age ~9 years. He also has other dysmorphic features, such as ear

Received January 3, 2001; accepted for publication February 13, 2001; electronically published March 9, 2001.

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0002-9297/2001/6804-0007$02.00

anomalies, but no severe mental retardation, and none of his relatives exhibit symptoms of GTS. Whereas most of the additional clinical signs have been reported in other cases with duplications of approximately the same chromosomal segment (Megarbane et al. 2000), neither vocal nor motor tics have been described in those cases.

There are several possible scenarios whereby the duplication could contribute to the GTS phenotype: (1) the extra copy of a gene or genes within the duplicated region leads to 50% increased gene dosage; (2) single disruption of a gene at the breakpoint in the duplicated fragment or at the insertion site would not affect gene dosage; however, a gene that is disrupted by both a breakpoint in the duplicated fragment and at the insertion site would lead to a 50% decrease in gene dosage; (3) alternatively, a breakpoint disrupting a gene could lead to a fusion protein, resulting in a possible gain of function. To further understand the phenotype in this patient, physical mapping and positional cloning experiments were undertaken to characterize the breakpoint region in 7q31 in detail and to search for genes possibly affected by the rearrangement.

Subjects and Methods

Case Report

The 13-year-old male propositus, whose parents were nonconsanguineous, demonstrates slight mental and physical retardation. DSM-III-R criteria were used to diagnose GTS. In addition to GTS features, such as involuntary motor and vocal tics, he had reduced speech development, depression, strabismus convergens, a malformed left ear, meatus acusticus stenosis, slight microgenia, and considerable gynecomastia. His karyotype was 46,XY,dup(7)(pter-q31.1::q31.1-q22.1::q31.1-qter) de novo. No history of GTS could be found in the family of the proband. A detailed clinical report of the patient and the cytogenetic findings may be found elsewhere (Kroisel et al., in press).

FISH

Routine chromosome preparations and high-resolution chromosomes were made from peripheral blood of the patient, his parents, and normal controls, using standard methods. Degenerate oligonucleotide primed–PCR (Telenius et al. 1992) was used to generate FISH probes from a number of chromosome 7q YAC and bacterial artificial chromosome (BAC) clones from CEPH, the Toronto Hospital for Sick Children (Cystic Fibrosis Mutation Data Base), and Research Genetics. Probe labeling with digoxigenin 11-dUTP (Roche) and FluorX (Amersham) and DNA hybridization were performed as described elsewhere (Petek et al. 1997). RxFISH was performed according to the recommendations of the supplier (Applied Imaging). Images were recorded with the use of a Zeiss Axiophot 2 microscope equipped with a cooled CCD camera (Photometrics). Digitized images were captured and processed on a CytoVision Ultra workstation (Applied Imaging).

Southern Blot Analysis

Genomic DNAs from immortalized cell lines of the patient and normal controls were purified using standard procedures. Restriction-enzyme digests were performed according to the manufacturer's specifications; digests were electrophoresed on 1.0% agarose gels, and the DNA was subsequently transferred to Hybond N+ filter membranes (Amersham), with 10 × SSC as transferring buffer. Probes were labeled, by random priming, with ^{32}P-dCTP. Membranes were hybridized overnight at 65°C. Final washing of the membranes was performed with 0.1 × SSC at 65°C. The results were analyzed with the STORM Phosphor Imaging System.

Cloning and Sequencing

The *Sac*I restriction fragments in the size of the junction fragment of the patient's DNA were isolated by gel purification (Machery-Nagel) prior to being cloned into the Litmus 28b vector (MBI). Junction fragment–positive clones were identified by means of the Tourette syndrome probe (TSP) forward primer (see sequence information in the legend to fig. 4) and the reverse primer from the vector polylinker site. Subsequent plasmid DNA sequencing reactions were performed using the SequiTherm DNA sequencing kit (Epicentre Technologies) and 20 ng of primer. Products were separated on a LI-COR DNA sequencer, according to the manufacturer's instructions.

cDNA Amplification

Marathon-Ready cDNA (Clontech) was used to confirm the 5′ sequence of human *IMMP2L* generated by expressed sequence tags (EST) sequence analysis. Primers used in this study were, in the first round, Adaptor primer 1 (Clontech) + AGATGTGACCACGGGGGACTTTGAC; and nested PCR, in the second round, Adaptor primer 2 (Clontech) + CCAGTGGTTCAAAAGCACCACATCA (Primer 3 Software Distribution). PCR conditions for both amplification steps were as follows: 95°C for 2 min, followed by 25 cycles of 94°C for 1 min and 70°C for 2 min, in a 20-μl reaction with 1.5 mM $MgCl_2$. For the nested PCR, 1 μl of a 1:20 dilution of the previous amplification reaction was used as a template. PCR products were separated through a 1% agarose gel. The amplification products (~600 bp) were gel purified and reamplified with 5′ end *IMMP2L* primers GTGATCGCGAGCATGTGT and CATGTTGTTCAAGCCTGACG (annealing temperature: 57°C; 1.5 mM

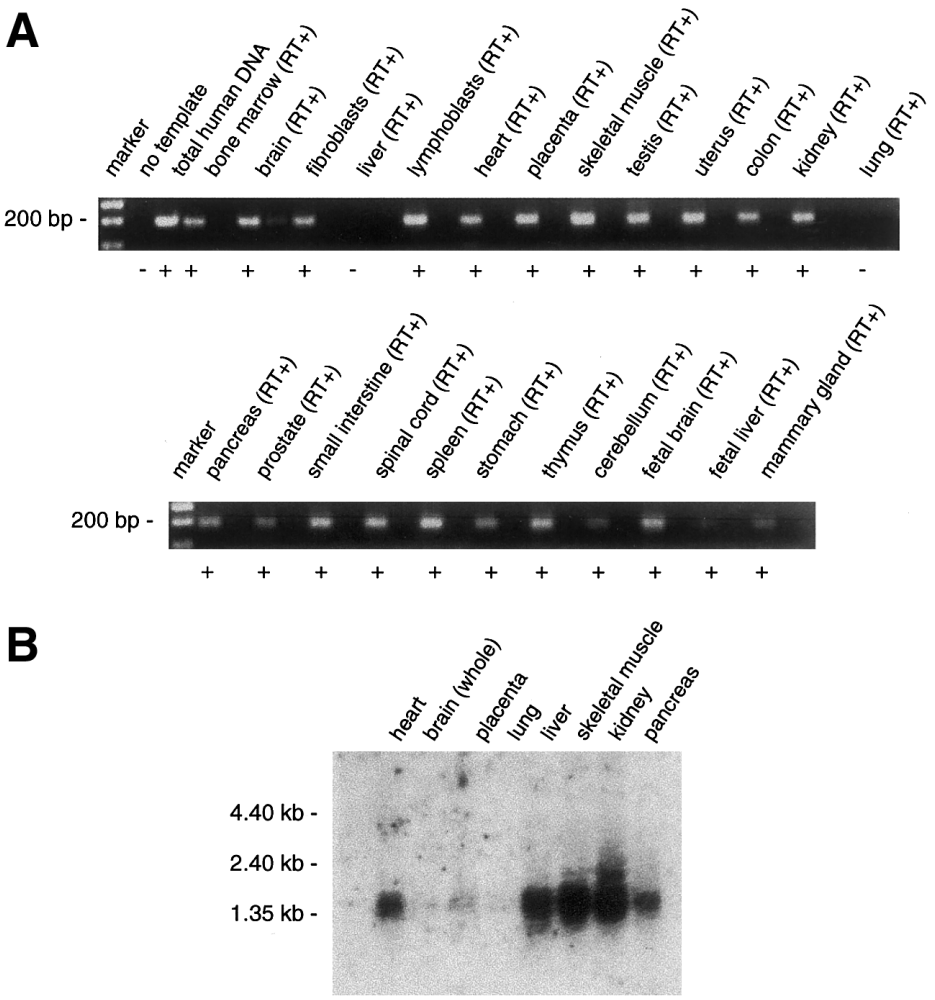

Figure 1 *A,* RT-PCR analysis of *IMMP2L* expression, using multiple-tissue RNA (Clontech). Lanes with RT reactions done with reverse transcriptase (RT+) are always followed by the same-tissue control reactions without reverse transcriptase. The *IMMP2L*-specific primers and the PCR conditions are described in the Subjects and Methods section. A 198-bp product is the expected product from the 3′ *IMMP2L* sequence. *B,* Northern blot analysis of human *IMMP2L*. Multiple-tissue northern blot filters, containing adult human poly (A)+ RNAs (2g/lane), were purchased from Clontech. Hybridization and washing were performed following the manufacturer's instructions. The insert from IMAGE clone 1714060 was labeled with $\alpha[^{32}P]$ dCTP and used as a hybridization probe. Two close major bands around 1.5 kb could be seen. Strong expression was shown in the heart, liver, skeletal muscle, kidney, and pancreas. Weak expression could be observed in the brain, placenta, and lung.

$MgCl_2$). Sequencing of the final product was performed as described above.

RNA Transcription Analysis

Human total RNAs (Clontech) from 23 tissues were used for reverse-transcription (RT)–PCR analysis of the *IMMP2L* gene (see fig. 1*A*). RT reactions, to control for DNA contamination within the RNA sample, were complete with and without reverse transcriptase. By means of random hexamers to the RNA strand, ~2 μg of total RNA was reverse transcribed in PCR buffer (Gibco BRL). The resulting cDNA samples were diluted 1:4, and 1 μl was subjected to PCR with gene-specific primers (forward primer: CAATATGATGCTGTGCGAGAA; reverse primer: AGCCCCATTAAGACATGTGG; annealing temperature: 57°C; 1.5 mM $MgCl_2$). PCR conditions were 95°C for 2 min, followed by 40 cycles of 94°C for 15 s, 57°C for 15 s, and 72°C for 1 min, in a 50-μl reaction with 1.5 mM $MgCl_2$.

Results

Localization of Duplication Breakpoints by FISH Analysis

Using RxFISH and a whole–chromosome 7 painting (WCP), the entire additional segment could be shown to be of chromosome 7 origin, and no further structural anomalies in the remainder of the chromosomes could be found (fig. 2*A*).

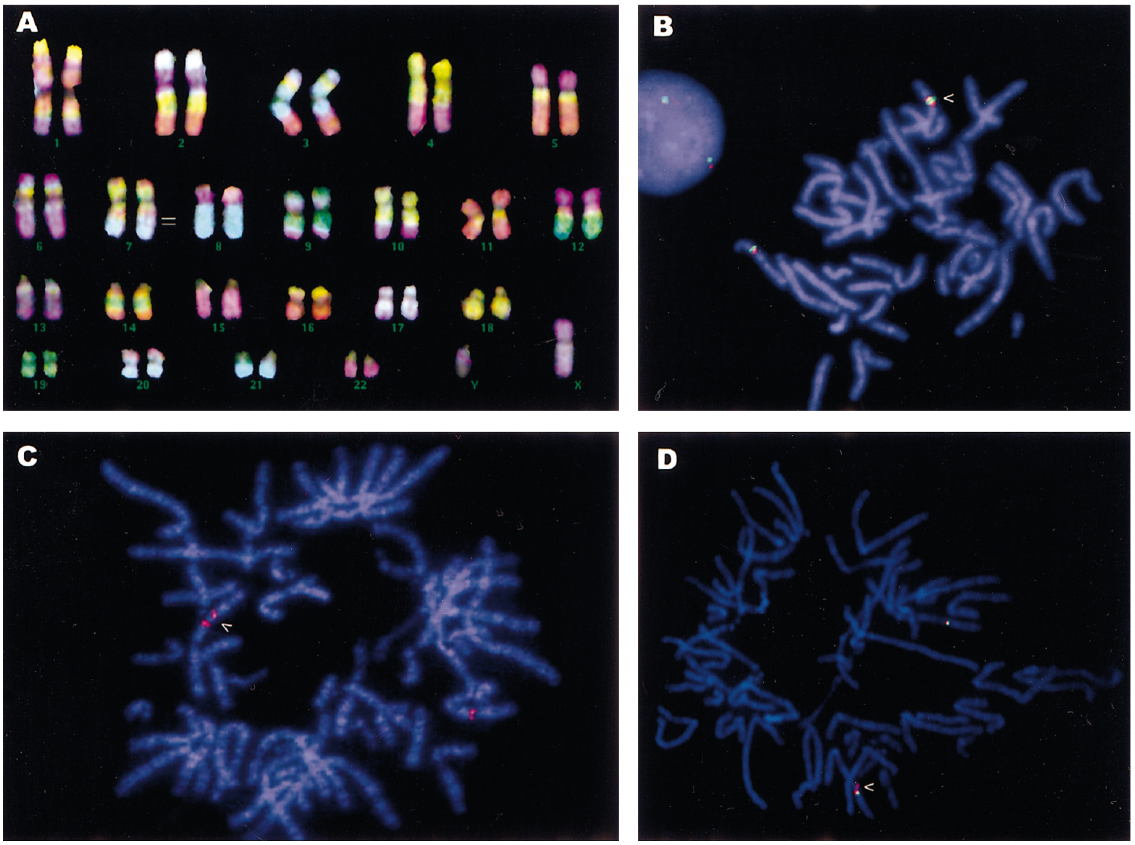

Figure 2 *A,* RxFISH karyotype of the propositus. The two parallel lines at the derivative chromosome 7 show the extent of the additional chromosomal material. No further changes in the remainder of the chromosomes could be recognized. *B,* Two-color FISH, using YAC clones C898h11 (red) and C805d11 (green), both mapped within the duplicated segment. The distribution of the signals on the der(7) confirm the presence of an inverted duplication. *C,* FISH with chromosome 7q22 YAC HSC7E737. This YAC was shown to be the most centromeric clone of a contig, revealing signals within and proximal to the duplicated segment. *D,* Two-color FISH using neighboring BAC clones H_RG118D7 (red) and H_DJ0777B17 (green). H_RG118D7 was shown to be the most distal BAC clone, revealing signals within and distal to the duplication. The der(7) are indicated by open arrows.

FISH analysis, by means of YACs hybridized against prometaphase chromosomes of the patient, was first used to delineate the rearrangement sites involved in the duplication event (table 1). By using two-color FISH of YACs C898h11 (7q22) and C805d11 (7q31), the additional segment on 7q was demonstrated to be inverted (fig. 2*B*). The duplication was found to encompass ~15 Mb of DNA. The CEPH YACs C900b6 and C857a12, both mapping to Whitehead contig WC7.6 on 7q31, were shown to span the distal breakpoint, revealing signals telomeric and within the duplicated segment. Because of the inversion of the duplicated segment, no difference between a clone spanning the proximal translocation breakpoint or just flanking it distally can be recognized by FISH analysis. The YAC HSC7E737 could, however, be shown as the most proximal clone of a contig in 7q22, revealing two signals on the derivative chromosome 7 (fig. 2*C*).

FISH using overlapping BACs, sharing sequence-tagged site (STS) markers with YAC clone C900b6, demonstrated that the clones H_RG118D7, H_NH0452K21, H_NH0520M18, and H_RG17K18 reside, at least in part, distal and within the duplicated segment (fig. 2*D*). These clones are part of the ~700-kb contig NT_000467, for which the complete DNA sequence is known (GenBank accession number GI:5868465).

The distal breakpoint was determined to reside in the same region (D7S515 to D7S522) as in the 7;18 translocation described elsewhere (Boghosian-Sell et al. 1996).

Cloning and Characterization of a Junction Fragment at 7q31

As a result of the inversion of the duplicated segment, two 7q31 breakpoints (the breakpoint of the duplication and the insertion site) were linked (fig. 3). Because these breakpoints were in the same general region as that of another patient with GTS (Boghosian-Sell et al. 1996), we characterized them further. Using DNA sequence in-

Table 1

Summary of FISH Analysis

Marker	YACs	BACs	Cytogenetic Localization[a]	Signals on der(7)[b]
WI-6095	C846g10		7q21	Proximal
swss3705	HSC7E79		7q22	Proximal
	HSC7E491		7q22	Proximal
D7S1619	C783B4		7q22	Proximal
D7S2480	HSC7E108		7q22	Proximal
D7S734	HSC7E47		7q22	Proximal
swss1304	HSC7E737		7q22	Proximal + WD
D7S1410	HSCE98		7q22	WD
D7S515	HSC7E1058		7q22	WD
...	C887d10		7q22	WD
D7S2504	C898h11		7q22	WD
D7S2182	C758g11		7q22	WD
D7S2453	C958g7		7q22	WD
D7S2459	C761c10		7q31	WD
D7S2425	C805d11		7q31	WD
swss3545	C857a12	H_RG017K18	7q31	Distal + WD
swss2098	C900b6	H_NH0520M18	7q31	Distal + WD
		H_NH0452K21	7q31	Distal + WD
swss2779		H_RG118D07	7q31	Distal + WD
		H_DJ0777B17	7q31	Distal
		H_NH0365F08	7q31	Distal
swss574		H_RG007J15	7q31	Distal
swss2736		H_DJ0905M06	7q31	Distal
swss364		H_GS034D21	7q31	Distal
D7S2769	C912d9		7q31	Distal

[a] Underlining indicates the YAC and BAC clones localized within the duplicated segment.

[b] "Proximal" indicates proximal to duplication, "WD" indicates within duplication, and "Distal" indicates distal to duplication.

formation obtained from analysis of the NT_000467 contig, we generated several PCR-derived probes from repeat-free regions. One of the probes, TSP, is a 1,414-bp PCR product, located at position 355,977 bp–357,390 bp in the contig. In Southern blots with DNA from the patient and a normal control digested with restriction enzymes *Eco*RI and *Sac*I, an additional rearrangement fragment in the patient's DNA could be detected by TSP, indicating that the breakpoints must be within this restriction fragment. According to these results, we could narrow the breakpoint regions to ~5 kb (fig. 4*A*). The experimental results from Southern blot analysis are shown in figure 4*B*.

The junction fragment was subcloned and sequenced, and the exact position of each breakpoint was identified. One breakpoint (DBP-1) was shown to be located in clone H_RG118D7 at position 46743 bp (AC004142), whereas the second break (DBP-2) occurred ~500 kb more proximal in clone H_NH0358A10, at position 25,141 bp of the working draft sequence AC073326.3 (fig. 4*A* and 4*C*). With the use of the included marker stsG3600, H_NH0358A10 could be mapped between contigs NT_000467 and NT_000469. By means of sequence comparison (BLAST), we were able to anchor this clone to contig NT_000469 through clones CTB-3083H11 (AC079453), 729J15 (AC032042), CTB-9H2 (AC003081), CTA-257F18 (AC006339), and H_RG119P24 (AC003088) (fig. 4*A*).

DNA Sequence Annotation and Characterization of IMMP2L

EST database–search analysis using BLAST (National Center for Biotechnology Information) did not reveal any transcripts immediately near the chromosome 7q31 breakpoint DBP-1 in H_ RG118D7, except for the gene encoding for the Leu-Rch Rep protein ~75 kb proximal to the breakpoint. However, analysis of the DBP-2 breakpoint region, encompassed by BAC H_NH0358A10, led to the identification of several ESTs from the UniGene cluster Hs.13058. Further characterization of HS.13058 indicated that it was part of a large gene (~860 kb) that was disrupted directly by DBP-1 and DBP-2 (fig. 4*A*). We have named the partial cDNA *IMMP2L,* because of its homology to *IMP2* of *Saccharomyces cerevisiae* (42%). By a combination of sequencing IMAGE clones (numbers 1714060 [AI128376] and 136260 [R33650]; Research Genetics), EST data-

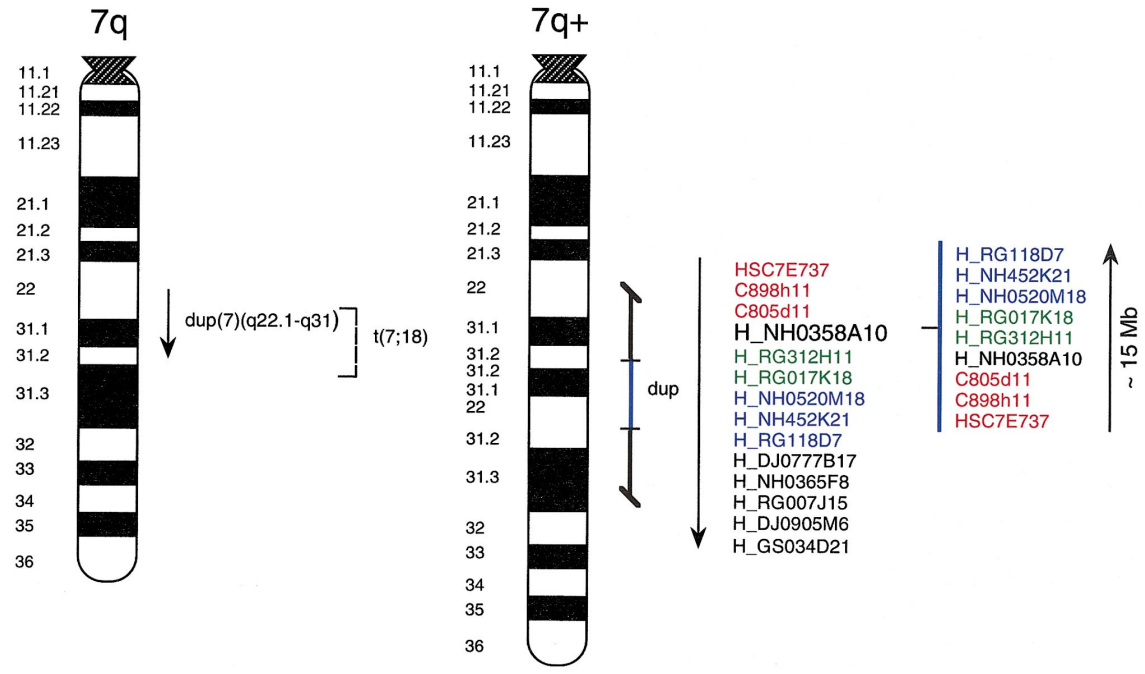

Figure 3 Ideogram of the derivative and normal long arm of chromosome 7. On the left side, the minimal breakpoint region of the familial 7;18 translocation associated with GTS, reported elsewhere, and the duplicated segment of the present case are indicated. The ideogram of the der(7), on the right side, shows the relative position of YAC and BAC clones used, in part, for FISH analysis. The inverted duplication, spanning a region of ~15 Mb, was found to be inserted in clone H_NH0358A10.

base screening, and cDNA amplification, we assembled a consensus transcript 1,522 nt in length (fig. 5). Clone number 1714060 contained a 1,153-bp insert spanning nucleotides −74 to 1079; clone number 136260 contained a 410-bp insert spanning nucleotides 510–919 and using an alternative polyadenylation signal at nucleotides 890–895; EST clone AW449560 spanned nucleotides −443 to −202. RT-PCR on testis cDNA (Clontech), followed by sequencing, was used to confirm that the 5′ and 3′ EST sequences were part of the same transcript. Several other EST clones having the alternative 3′ end splicing, using a poly(A) site ~170 bp upstream, could be recognized (fig. 5). Alignment of the cDNA sequence with the human genomic DNA sequence in 7q31 revealed six exons spanning 860 kb genomic DNA (fig. 4*A*). Related to the cDNA size, it is, to our knowledge, the largest gene that has been described so far. The first 281 bp of the cDNA are within a gap of the sequenced BAC contig. However, by amplification and sequencing of this region from total human DNA, no further intron-exon structure could be demonstrated.

The putative gene predicted a continuous open reading frame (ORF) of 175 amino acids (aa) (fig. 5; ORF Finder). Seventy-eight nucleotides upstream of the initiation methionine, an in-frame stop codon (TAG) could be found. Moreover, a CpG island encompassing the first 464 bp of the *IMMP2L* gene was identified (CpG island prediction). Besides the *Leu-Rch Rep* gene, no other transcription units could be found in the introns of *IMMP2L*. To determine the RNA expression distribution, we performed RT-PCR analysis. *IMMP2L* was found to be expressed in a wide range of tissues but not in the adult liver and lung. RT-PCR analysis of *IMMP2L* expression is summarized in figure 1.

On the basis of hydropathy analysis using the Web-based prediction programs HMMTOP and Tmpred, the IMMP2L protein contains one predicted N-terminal transmembrane domain (residues 11–30). A protein-sequence search, using the Protein families database of alignments (Pfam), indicates that residues 1–137 resemble signal peptidase I (SPase I). SPase I is a bacterial leader peptidase, anchored in the cytoplasmic membrane by one or two N-terminal transmembrane domains, the function of which is to remove the signal peptides from secretory proteins (Dalbey and von Heijne 1992). Moreover, Nunnari et al. (1993) have shown that SPase I is evolutionarily related to the yeast mitochondrial inner-membrane protease subunits 1 and 2 (genes *IMP1* and *IMP2*).

Within the broader 7q31 breakpoint region, there are several other brain-expressed transcripts, including neuronal cell adhesion molecule (NRCAM), KIAA0716, and CAGH44 (polyglutamine repeat protein from brain) (fig. 4*A*). Interestingly, the gene coding for the Reelin

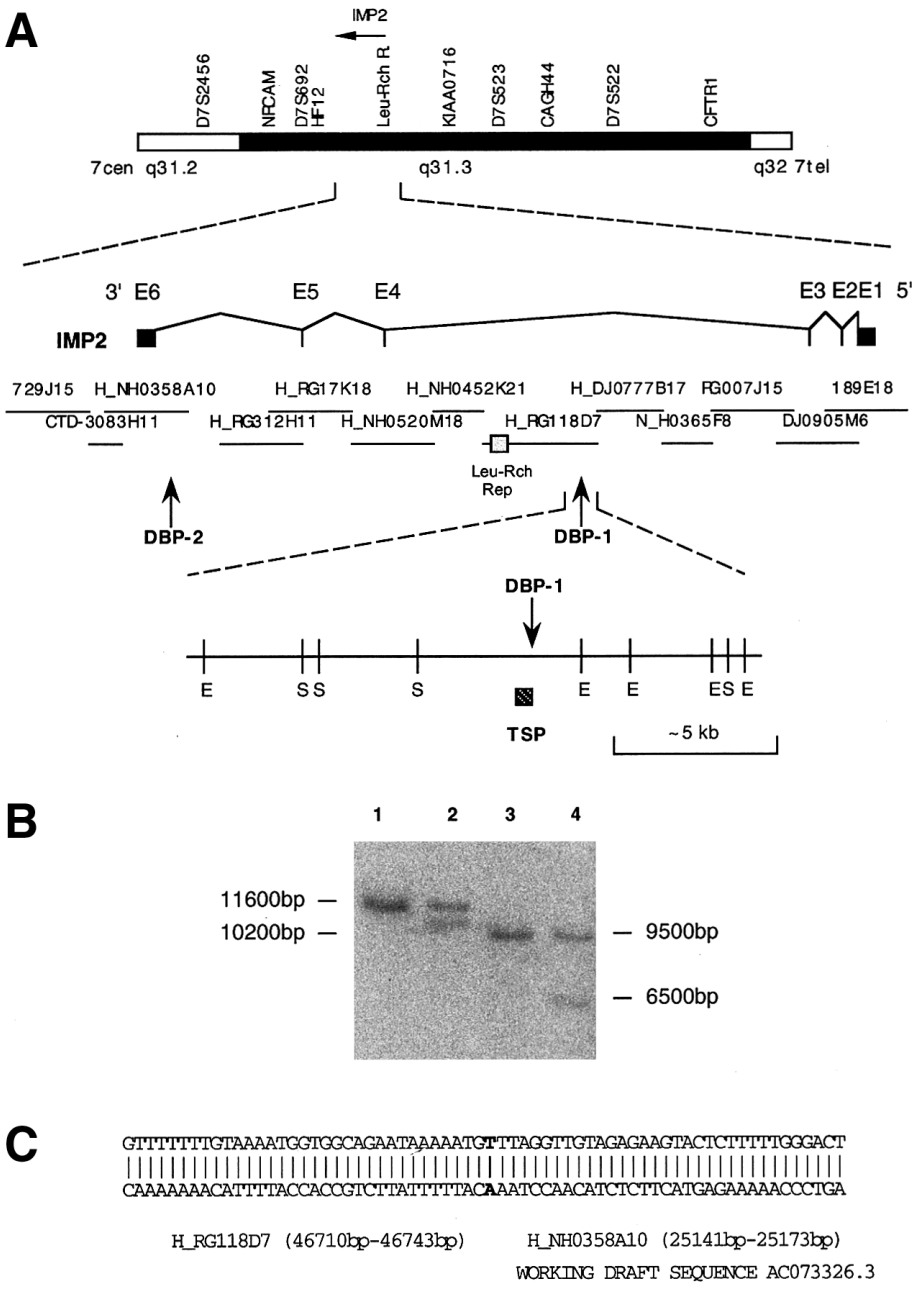

Figure 4 *A*, Refined physical BAC map of the 7q31 breakpoint region and genomic organization of *IMMP2L*. Breakpoints DBP-1 and DBP-2 are indicated by arrows. The probe TSP, used for Southern blot hybridization, is also shown, E: *Eco*RI, S: *Sac*I. The first 281 bp of exon 1 are within a sequence gap. Amplification (forward primer: GTGATCGCGAGCATGTGT; reverse primer: CATGTTGTTCAAGCCTGACG; annealing temperature: 57°C; 1.5 mM $MgCl_2$) and sequencing of this region did not show intron-exon structure. By use of a Web-based repeat masker (REPEATMASKER WEB SERVER), the DBP-1 breakpoint region (~5 kb) was shown to contain four Alu elements, two MaLR elements, one L2 element, one MIR element, and one simple (CAAA)n repeat. Around DBP-2, one Alu element, four L1 elements, and one simple (CAAA)n repeat could be identified. *B*, Southern blot analysis of DNA digested with either *Eco*RI or *Sac*I and probed with the 1,414-bp TSP amplification product (forward primer: GCAACAATAACAAAGAAGGAGG; reverse primer: GCAAAAGGAAATTACAGGGAAC; annealing temperature: 52°C; 1.5 mM $MgCl_2$). Lanes 1 and 3 contain total human control DNA, and lanes 2 and 4 contain total human DNA of the proband. For the *Eco*RI digests (*lanes 1 and 2*), the 11.6-kb band is derived from the normal chromosome 7 and the 10.2-kb rearranged band from the der(7) in the proband. Restriction digestion with *Sac*I (*lanes 3 and 4*) produces a normal 9.5-kb band and a rearranged 6.5-kb band seen only in the proband. *C*, Nucleotide sequence of the subclone from der(7) spanning the breakpoint in 7q31. T–A, in boldface, indicates the base pair where the break occurred.

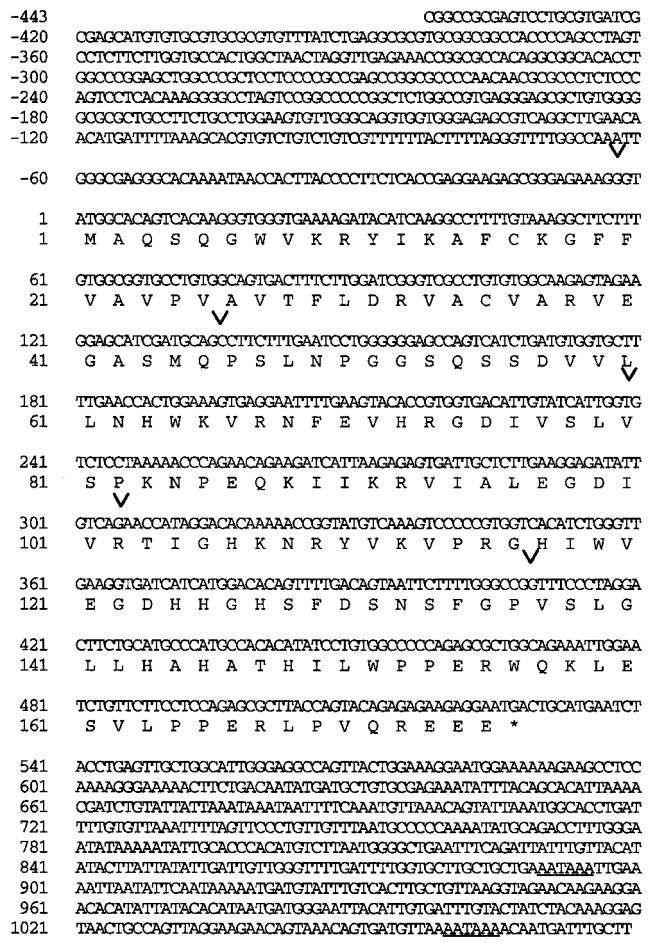

Figure 5 The nucleotide and deduced amino acid sequences of human *IMMP2L* cDNA. The position of the stop codon is indicated by the asterisk, and the two alternative polyadenylation signals are underlined. Exon boundaries are indicated by open arrows.

protein, which was recently reported to cause lissencephaly when mutated (Hong et al. 2000), could be found within the duplicated region.

Identification of the Mouse Homologue of IMMP2L

The mouse gene corresponding to human *IMMP2L* was identified by EST analysis. The complete coding region could be covered by joining the 5′ EST clone BE656276 and the EST clone AI854557, which contained the poly(A)site and 3′ end of the gene. With this as a consensus, an ORF of 175 aa could be identified, which demonstrated 90% aa identity to the predicted human protein (fig. 6). The human IMMP2L protein is also 58% homologous to the *Drosophila melanogaster* IMMP2L protein and 42% homologous to the *Caenorhabditis elegans* IMMP2L protein (fig. 6).

Discussion

We have cloned and sequenced the distal breakpoint and narrowed down the proximal breakpoint of a dup(7)(q22.1-q31.1) associated with GTS and related minor expression of depressions. The distal breakpoint occurred within a region on 7q, common to that described in a familial form of GTS cosegregating with a t(7;18)(q22-q31;q22.3) translocation. Some of these translocation carriers also experienced panic attacks and exhibited aggressive behavioral features. Sequence analysis of the 7q31 breakpoint in our patient confirmed that the rearrangement occurred within a novel gene named "inner mitochondrial membrane peptidase 2 (*IMP2, S. cerevisiae*)–like" (*IMMP2L*) as a result of its similarity to yeast IMP2.

The human *IMMP2L* gene encodes 175 aa and spans ~860 kb of genomic DNA. Ubiquitous expression could be demonstrated by RT-PCR, except in lung and adult liver. Northern analysis showed only very weak transcription in the brain, whereas RT-PCR analysis suggests transcription levels in the brain that are similar to other tissues, such as those of the heart or kidney. Northern analysis also shows strong transcription levels in the liver, whereas RT-PCR showed no liver transcription. According to Nunnari et al. (1993), yeast IMP2 is required for processing of i-cytochrome c1 and for the stable expression of IMP1, which, in turn, is the protease required for the processing of pre-COXII (cytochrome c oxidase subunit II) and i-cytochrome b2. Interestingly, mitochondrial proteins, including COXII, have been associated with the appearance of neurodegenerative disorders such as Friedreich ataxia; Leigh syndrome; myoclonic epilepsy associated with ragged-red fibers; and mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes (Graeber and Muller 1998; Leonard and Schapira 2000) and have been hypothesized in neuropsychiatric disorders (Whatley et al. 1996).

The original copy of the *IMMP2L* gene on the derivative chromosome 7 is directly disrupted by the insertion site (DBP-2), and the duplicated copy of *IMMP2L* is disrupted by the duplication breakpoint (DBP-1). Therefore, only the allele on the normal chromosome 7 can transcribe a full-length mRNA for this gene. The decrease in the *IMMP2L* gene dosage could be responsible for the appearance of GTS features that were in agreement with the proposed autosomal dominant mode of inheritance of GTS (Carter et al. 1994). Although one disrupted *IMMP2L* transcribed potentially encodes 137 aa, it is unlikely that this mRNA would be translated and would remain stable. Confirmation of whether a defective *IMMP2L* gene may predispose to GTS or related disorders should come from

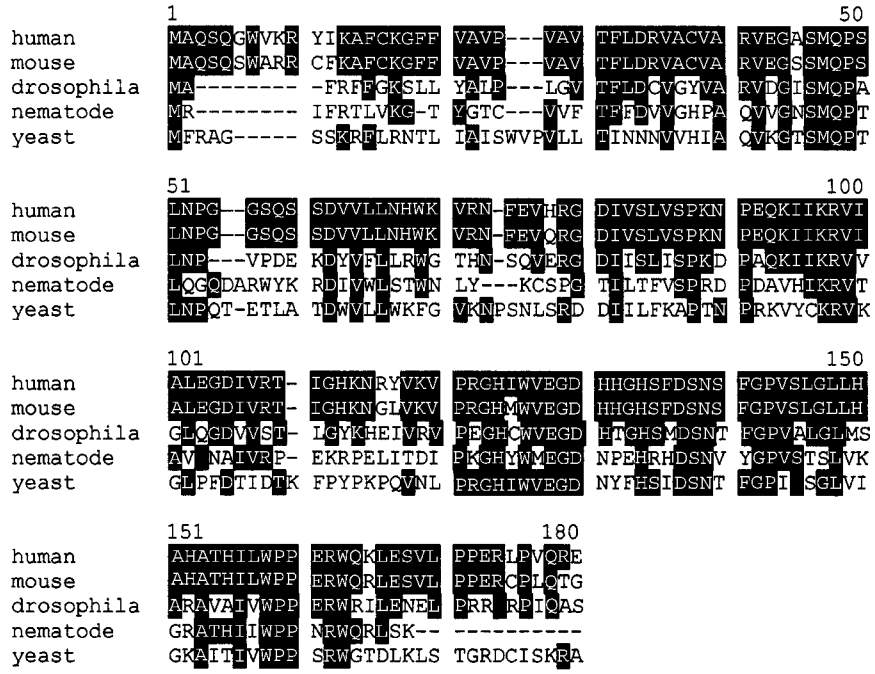

Figure 6 Alignment of human *IMMP2L, Mus musculus IMMP2L, Drosophila melanogaster IMMP2L, Caenorhabditis elegans IMMP2L,* and *Saccharomyces cerevisiae IMP2.* Identical amino acid residues are printed in white on black background. Gaps are introduced to maximize the alignment.

examination of unrelated patients with GTS for point mutations in the *IMMP2L* gene.

Baron-Cohen et al. (1999) presented a large-scale study in the comorbidity of autism and GTS. They concluded that the rate of GTS symptoms in patients with autism disorder (AD) is 6.5%, much higher than would be expected by chance. This is of interest, since it has been shown that gene(s) in the q31 region of chromosome 7 may be involved in the etiology of AD and speech-language disorder (SPCH1). In addition to familial linkage data that suggest an autism susceptibility locus at 7q31, chromosomal breakpoints in the same region in patients were observed (Lai et al. 2000; Vincent et al. 2000; Warburton et al. 2000). Our patient, however, did not show obvious autistic signs and presented only a moderate delay in his speech development, which could be the result of mental retardation. The order of genes and breakpoints relevant to the study of SPCH1 and autism at 7q31 is *D7S2459,* proximal to the *SPCH1* region; *IMMP2L,* dup(7);GTS; *CAGH44,* t(*5*;7);SPCH; *RAY1,* t(7;13);AD; *CFTR,* t(2;7);SPCH; and *D7S643,* distal to the *SPCH1* region.

Several other brain-expressed transcripts could be identified from the autism region (Lai et al. 2000; Vincent et al. 2000). The *Leu-Rch Rep* gene, which is strongly expressed in the cerebellum, could be found ~75 kb proximal to DBP-1 in the nonduplicated region between both 7q31 breakpoints of the derivative chromosome 7. Leu-Rch Rep is similar to a murine leucine-rich repeat protein, which is suggested to play a role in the development of the nervous system by protein-protein interactions (Taniguchi et al. 1996).

At ~1 mb proximal to DBP-2, we found the gene encoding for the NRCAM, a member of the cell adhesion molecules (CAMs), which are a subset of the immunoglobin (Ig) superfamily found in the nervous systems. Interestingly, other members of the CAM family, such as the gene for neural cell adhesion molecule (NCAM) and the opioid-binding cell adhesion molecule (OBCAM), map to 11q22-q23 (Shark and Lee 1995). This chromosomal region is already known to show significant linkage to GTS in a large French Canadian family (Merette et al. 2000). CAGH44 maps ~1 mb distal to the DBP-1 breakpoint. Because of its proximity to a translocation breakpoint segregating with SPCH1, patients were already screened for mutations. However, no expansion of the intragenic CAG repeats or other DNA mutations causing SPCH1 were identified on the currently available partial cDNA of this gene (Lai et al. 2000).

Reelin, a glycoprotein, was found in the duplicated region. This gene is involved in the layering of neurons in the cerebral cortex and cerebellum maps within the duplication in 7q22. Recently, two independent splicing

mutations of this gene have been found to cause lissencephaly when appearing as homozygous (Hong et al. 2000). Impagnatiello et al. (1998) suggested that *Reelin* may play a role in the etiology of schizophrenia. Our proband carries three copies of this gene. The effects of an overexpression of *Reelin* on the development of the brain have not yet been investigated. Further studies are required, to determine what roles *IMMP2L* and the genes within the duplication or near the breakpoints play in the etiology of GTS.

Acknowledgments

We thank Andrea Renner and Jennifer Skaug for their excellent technical assistance. This work was supported by grant OENB 7872 from the Oesterreichische Nationalbank (to E.P.) and by a grant from the Cure Autism Now (CAN) Foundation (to S.W.S.).

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

BLAST, http://www.ncbi.nlm.nih.gov/BLAST/ (for sequence comparison)

CpG island prediction, http://l25.itba.mi.cnr.it/genebin/wwwcpg.pl (for human *IMMP2L*)

Cystic Fibrosis Mutation Data Base, http://www.genet.sickkids.on.ca/ (for YAC and BAC clone information)

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for BACs, ESTs, and gene sequences)

HMMTOP: Prediction of transmembrane helices and topology of proteins, http://www.enzim.hu/hmmtop/

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

ORF Finder (Open Reading Frame Finder), http://www.ncbi.nlm.nih.gov/gorf/gorf.html (for identifying ORFs in cloned cDNA)

Primer 3 Software Distribution, http://www.genome.wi.mit.edu/genome_software/other/primer3.html (for primer development)

Protein families database of alignments and HMMs, http://www.sanger.ac.uk/Pfam/

REPEATMASKER WEB SERVER, http://ftp.genome.washington.edu/cgi-bin/RepeatMasker (for repeat masking of BAC clones)

TMpred: Prediction of Transmembrane Regions and Orientation, http://www.ch.embnet.org/software/TMPRED_form.html

UniGene Resources, http://www.ncbi.nlm.nih.gov/UniGene/ (for Hs.13058)

References

Baron-Cohen S, Scahill VL, Izaguirre J, Hornsey H, Robertson MM (1999) The prevalence of Gilles de la Tourette syndrome in children and adolescents with autism: a large scale study. Psychol Med 29:1151–1159

Barr CL, Sandor P (1998) Current status of genetic studies of Gilles de la Tourette syndrome. Can J Psychiatry 43: 351–357

Boghosian-Sell L, Comings DE, Overhauser J (1996) Tourette syndrome in a pedigree with a 7;18 translocation: identification of a YAC spanning the translocation breakpoint at 18q22.3. Am J Hum Genet 59:999–1005

Carter AS, Pauls DL, Leckman JF, Cohen DJ (1994) A prospective longitudinal study of Gilles de la Tourette's syndrome. J Am Acad Child Adolesc Psychiatry 33:377–385

Curtis D, Robertson MM, Gurling HM (1992) Autosomal dominant gene transmission in a large kindred with Gilles de la Tourette syndrome. Br J Psychiatry 160:845–849

Dalbey RE, von Heijne G (1992) Signal peptidases in prokaryotes and eukaryotes: a new protease family. Trends Biochem Sci 17:474–478

Donnai D (1987) Gene location in Tourette syndrome. Lancet 1(8533):627

Freeman RD, Fast DK, Burd L, Kerbeshian J, Robertson MM, Sandor P (2000) An international perspective on Tourette syndrome: selected findings from 3,500 individuals in 22 countries. Dev Med Child Neurol 42:436–447

Graeber MB, Muller U (1998) Recent developments in the molecular genetics of mitochondrial disorders. J Neurol Sci 153:251–263

Hong SE, Shugart YY, Huang DT, Shahwan SA, Grant PE, Hourihane JO, Martin ND, Walsh CA (2000) Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. Nat Genet 26:93–96

Impagnatiello F, Guidotti AR, Pesold C, Dwivedi Y, Caruncho H, Pisu MG, Uzunov DP, Smalheiser NR, Davis JM, Pandey GN, Pappas GD, Tueting P, Sharma RP, Costa E (1998) A decrease of reelin expression as a putative vulnerability factor in schizophrenia. Proc Natl Acad Sci USA 95: 15718–15723

Kroisel PM, Petek E, Emberger W, Windpassinger C, Wladika W, and Wagner K. Candidate region for Gilles de la Tourette syndrome at 7q31. Am J Med Genet (in press)

Lai CS, Fisher SE, Hurst JA, Levy ER, Hodgson S, Fox M, Jeremiah S, Povey S, Jamison DC, Green ED, Vargha-Khadem F, Monaco AP (2000) The SPCH1 region on human 7q31: genomic characterization of the critical interval and localization of translocations associated with speech and language disorder. Am J Hum Genet 67:357–368

Leonard JV, Schapira AH (2000) Mitochondrial respiratory chain disorders II: neurodegenerative disorders and nuclear gene defects. Lancet 355(9201):389–394

Megarbane A, Gosset P, Souraty N, Lapierre JM, Turleau C, Vekemans M, Loiselet J, Prieur M (2000) Chromosome 7q22-q31 duplication: report of a new case and review. Am J Med Genet 95:164–168

Merette C, Brassard A, Potvin A, Bouvier H, Rousseau F, Emond C, Bissonnette L, Roy MA, Maziade M, Ott J, Caron C (2000) Significant linkage for Tourette syndrome in a large French Canadian family. Am J Hum Genet 67:1008–1013

Nunnari J, Fox TD, Walter P (1993) A mitochondrial protease with two catalytic subunits of nonoverlapping specificities. Science 262:1997–2004

Petek E, Kroisel PM, Wagner K (1997) Isolation of site-specific insert probes from chimeric YACs. Biotechniques 23:72 ,74, 77

Robertson MM (1994) Annotation: Gilles de la Tourette syndrome: an update. J Child Psychol Psychiatry 35:597–611

Shark KB, Lee NM (1995) Cloning, sequencing and localization to chromosome 11 of a cDNA encoding a human opioid-binding cell adhesion molecule (OBCAM). Gene 155: 213–217

Taniguchi H, Tohyama M, Takagi T (1996) Cloning and expression of a novel gene for a protein with leucine-rich repeats in the developing mouse nervous system. Brain Res Mol Brain Res 36:45–52

Telenius H, Carter NP, Bebb CE, Nordenskjold M, Ponder BA, Tunnacliffe A (1992) Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics 13:718–725

Vincent JB, Herbrick JA, Gurling HM, Bolton PF, Roberts W, Scherer SW (2000) Identification of a novel gene on chromosome 7q31 that is interrupted by a translocation breakpoint in an autistic individual. Am J Hum Genet 67: 510–514

Warburton P, Baird G, Chen W, Morris K, Jacobs BW, Hodgson S, Docherty Z (2000) Support for linkage of autism and specific language impairment to 7q3 from two chromosome rearrangements involving band 7q31. Am J Med Genet 96: 228–234

Whatley SA, Curti D, Marchbanks RM (1996) Mitochondrial involvement in schizophrenia and other functional psychoses. Neurochem Res 21:995–1004