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ARTICLE

Breakpoint sequences of an 1;8 translocation in a family with Gilles de la Tourette syndrome

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Gilles de la Tourette syndrome (GTS) is a common, heritable neurological disorder manifested by chronic motor and vocal tics with childhood onset. Previous extensive linkage analysis failed to identify a GTS gene based on an autosomal dominant pattern of inheritance. Recently, a family was reported with a balanced chromosomal translocation t(1;8)(q21.1;q22.1) in family members with GTS or tics. Chromosome 8q22.1 was previously implicated in GTS by both association and linkage results. We therefore cloned and sequenced both translocation breakpoints from this family. The *CBFA2T1* gene was identified 11 kb distal to the 8q22.1 breakpoint. Sequencing of *CBFA2TI* exons within 37 unrelated GTS patients failed to identify any mutations. However, it is possible that the translocation altered the expression of this gene or another nearby gene. Examination of the breakpoint sequences revealed a duplication of six nucleotides from chromosome 8 but no change in the chromosome 1 sequence. The sequences immediately flanking the breakpoints on the two chromosomes were modestly similar, but the breakpoints did not occur within known interspersed repeats. Our results add to our knowledge of the genetics of GTS and the mechanisms of balanced chromosomal translocations. *European Journal of Human Genetics* (2000) **8**, 875–883.

Keywords: Gilles de la Tourette syndrome; chromosomal translocation; positional cloning; chromosome 8; *CBFA2T1*

Introduction

Gilles de la Tourette Syndrome (GTS) (MIM137580) is characterized by chronic involuntary motor and vocal tics with childhood onset.[1] GTS can also present with obsessive-compulsive disorder and attention deficit disorder. Strong heritability of GTS has been concluded from both twin and family studies.[2,3] Concordances for full GTS were 53% or 56% for monozygotic (MZ) and 8% for dizygotic (DZ) twins. If chronic motor tics were included in the diagnoses, concordances would be 77% or 94% for MZ and 23% for DZ twins, respectively.[4,5] Initially, GTS was predicted to be inherited in an autosomal dominant fashion with incomplete penetrance and variable expression,[6–8] but extensive linkage analysis failed to identify a gene based on this mode of inheritance.[9–11] This could be due to uncertain phenotype spectrum, locus heterogeneity, or polygenic inheritance. Recent segregation studies suggested a complex model of inheritance.[12,13] Furthermore, clinical evidence of genomic imprinting and bilineal transmission have been reported.[14,15]

More recently, results of whole genome sib pair and association studies have been reported. Using 110 affected sib pairs, The Tourette Syndrome Genetic Consortium[16] reported evidence for linkage on chromosomes 8p and 4q. Simonic *et al*[17] reported association of GTS among South African Afrikaners to chromosomes 2, 6p, 8q, 11q, 14q, 20q and 21q.

At least five chromosomal abnormalities have been reported in GTS patients. These include a 9p deletion,[18] 18q22.2 deletion,[19] t(7;18)(q22;q22.3),[20] t(3;8) (p21.3; q24.1),[21] and most recently a t(1;8)(q21.1;q22.1).[22] The last report involved a nuclear family in which the father and six of the seven children shared the balanced (1;8) translocation. Of the six children with the translocation, one had full blown GTS, one displayed modest tics and Asperger's syndrome, two

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had mild tics, and the two youngest were unaffected but were also below the average age of onset.

The site of the chromosome 8q22.1 breakpoint within the t(1;8) family was the same, within confidence intervals, as the 8q association region reported by Simonic *et al*.[17] This same region was also implicated by linkage analysis in a large Utah GTS kindred.[23] We therefore set out to map the 8q22.1 breakpoint within the translocation family. We report here the cloning and sequencing of both the chromosome 1 and 8 breakpoints from this family and the identification of the *CBFA2T1* gene 11 kb distal of the 8q breakpoint.

Materials and methods

Fluorescence *in situ* hybridization

FISH using BAC, cosmid and phage DNA was performed on metaphases of the patients with the 1;8 translocation and a normal control. Chromosome preparations were made from lymphoblastoid cell lines by conventional methods. Slides were incubated in 2× SSC at 37°C for 30 min, serially dehydrated in 70%, 80%, and 95% ethanol at room temperature, denatured in 70% formamide/2× SSC at 72°C for 2 min, then serially dehydrated at –20°C in 70%, 80%, 90%, and 100% ethanol. Clone DNAs were labeled with digoxigenin-11-dTUP (Boehringer-Mannheim, Indianapolis, IN, USA) by nick translation, precipitated in ethanol with a 50X excess of human Cot-1 and herring testis DNA (Gibco–BRL, Gaithersburg, MD, USA), and resuspended to a final concentration of 20 ng/μl in hybridization solution (50% formamide, 2× SSC, 10% dextran sulfate). Probes were denatured at 76°C for 10 min and preassociated at 37°C for 15 min prior to hybridization. The probe–hybridization mix (10 μl) was applied under a 22 mm × 22 mm coverslip and slides were incubated in a moist chamber for 16 h at 37°C, then washed as previously described.[24] Probes were detected at 50 μl of rhodamine anti-digoxigenin (Boehringer-Mannheim) at 1 μg/μl. Slides were washed three times in 4× SSC, 0.1% Tween-20 at 45°C and mounted in antifade solution (Vector, Burlingame, NY, USA) containing DAPI. Analysis was performed using a (Zeiss, Thornwood, NY, USA) Axiophot microscope equipped with filters to detect DAPI and rhodamine separately, as well as a triple band pass filter (Chroma Technology Corporation, Brattleboro, VT, USA) to detect signals simultaneously. Images were collected and merged using a cooled CCD camera (KAF 1400, Photometrics, Tucson, AZ, USA) and Quips M-FISH software (Vysis Inc, Downers Grove, IL, USA).

Isolation of BACs, cosmids, phages and plasmids

Human BAC libraries (Research Genetics, Huntsville, AL, USA; Genome Systems, St Louis, MO, USA) were screened using PCR. The size of BACs spanning the breakpoints was determined by pulsed-field gel electrophoresis analysis as described previously.[25]

Cosmid and phage sublibraries were prepared from BACs spanning the breakpoint. Purified BAC DNA was isolated using Qiagen Midi-Prep columns (Chatsworth, CA, USA) and digested partially with *Sau*3AI. The SuperCos1 cosmid vector was prepared according to manufacturer's instructions (Stratagene, La Jolla, CA, USA) and ligated to the digested BAC DNA overnight using T4 DNA ligase. Lambda FIXII/*Xho*I vector (Stratagene) after partial filling-in was also ligated to BAC DNA. Each ligation reaction was packaged using the Gigapack III gold extract (Stratagene) and transfected into XL1-Blue MR host cells (Stratagene) for cosmid or XLI-Blue MRA (P2) host cells (Stratagene) for phage, respectively.

Plasmid subclones for sample sequencing were also prepared from BAC DNA. BAC DNA was isolated using Qiagen Midi-Prep columns and sheared using an ultrasound sonicator. Fragments of 1–2 kb length were cut from an agarose gel, extracted with GenElute™ agarose spin columns (Supelco, Bellefonte, PA, USA), and end-polished with mung bean nuclease. Fragments were ligated into *Eco*RV-digested pBluescript SK(-) using T4 DNA ligase. Epicurian Coli SoloPack™ supercompetent cells (Stratagene) were used for transformation.

Construction of cosmid and phage contig

Cosmid DNAs were extracted by an automatic nucleic acid isolation system (AutoGen 740, Integrated Separation Systems, Natick, MA, USA), and phage DNAs were isolated using a Qiagen Lambda mini kit. A total of 24 cosmid clones and 50 phage clones were randomly chosen and used for construction of a contig covering the breakpoint. The contig was constructed by means of STS content mapping using PCR. New STSs were generated from clone end sequences and *CBFA2T1* exon-specific primers (GenBank accession numbers AF018270-AF018282).

Isolation of cosmids spanning the translocation breakpoint

High molecular weight genomic DNA was prepared from the GTS patient's lymphoblastoid cell line, partially digested with *Sau*3AI, and ligated to SuperCos1 vector. The ligated products were packaged using the Gigapack III gold extract, and transfected into XL1-Blue MR host cells (Stratagene). A 2.5-genome fold library was plated on 130 mm dishes, transferred to nylon membranes, and hybridized with two probes flanking the 8q22.1 breakpoint. These probes were PCR products prepared using the following two pairs of primers: 41781F3 (5'-TTT CAC TCT ACC TCT GAC AG-3')/WI-13899-W1 (5'-GCA TAA GTA AAC CAG CCT TG-3') and 4.7 kb-W1 (5'-TCT TCC CAA TTA CTA ACT AG-3')/cos9T3-W7 (5'-TCA TGA GGC TTA AAG GAG GG-3'). Primers 41781F3/WI-13899-W1 turned out to be 9.1 kb telomeric to the breakpoint, and primers 4.7 kb-W1/cos9T3-W7 were 8.0 kb centromeric to the breakpoint. Hybridization was carried out in ExpressHyb™ Hybridization Solution (Clontech, Palo

Alto, CA, USA) according to the manufacturer's protocol. Blots were washed in 0.1 × SSC/0.1 × SDS at 50°C.

DNA sequencing

BAC, cosmid, and plasmid DNAs were extracted with the AutoGen 740 and purified with Microcon 100 spin columns (Amicon Inc, Beverly, MA, USA). Phage DNA was extracted using a Qiagen lambda mini kit. PCR products were purified using the QIAquick PCR purification kit (Qiagen). Sequencing reactions were carried out with the ABI Prism™ BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems Inc, Foster, CA, USA) using 500 ng of BAC, cosmid, phage, or plasmid DNA or 90 ng of DNA from PCR product as template and 40 pmol (BAC, cosmid, and phage) or 4 pmol (plasmid and PCR product) primer. Products were analyzed on an ABI 377 automated sequencer. Cycle sequencing was performed for 50 cycles (BAC, cosmid, and phage) or 25 cycles (plasmid and PCR) at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. For complete sequencing of cosmids spanning the breakpoints, a primer walking strategy was adopted. For clone end-sequencing, T7 and SP6 primers for BAC, or T7 and T3 for cosmid, phage, and plasmid were used.

Computational analysis of sequences

Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) was used for generating new STSs from the sequences obtained from clone ends after excluding repetitive DNA sequences such as Alu or L1 with BLASTN (http: // www.ncbi.nlm.nih.gov / cgi-bin / BLAST / nph-newblast?Jform = 0). For analysis of entire cosmid sequences, RepeatMasker (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker) was first used for elimination of the repetitive sequences. Then the exon prediction programs GENSCAN (http://ccr-081.mit.edu/GENSCANMIT.html), FGENE (http://genomic.sanger.ac.uk/gf/gf.shtml), and GRAIL2 (http://dmg.nott.ac.uk/molbio/replaced1198/INTRON.HTM) were used to detect potential exons. BLASTN searches were done in GenBank + EMBL + DDBJ sequence databases along with dbEST. BLAST2 analysis (http://www.ncbi.nlm.nih.gov/gorf/b12.html) was performed for comparison of sequences of two cosmids spanning 1q21.1 and 8q22.1 breakpoints.

Results

Isolation of BACs spanning the 8q22.1 breakpoint

To identify BACs spanning the chromosome 8 translocation breakpoint in the GTS family, we isolated at least one BAC clone encompassing each of 10 short tandem repeat polymorphisms (STRPs) near 8q22.1: ATA19G07 (D8S1119), Mfd185A (D8S167), Mfd45A (D8S88), AFM165yb10 (D8S271), AFM179yf6 (D8S273), AFM287wa5 (D8S1800), AFM165xh4 (D8S270), GATA28F12, GAAT1A4, and GATA26A08 (D8S1131) (www.marshmed.org/genetics). FISH was then performed by hybridizing DNA from each of these BACs to metaphase chromosome preparations from the GTS patient. Six BACs encompassing ATA19G07, Mfd185A, Mfd45A, AFM165yb10, AFM179yf6, and AFM287wa5 were mapped proximal to the breakpoint; three BACs encompassing GATA28F12, GAAT1A4, and GATA26A08 were distal, and two BACs (RG302N1 and RG127D17) encompassing AFM165xh4 spanned the 8q22.1 breakpoint (Figure 1a). These two BACs were also shown to span the chromosome 8 breakpoint in the father and the child with Asperger's syndrome. The DNA inserts of RG302N1 and RG127D17 were 140 kb and 120 kb, respectively.

Identification of ESTs within the chromosome 8 breakpoint BACs

ESTs which map close to AFM165xh4 were identified using the NCBI GeneMap'99 resource (www.ncbi.nlm.nih.gov/genemap/). PCR was used to confirm the presence of the *CBFA2T1* gene (also known as *MTG8*) and WI-13899 (Unigene Hs.144995) on the two BACs spanning the chromosome 8 breakpoint. The *CBFA2T1* gene encodes a putative transcription factor and is often joined to the *AML1* gene on chromosome 21 in acute myeloid leukemias.[26,27] The genomic structure of *CBFA2T1* has previously been characterized.[28] The gene has a total of 13 exons distributed over 87 kb of genomic DNA with transcription orientation from telomere to centromere.[26] We examined which portions of this gene were present on the two breakpoint BACs by PCR using primers specific for each exon (GenBank accession nos AF018270–AF018282). RG302N1 contained *CBFA2T1* exons 1a to 11, and RG127D17 had exons 2 to 11. We also characterized one WI-13899 cDNA clone, IMAGE 37404. The 1.6 kb long sequence of IMAGE 37404 did not show any long open reading frames (ORFs). This EST also did not have any introns within the genomic sequence of BAC RG127D17 (see below). Sample sequencing from RG127D17 failed to identify any additional genes. A total of 96 shotgun plasmid clones was sequenced at both ends, and a BLASTN search was performed, but no genes other than *CBFA2T1* were found.

Construction of a cosmid and phage contig for BAC RG127D17

A complete cosmid and phage contig for BAC clone RG127D17 was constructed by means of STS content mapping using PCR (Figure 2). Besides WI-13899-3', and *CBFA2T1* exons, 2, 5, 6, 8, 10 and 11, six new STSs, RG127D17SP6, RG127D17T7, P18T3, P24T3, cos9T3, and cos4T7, were developed from end sequences of cosmid and phage subclones. FISH mapping revealed that cosmid 9 and phages 18, 21 and 24 were located proximal to the translocation breakpoint; cosmid 4 and phages 1, 3, 15, 30 and 36 were distal; and cosmid 10 spanned the breakpoint.

Characterization of the cosmid 10 sequence

The complete 32 884 bp sequence of cosmid 10 was determined (GenBank Accession AF198490) and was characterized by computational methods (Figure 3a). Human endogenous

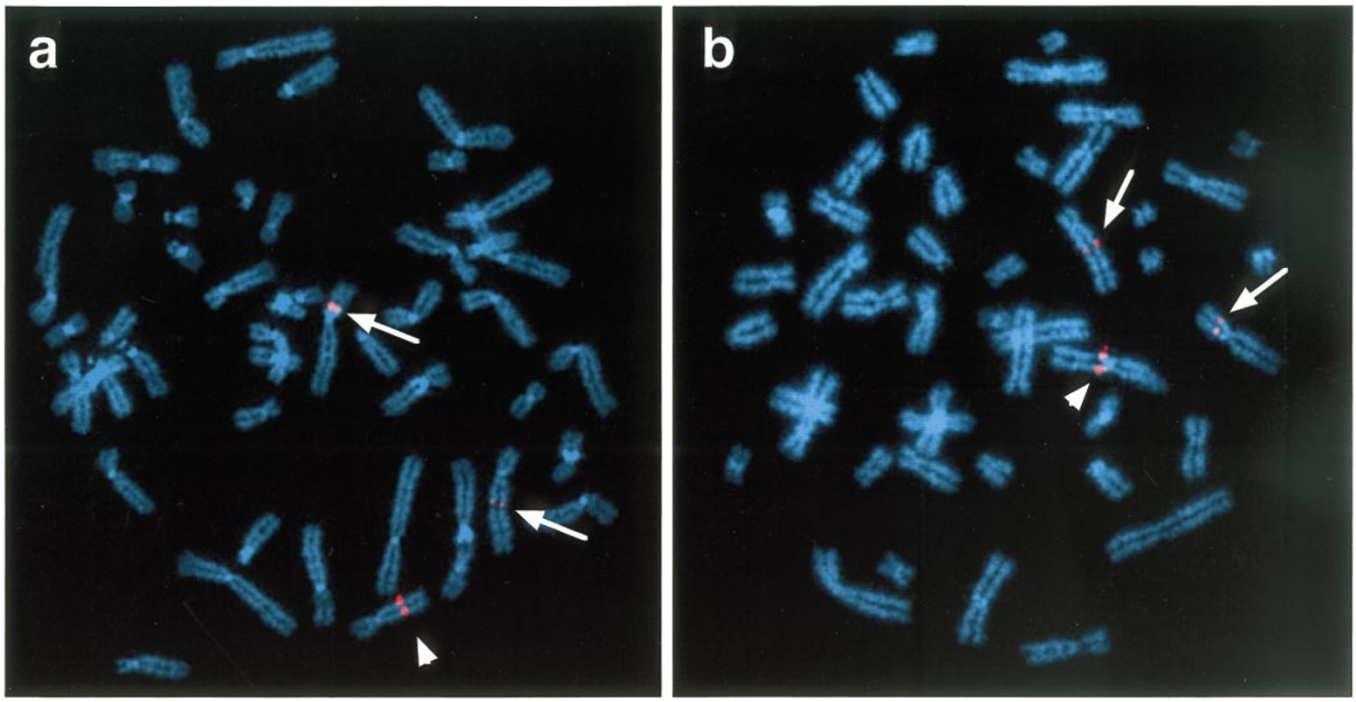

Figure 1 FISH analysis of BACs spanning the breakpoint on the GTS patient. Arrowheads show normal chromosome 8 or 1, arrows indicate derivative chromosomes. The probes are **a** RG127D17 spanning the 8q22.1 breakpoint, and **b** GS156E10 spanning the 1q21.1 breakpoint.

retroviral element H (HERVH) was identified by RepeatMasker. Exon prediction programs indicated several potential exons, but none of them could be confirmed by BLASTN searches to dbEST except for the *CBFA2T1* exon 11. The whole cosmid sequence after masking repetitive sequences with RepeatMasker was used for BLASTN search to dbEST. Hits on two Unigene clusters, Hs. 90858 and Hs. 144995 were obtained. Hs. 90858 and Hs. 14495 are located 8.7 kb and 7.2 kb distal, respectively, from the breakpoint (see below). A total of 5 IMAGE clones (clones IMAGE 783857, IMAGE 41781, IMAGE 25023 and IMAGE 1692252 from Hs. 90858 and clone IMAGE 37404 from Hs. 144995) were sequenced. These sequences showed no long ORFs, and were identical to the corresponding genomic sequences from cosmid 10 (data not shown). Furthermore, we did not find any expression in fetal tissues by northern analysis using PCR probes from these two Unigene clusters (data not shown). Thus we could not identify any genes spanning the 8q22.1 breakpoint. The last *CBFA2T1* exon (exon 11) was 11 kb distal to the breakpoint.

Cloning of the cosmid containing the 8q22.1 junction fragment

A cosmid library was constructed from genomic DNA of the GTS patient and screened with two PCR probes, 41781F3/WI-13899-W1 and 4.7 kb-W1/cos9T3-W7 (see Methods). One cosmid clone, cos1GTS, was identified which contained the 4.7 kb-W1/cos9T3-W7 probe. Partial sequence analysis of cos1GTS using the same walking primers used for cosmid 10 sequencing revealed that cos1GTS contained some sequence completely different from sequences of cosmid 10 and the cosmid vector. PCR primers were designed using this novel sequence, ch1-W1-F(5'-GCG TTC ATG AAT GAG GTC CC-3')/ch1-W1-R(5'-CTG TTT GAA AAT GCC CAA AA-3'), and were used to screen a panel of monochromosomal hybrid DNAs. This screen was positive only for chromosome 1 DNA (data not shown). Furthermore, FISH analysis of the cos1GTS on normal controls showed signals both at 1q21.1 and 8q22.1 (data not shown).

Identification and characterization of BAC and cosmid clones spanning the 1q21.1 breakpoint

A total of three BAC cones, GS68O16, GS141F1, and GS156E10 were isolated by PCR screening of a normal human BAC library with chromosome 1 primers from the cos1GTS (ch1-W1-F/ch1-W1-R). FISH analysis of these three clones on the patient's metaphases showed that all clones spanned the 1q21.1 breakpoint (Figure 1b). The insert DNAs of GS68O16, GS141F1, and GS156E10 were 40, 110, and 130 kb, respectively. A cosmid sublibrary was constructed from GS156E10 DNA. From 24 randomly chosen cosmid clones, one (1cos4) was identified by PCR primers using primers ch1-W1-F/ch1-W1-R and confirmed to span the

breakpoint by FISH. Note that 1cos4 is from normal chromosome 1 and is different from chromosome 8 cosmid 4 shown in Figure 2.

Cosmid 1cos4 was sequenced completely by a primer walking strategy (GenBank Accession AF198494). Partial sequence from cos1GTS was also added to the 1cos4 sequence. A 36 972 bp sequence covering the 1q21.1 breakpoint was thereby obtained and characterized (Figure 3b). A sequence similar to the *KIAA0454* gene (which also maps to chromosome 1) was found 21 kb proximal to the 1q21.1 breakpoint. This homologue was identified by FGENE and GENSCAN exon-finding programs but not by Grail 2. The *KIAA0454* gene was originally isolated from a size-fractionated human brain cDNA library.[29] The sequence from 1cos4 of 766 bp of connected exons showed 97% identity with *KIAA0454* at the nucleotide level, and its assumed translation product showed 97% identity with KIAA0454 over 237 amino acids. BLASTN search of this 766 bp sequence to dbEST failed to indentify any perfect match ESTs. Furthermore, expression of the homologue was not confirmed in fetal brain cDNAs by RT-PCR analysis, even though *KIAA0454* expression was easily detected (data not shown). This homologue may therefore be an unprocessed pseudogene of *KIAA0454*.

Sequence comparison of the breakpoints of der(1) and der(8) chromosomes

Primers cos10T7-W7 (5'-GTT TCC TTC TAA GCA ACA CA-3') at 8q22.1 and der(1)R (5'-GAG GTA ACT TAA CAC TTT CAG-3') at 1q21.1 were used for PCR amplification of the der(1) breakpoint. PCR product was obtained and sequenced from the GTS patient. As expected, no product could be amplified from a normal control. The der(8) breakpoint sequence was obtained from cos1GTS as described above. Sequences of normal chromosome 1, 8, der(1), and der(8) are aligned in Figure 4a. A six nucleotide sequence, 5'-CTTCAC-3', from chromosome 8 immediately flanking the breakpoints was duplicated during the translocation process (underlined in Figure 4a). Breakpoint sequences from the father in the translocation family, the GTS patient, and the child with Asperger's syndrome were also compared by PCR direct sequencing. Two sets of primers, cos10T7-W7/der(1)R for the der(1) breakpoint, and ch1F (5'-TGT CAA TAT GCA ATT AAA

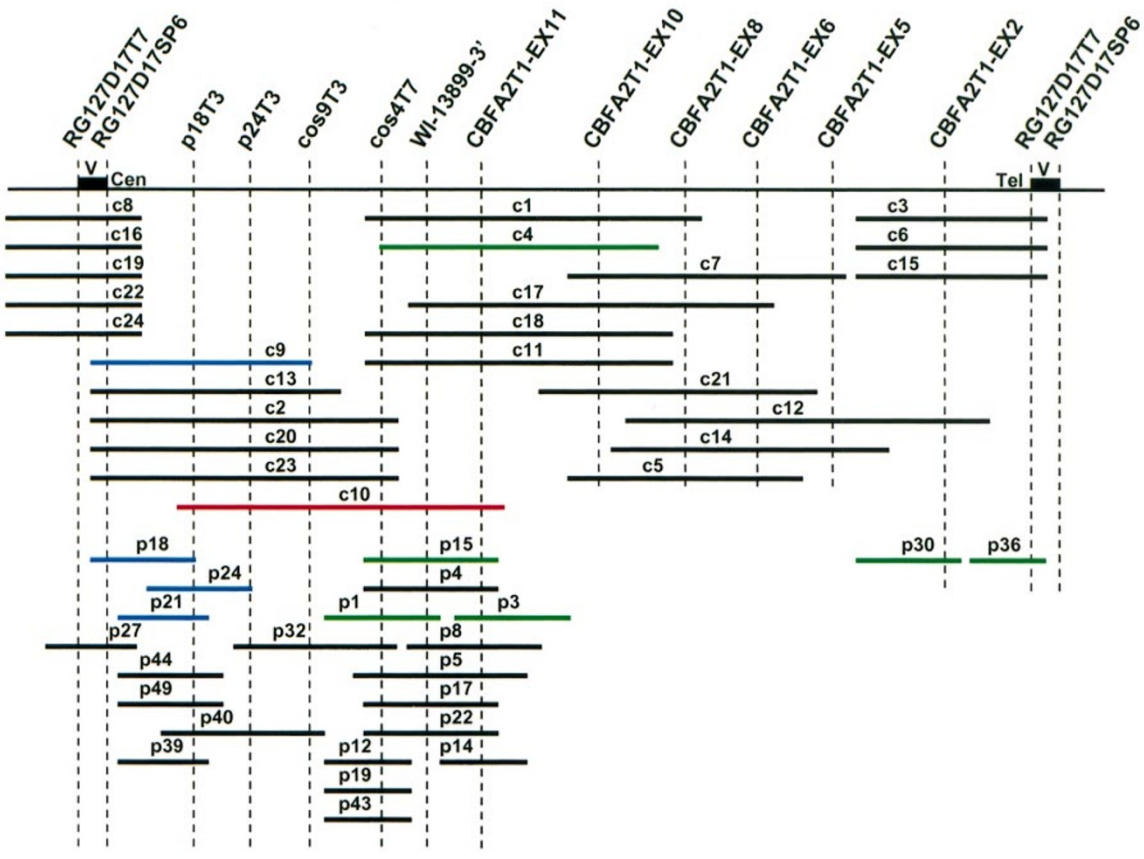

Figure 2 Cosmid and phage contig crossing the 8q22.1 breakpoint. Markers including STS and *CBFA2T1* exons are indicated at the top. Clone numbers beginning with c indicate cosmid, p indicates phage. FISH experiments showed cosmid 9 and phages 18, 21 and 24 (shown in blue) were mapped proximal to the breakpoint, cosmid 4 and phages 1, 3, 15, 30 and 36 (green) mapped distal, and cosmid 10 (red) spanned the breakpoint. V: BAC vector sequence, Cen: centromere, Tel: telomere.

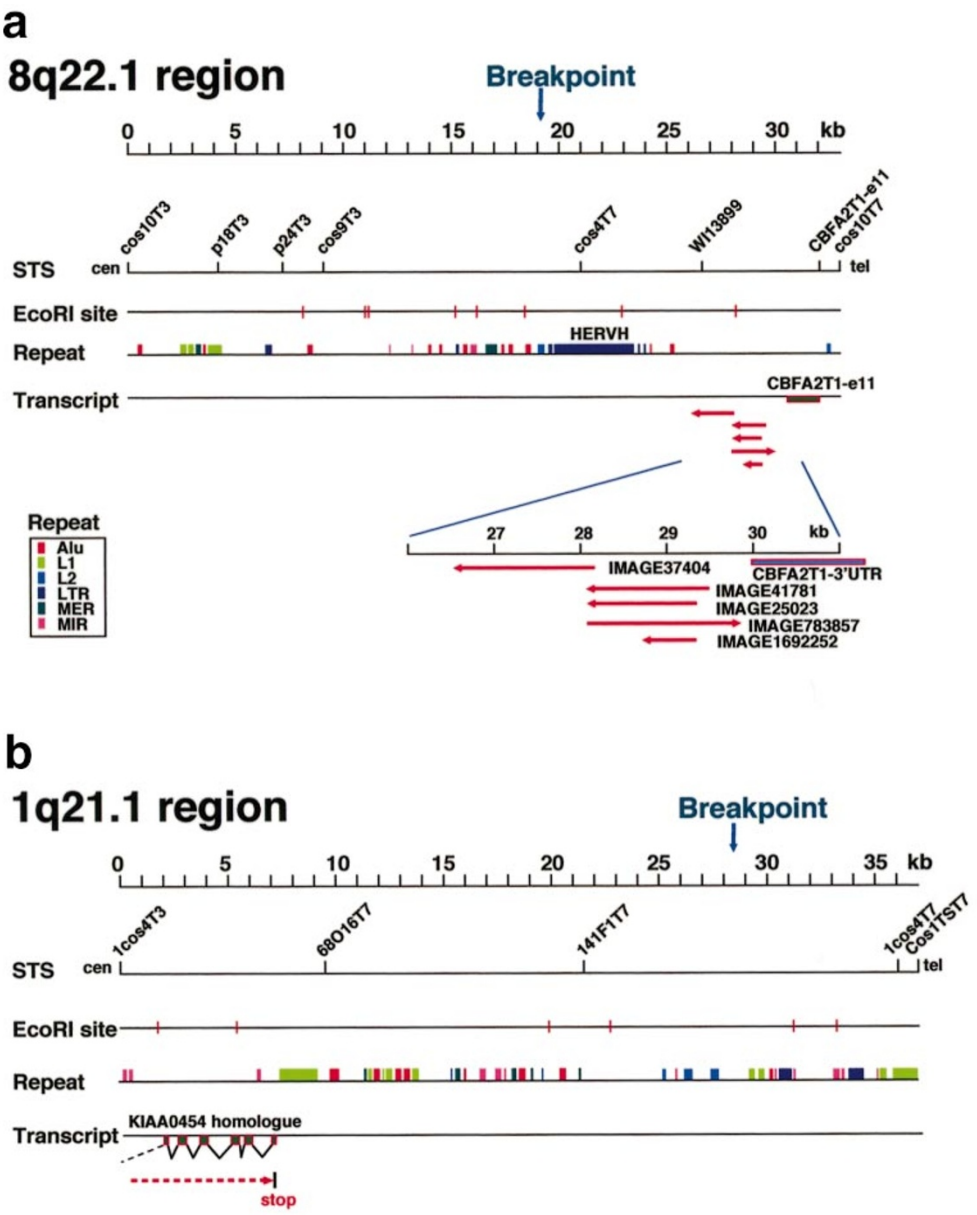

Figure 3 **a** Sequence characterization of cosmid 10 spanning the 8q22.1 breakpoint. Markers, *Eco*RI sites, repetitive sequences identified by RepeatMasker (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker), and transcripts are indicated separately. The *CBFA2T1* gene (exon 11) was 11 kb distal to the breakpoint **b** Sequence characterization of the 1cos4 spanning the 1q21.1 breakpoint. The same colors as in **a** were used to show repetitive sequences. A new gene homologous to *KIAA0454* was found 21 kb proximal to the breakpoint.

TAG AAG AGG-3')/der(8)R (5'-AGC TTT ACA GCC ACT TCC CT-3') for the der(8) breakpoint were used. Breakpoint sequences of both der(1) and der(8) for all three individuals were identical and therefore appear to be stable in this family. BLAST2 comparison of cosmid 10 (8q22.1) and 1cos4 (1q21.1) sequences revealed several interspersed repetitive elements, such as Alu and L1 repeats, which were shared by both clones, but none of these repeats were close to the breakpoints. The two chromosomes did, however, show moderate similarity (62–69%) in short unique sequences (26–29 bp) immediately flanking the breakpoints (Figure 4b).

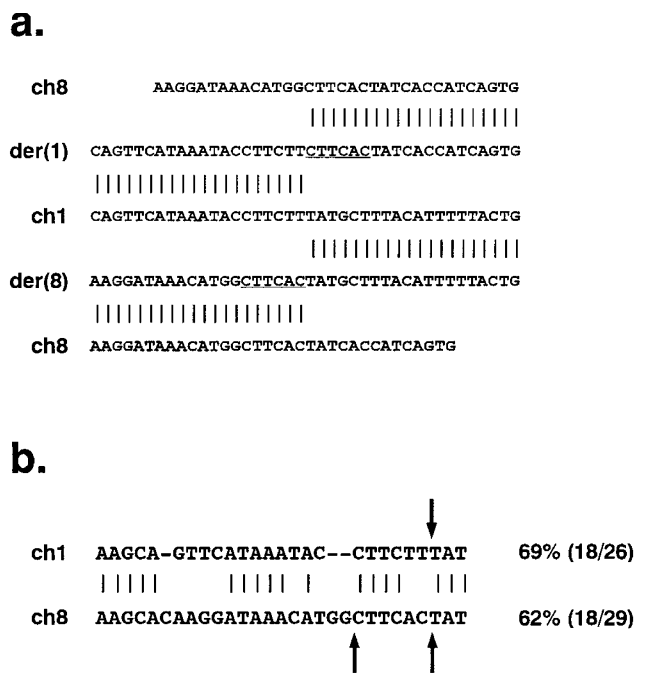

Figure 4 **a** Sequence comparison of normal chromosomes 1 and 8, der(1), and der(8) at translocation breakpoints. Underlining indicates a duplication of six nucleotides from chromosome 8 **b** Short sequence (26–29 bp) of similarity at breakpoints. Arrows mark chromosomal breakage sites.

Mutation analysis of the *CBFA2T1* gene in 37 GTS patients

DNA from a total of 37 unrelated GTS patients was screened for mutations within all *CBFA2T1* exons. The patients came from the Tourette Syndrome Association affected sib pair study[16] and from the South African Afrikaner study.[17] No nucleotide substitutions, deletions, or insertions were identified in the coding regions. One polymorphism, a T/C transition in intron 10, 35 bp upstream of exon 11, was found (nucleotide 67 within GenBank sequence AF018282: AGTGTATTAA [T/C] TTGGGATCTT). However, the allele frequencies for this polymorphism were not significantly different in patients and controls. T allele frequencies were 45% in patients and 50% in controls.

Discussion

We have characterized the translocation breakpoints from a GTS family with a balanced (1;8)(q21.1;q22.1) translocation. Although we did not find any genes that were directly disrupted by the breakpoints, we did identify the *CBFA2T1* gene 11 kb distal to the 8q22.1 breakpoint and a sequence closely related to the *KIAA0454* gene 21 kb proximal to the 1q21.1 breakpoint. By sequencing the junction fragments, we also found that the translocation process involved the duplication of six nucleotides from chromosome 8.

The chromosome 1 *KIAA0454* gene homologue is an unlikely candidate for a GTS gene. *KIAA0454* encodes a non-muscle myosin heavy chain A (NMMHC-A)-like protein which might play a role in smooth muscle cell proliferation.[30,31] The related 1q21.1 sequence showed 97% identity at a nucleotide level and 97% identity at an amino acid level with *KIAA0454*. However, the 1q21.1 sequence did not have any representative ESTs in dbEST as determined by BLASTN analysis. In addition, expression of this sequence was not observed in fetal brain cDNAs even though *KIAA0454* expression was readily detected. The 1q21.1 sequence related to *KIAA0454* may therefore not be a functional gene. Other GTS gene mapping studies have also failed to implicate the 1q21.1 locus in this disorder.

In contrast, chromosome 8q22.1 remains an attractive location for a GTS gene. Leppert and colleagues[23] obtained some of their best parametric lod scores at this site using a large, unilineal Utah GTS kindred. Simonic *et al*[17] also found some of their most promising case/control association results using the isolated Afrikaner population of South Africa at this location. The Afrikaner results for 8q22.1 have recently been confirmed by the Transmission Distortion Test (TDT) (unpublished results).

The *CBFA2T1* gene remains a viable GTS candidate. *CBFA2T1* was originally identified through studies of chromosome 8;21 translocations which frequently are found in acute myeloid leukemias.[26,27] The hybrid protein resulting from the fusion of *AML1* at 21q21.3 and *CBFA2T1* is believed to contribute to malignant transformation. The CBFA2T1 protein is a likely transcription factor because it has characteristic zinc finger DNA binding motifs and proline/serine/threonine rich regions.[32,33] *CBFA2T1* is expressed at high levels in fetal and adult brain, heart, and skeletal muscle. *CBFA2T1* has a total of 13 exons spread over about 87 kb of genomic DNA.[28] The *nervy* gene in *Drosophila* is similar to *CBFA2T1* in sequence and is a target of the ultrabithorax homeotic gene product.[34,35] *CBFA2T1* coding, 5' and 3' sequences are very highly conserved between humans and mice.[36]

Although *CBFA2T1* was not directly disrupted by the translocation breakpoint in the GTS family we studied, it is still possible that the translocation significantly altered the expression of *CBFA2T1* (or another nearby gene). Position effects of translocations have been reported for more than 10 human diseases (reviewed by Kleinjan *et al*[37]) Breakpoints have been found at both 5' and 3' sides of causative genes. Aniridia, Greig cephalopolysyndactyly, Saethre-Chotzen syndrome, sex reversal, facio-scapulo-humural muscular dystrophy, and split hand/split foot malformation have all been reported to be caused by translocations 3–450 kb downstream (3') of the genes.[38–43]

The sequences of the junction fragments for the translocation in the GTS family contribute to our knowledge of reciprocal translocations. Since the *CBFA2T1* breakpoints in leukemias usually occur between exons 1a and 2, the 8q22.1 breakpoint in the GTS family is completely different. Although Alu elements and other interspersed repeats are

often involved in chromosomal rearrangements,[44] no repeat sequences were found which could explain the translocation in the GTS family. The breakpoint regions of 1q21.1 and 8q22.1 did, however, show moderate similarity in short sequences (26–29 bp) flanking the breakpoints which may have mediated chromosomal pairing.

Six nucleotides from chromosome 8 but none from chromosome 1 were duplicated during generation of the translocation. The breakpoint sequences of several constitutional balanced translocations in human have now been determined.[45–54] Most translocations appear to involve either deletion or duplication of at least a few bases at the breakpoints. Some translocations involve the insertion of bases unrelated to either of the parental chromosomes. The duplication of a few bases at the breakpoint is also reminiscent of transposition of Alu elements and other retroposons.[44] It appears that translocations can occur by at least several quite different mechanisms. Compilation of additional examples will eventually improve our understanding.

Although the evidence is overwhelming that Gilles de la Tourette syndrome is highly heritable, it is also becoming clear that the genetics of GTS are much more complex than originally anticipated. A simple autosomal dominant mode of inheritance with reduced penetrance is no longer seriously considered. Candidate gene studies, parametric linkage mapping using large kindreds, an affected sib pair study and a genome-wide association study have all implicated quite different loci. Multiple genes as well as non-heritable factors almost certainly interact in complex ways to produce the GTS phenotype. Identifying the various GTS genes will be difficult. However, chromosomal rearrangements occurring in GTS patients like the chromosome 1;8 translocation studied here may facilitate identification of some of these genes.

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