No evidence for genetic linkage of Gilles de la Tourette syndrome on chromosomes 7 and 18

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Abstract

Gilles de la Tourette syndrome is a heritable neuropsychiatric disorder. In order to determine the chromosomal localisation of the locus involved, genetic linkage studies were initiated in six extended families. The Gilles de la Tourette gene has been tentatively assigned to chromosome 18q22.1. In our present study no evidence for genetic linkage on chromosome 18 and chromosome 7 was obtained. Data from the markers tested made it possible to exclude the whole of chromosome 18 and the chromosome 7q21.3-qter region as a site for the Gilles de la Tourette gene.

Gilles de la Tourette syndrome (GTS) is a neuropsychiatric disorder with an unknown aetiology. The syndrome is characterised by recurrent, involuntary, repetitive, multiple motor and vocal tics. In many patients associated behavioural problems like

obsessive compulsive behaviour and copro- and echo-phenomena are observed.¹

There is strong evidence that GTS is genetically determined. The exact mode of inheritance, however, is still a matter of discussion. Both single major locus and multifactorial models have been proposed.¹⁻⁶ The most widely held hypothesis states that a single autosomal dominant gene with reduced penetrance is involved.⁶ However, it has been discussed that associated behavioural symptoms should be considered as variant expressions of the presumed genetic defect responsible for GTS. There is general agreement that the chronic tic syndromes, according to DSMIII-R criteria,⁷ in families afflicted with GTS are variant phenotypes of the GTS gene defect.²

Comings *et al*⁸ presented evidence for the localisation of the GTS gene. They reported a 46,t(7;18)(q22;q22.1) reciprocal translocation in six relatives suffering from GTS. No support for linkage was found between the breakpoint on chromosome 7q22 and the COLA1 locus at 7q21.3-q22.1, suggesting a localisation of the GTS gene near the 18q22.1 breakpoint. Donnai⁹ reported a GTS patient with a deletion of the long arm of chromosome 18 at 18q22.2. These findings led to the tentative assignment of the GTS gene to chromosome 18q22.1. At 18q21, a candidate gene, gastrin releasing peptide (GRP), has been localised coding for a neuropeptide-like protein.^{10 11}

In order to determine the chromosomal location of the GTS gene, we started genetic linkage studies in five families of Dutch origin and in one family of Norwegian origin. In our present study we found no support for linkage on either chromosome 18 or chromosome 7, including the COLA1 locus (7q21.3-q22.1) near the translocation breakpoint.

Material and methods

FAMILY MATERIAL

Clinical and genetic studies were performed in five Dutch families and one Norwegian family. Complete pedigree data and methods of ascertainment will be published elsewhere (van de Wetering *et al*, in preparation) and are briefly summarised here.

All subjects were investigated using a standardised

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psychiatric interview with an added section on GTS and tics (Yale Scale, Dr D L Pauls, New Haven). Only those with GTS or tic syndrome, according to DSMIII-R criteria,⁷ were regarded as affected. The interviews were reviewed by an independent psychiatrist and a neurologist without previous knowledge of the family history. A total of 236 subjects was investigated, of whom 48 were considered to be affected.

Pedigrees of the families used in this study are not shown to protect the privacy of the subjects that collaborated in this study. However, pedigrees can be sent to investigators only, on request.

DNA ANALYSIS

DNA was isolated from peripheral blood lymphocytes of family members as described by Miller *et al.*¹² DNA was digested with various restriction enzymes (Boehringer, Pharmacia, BRL) according to the manufacturer's instructions. Gel electrophoresis of 8 µg DNA samples on 0.7% agarose gels, and DNA immobilisation by alkaline blotting onto nylon membranes (Gene Screen plus), were performed according to standard procedures.¹³ Hybridisation conditions were as described by Maniatis *et al.*¹³ and washing was performed at 65°C in 0.1×SSC final stringency. DNA was labelled by random hexamer priming according to Feinberg and Vogelstein.¹⁴

Markers B74 (*D18S3*), OLVIIA8 (*D18S7*), OS-4 (*D18S5*), pHF12-62 (*D18S1*), and pERT25 (*D18S11*) were used as reference points for chromosome 18, as they had been mapped previously by physical methods, and were used for the construction of a continuous linkage map for chromosome 18.¹⁵ They were kindly provided by Drs J L Mandel, H Olek, H Tateishi, R White, and U Müller, respectively. Markers OLVIIE10 and GRP have previously been described^{16 17} and shown to map to chromosome 18.

Markers pJ2 (*TCRG*), Cgamma (*TCRG*), pMetH (*MET*), NJ3 (*COLA1*), pTHH28 (*D7S371*), pRMU7.4 (*D7S370*), pYNB3.1R (*D7S372*), C33 (*D7S126*), TM102L (*D7S135*), and pB79A (*D7S13*) have previously been mapped on chromosome 7 and used in a linkage map.¹⁸ In our present study they were used as reference points for chromosome 7. These markers were kindly provided by Drs T W Mak, R White, P Tsipouras, Y Nakamura, L Tsui, and J Schmidtke, respectively. Markers pXV-2C (*D7S23*) and TN127 (*D7S144*) have been described previously and shown to map to chromosome 7.^{19 20}

LINKAGE ANALYSIS

Linkage analysis was performed using the LINKAGE program package version 5.03.^{21 22} The GTS gene frequency was estimated to be 0.003, with a male penetrance of 0.999 and a female penetrance of 0.56.

The correction for possible phenocopies was 0.0002.⁶ Two point linkage analysis was performed with the MLINK program and multipoint analysis with the LINKMAP program using Haldane's mapping function for interference. No allowance was made for spontaneous mutations. In the multipoint analysis we assumed a constant sex ratio for crossing over of 2:1 (female/male) for chromosome 18.¹⁵ For chromosome 7 we assumed a constant sex ratio for crossing over of 2:0.¹⁸ A lod score of at least 3.0 was considered as evidence for genetic linkage, and a lod score of -2.0 was considered as evidence for exclusion of linkage, for the assumed model of a single dominant gene with reduced penetrance.²³

Results and discussion

Chromosome 18 markers were tested in six extended families and a linkage map was constructed using the continuous linkage map of O'Connell *et al.*¹⁵ as a basis. Mapping the genetic distances of corresponding markers B74, pHF12-62, OLVIIA8, OS-4, and pERT25 with our own material differed only marginally from the O'Connell mapping distances. Additional markers OLVIIE10 and GRP were then mapped, with our family material, onto the fixed O'Connell linkage map.

Comings *et al.*⁸ and Donnai⁹ postulated that the gene responsible for GTS resides on chromosome 18q22.1. Markers OLVIIE10 at 18q21.3, GRP at 18q21, OS-4 at 18q21-qter, and pERT25 at 18q23

Figure 1 Female specific genetic maps of chromosomes 7 and 18. Physical locations are indicated where known. Marker order and map distances are based on the linkage maps of O'Connell *et al.*^{15 18} and mapping studies with our family material. Map distances are presented as θ , assuming a constant female/male crossing over ratio of 2:0 for chromosome 7 and 2:1 for chromosome 18.

map in the region surrounding the t(7;18)(q22;q22.1) translocation breakpoint.²⁴ Given the linkage map and the cytogenetic maps^{15 24} (fig 1), the most likely localisation for the translocation breakpoint is between markers OLVIIE10 and OS-4.

Lod scores for the two point analysis of chromosome 18 between GTS and marker loci are shown in table 1. Only subjects presenting GTS or tic syndrome were included as affected in our present study. None of the markers tested showed evidence for linkage and they excluded linkage for the genetic distance mentioned in table 1. Using multipoint analysis (fig 2),

Figure 2 Multipoint linkage analysis showing the exclusion of the GTS locus from chromosome 7q21.3-qter (a) and chromosome 18 (b). Dotted line represents a value of -2.

we obtained lod scores of at least -5 for the translocation breakpoint region between markers OLVIIE10 and OS-4. A lod score of at least -2 was obtained for the complete linkage map of chromosome 18. This value is generally accepted as evidence for the exclusion of linkage.²³ We therefore conclude that chromosome 18 can be excluded as a site for the GTS gene. These results are not in agreement with the findings of Comings *et al*⁸ and Donnai.⁹

Another possible site for the GTS gene would be the breakpoint of the translocation on chromosome 7q22 reported by Comings *et al*.⁸ We have tested several RFLP markers on chromosome 7 with our family material, and a linkage map was constructed (table 2). Comparison of our linkage map with the primary linkage map of O'Connell *et al*¹⁸ gave marginal differences only, except for marker C33, which we mapped at $\theta=0.01$ telomeric to pMetH instead of $\theta=0.11$ in the O'Connell data. Our results are consistent with the findings of Rommens *et al*.¹⁹ To avoid possible mapping errors we did not include marker C33 in the multipoint analysis. Marker TN127, not on the primary linkage map of O'Connell *et al*,¹⁸ was mapped using our family material and added to the combined linkage map (fig 1).

For none of the markers tested on chromosome 7 did we find evidence for linkage, including the COLA1 locus which is located proximal to the EPO locus at 7q21 and thus must be located proximal to the presumed breakpoint of the translocation.²⁴ With multipoint analysis we have been able to exclude part of chromosome 7p and the 7q21.3-qter region including the translocation breakpoint region. The region around marker C33 is excluded by flanking markers pMetH, pXV2C, and TCRB in the two point analysis as well as in the multipoint analysis, even if we assume that marker C33 is localised at $\theta=0.11$ to pMetH.

With the exclusion of chromosome 18 as a possible site for the GTS gene and the exclusion of the 7q21.3-qter region of chromosome 7, we conclude that the 46,t(7;18)(q22;q22.1) reciprocal translocation is not linked to the gene responsible for the Gilles de la Tourette syndrome. However, genetic heterogeneity could mask a positive result. In this study, all families

Table 1 Two point linkage data for chromosome 18.

Probe	Recombination fraction (θ)							Exclusion*
	0.0	0.05	0.1	0.15	0.2	0.3	0.4	
B74	-23.46	-6.68	-4.24	-2.86	-1.96	-0.85	-0.26	25
pHF12-62	-15.89	-3.92	-2.18	-1.26	-0.69	-0.11	0.07	11
OLVIIA8	-14.33	-3.92	-2.22	-1.32	-0.77	-0.21	-0.02	11
OLVII10	-13.88	-5.39	-3.34	-2.20	-1.46	-0.62	-0.24	18
GRP	-10.26	-2.76	-1.84	-1.30	-0.91	-0.40	-0.11	5
OS4	-12.77	-4.32	-2.83	-1.93	-1.31	-0.51	-0.11	17
pERT25	-25.07	-8.04	-4.47	-2.61	-1.50	-0.44	-0.11	18

*Centimorgans definitely excluded on either site of the tested marker. A lod score of -2 or less was assumed as definite proof of exclusion.

Table 2 Two point linkage data for chromosome 7.

	Recombination fraction (θ)							
Probe	0.0	0.05	0.1	0.15	0.2	0.3	0.4	Exclusion*
Tm102L	-22.62	-5.09	-2.48	-1.17	-0.43	0.19	0.22	11
TCRG	-12.68	-4.90	-3.29	-2.29	-1.60	-0.73	-0.26	18
pRMU7.4	-21.01	-5.06	-2.92	-1.76	-1.04	-0.58	-0.30	11
pTHH28	-11.04	-2.78	-1.43	-0.75	-0.36	-0.01	0.07	5
COL1A	-13.62	-2.73	-1.26	-0.54	-0.13	0.23	0.23	5
pB79A	-12.28	-3.28	-1.75	-0.93	-0.44	0.02	0.10	5
pMetH	-17.36	-3.91	-1.95	-1.00	-0.47	-0.01	0.06	11
pXV2C	-25.28	-6.47	-3.46	-1.93	-1.02	-0.13	0.11	17
C33	-1.54	0.04	0.20	0.23	0.21	0.12	0.03	0
TCRB	-21.18	-6.15	-3.63	-2.20	-1.28	-0.30	0.04	18
TN127	-15.73	-4.98	-3.05	-1.99	-1.31	-0.52	-0.14	18
pYNB3.1	-7.28	-2.63	-1.45	-0.82	-0.43	-0.05	0.05	5

*Centimorgans definitely excluded on either site of the tested marker. A lod score of -2 or less was assumed as definite proof of exclusion.

contributed to the negative lod scores on both chromosomes.

Currently, we are performing collaborative genetic linkage studies on other parts of the genome in order to find the location of the GTS gene.²⁵ Chromosomal rearrangements in families suffering from GTS could facilitate the localisation of the GTS gene, but should be followed by extensive linkage studies in order to obtain definite proof for genetic linkage.

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