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Progress in the Search for Genetic Linkage with Tourette Syndrome: An Exclusion Map Covering More than 50% of the Autosomal Genome

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Summary

Gilles de la Tourette syndrome is a neuropsychiatric disorder with an autosomal dominant mode of inheritance and reduced penetrance at a single genetic locus. Several research groups have genetic linkage studies underway to detect the chromosomal location of the gene that predisposes for this disorder. Strong and clear evidence of linkage has not yet been produced for Tourette syndrome. This paper presents an overview of the methods and progress of the groups centered at Yale University and Erasmus University in excluding linkage from a large portion of the genome. Our labs have screened 228 genetic marker loci for linkage with a gene for this disorder in a series of affected families in the United States, Canada, The Netherlands, and Norway. More than 50% (and perhaps as much as 66%) of the autosomal genome has now been excluded on the assumption that genetic heterogeneity is not an important factor in the Tourette syndrome pedigrees pooled for this summary.

Introduction

Gilles de la Tourette Syndrome (GTS) is a familial, neuropsychiatric disorder with onset in childhood characterized by chronic, intermittent motor and vocal tics (Kidd et al. 1980; Pauls et al. 1981, 1984). In addition to tics, affected individuals frequently display symptoms such as attention-deficit hyperactivity disorder (ADHD) and/or obsessive compulsive disorder (OCD) and/or obsessive compulsive disorder (OCD). Coprolalia and echolalia, which are complex vocal tics, are often also associated with the syndrome. Sex and age affect the risk of expressing GTS. Genetic analyses of family data have been consistent with the hypothesis that susceptibility to the disorder is most likely due to a single genetic locus with a dominant mode of transmission and reduced penetrance. Recent reviews of the disorder and the genetic models that have been considered can be found in the work of Price et al. (1987) and Pauls et al. (1990). In families affected by GTS, the chronic multiple-tic syndrome (CMT) consisting of motor or vocal tics (but not both) is generally agreed to be a variant, milder phenotype of the GTS gene (Kurlan et al. 1987; Kurlan 1989). Research that has examined the relationship between GTS and OCD suggests that OCD is etiologically related to GTS and that it represents a variant phenotype of the disorder that may be more commonly expressed in females (Pauls and Leckman 1986). However, there is no general agreement on whether individuals suffering from OCD alone should be included as affected with GTS. The nature of the relationship between ADHD

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and GTS is less clear. In the present report we shall report the results of testing for genetic linkage with GTS by using a conservative diagnostic scheme in which affected status includes either the core GTS or chronic multiple tics.

Establishing strong evidence for genetic linkage between GTS and one or more marker loci would clearly demonstrate the existence of a major locus and help to obtain a better understanding of the pattern of inheritance of the syndrome, since it would then be possible to identify carriers of the disease-related gene independently of the observed clinical phenotype. Linkage studies can be a first step toward characterizing the products and function of the locus responsible for GTS during development and thus can lead to useful therapeutic remedies. Several research groups have started genetic linkage studies on GTS. Interim results of their work were presented at the Tourette Syndrome Association meeting in Cambridge, England, in August of 1989. In the present collaborative paper we present an overview of the genetic markers typed and analyzed so far by our research groups (led by K.K.K. at Yale University in New Haven, CT, and by B.A.O. at Erasmus University at Rotterdam).

Subjects and Methods

Families

In Rotterdam, clinical and genetic studies have been carried out in five families of Dutch origin and in one family of Norwegian origin. The complete pedigree data and methods of ascertainment will be published elsewhere (B. J. M. van de Wetering, unpublished data). In brief, 236 individuals have been investigated by using a structured questionnaire (Pauls et al. 1980) extended to include a section on GTS and CMT. Only individuals with GTS or CMT, according to DSM-III (R) criteria, have been regarded as being affected. The interviews were reviewed by an independent psychiatrist and by a neurologist, neither of whom had prior knowledge of the family history, Also, genetic studies were performed on a North American family which was investigated by using the same structured questionnaire (Pauls et al. 1980).

In New Haven, four large pedigrees have been collected with the help of R. Kurlan and D. L. Pauls. The three principal pedigrees which have contributed to the linkage results pooled together here include the Canadian (184 individuals in the pedigree structure analyzed) (Kurlan et al. 1986), Oregon (54 individuals), and Michigan (43 individuals) families. Pauls et al. (1990) can be consulted for descriptions of the methods of ascertainment and diagnosis.

DNA Analysis

At New Haven, lymphoblastoid cell lines have been established on some 230 of the 281 individuals who are included in the Canadian, Oregon, and Michigan pedigree structures analyzed for linkage. The standard methods followed in establishing cell lines, extracting DNA, and phenotyping the RFLP systems used have been described elsewhere (Kidd et al. 1986). The 176 RFLP systems and classical blood groups and serum proteins tested for linkage in New Haven can be found in table 1. The laboratory of R. S. Sparkes typed the classical genetic markers by employing standard methods (Spence et al. 1977).

RFLP analyses at Rotterdam followed standard procedures (Maniatis et al. 1982) on genomic DNA isolated from peripheral blood lymphocytes of family members (Miller et al. 1988). DNA was labeled by primed synthesis according to the protocol of Feinberg and Vogelstein (1983). Those markers whose names begin with "mfd" were typed by the group of J. Weber according to procedures described by Weber and May (1989). Markers were typed on five Dutch families and on the Norwegian family, except for the mfd markers, which were typed on the North American family and at least one of the larger Dutch families. The 67 genetic markers tested in Rotterdam, their cytogenetic map localization, and source are also listed in table 1.

Our two labs overlap only in the typing of 15 markers. As can be seen from the distribution of typings by lab and across chromosomes (table 1), our labs have generally concentrated on rather different chromosomes. So far the Rotterdam group has been primarily active on chromosomes 7, 12, 18, and 19, while the New Haven group so far has focused on chromosomes 1, 2, 5, 10 and 17.

Linkage Analysis

Pairwise linkage analyses (at New Haven) were carried out using the LIPED program of Ott (1974), with test points generated on a 64-point test grid allowing male and female recombination frequencies to vary with the same eight recombination fractions (.0, .001, .05, .1, .2, .3, .4, and .5) on each axis. Pairwise and multipoint linkage analyses have been performed using the linkage analysis package (version 5.03) of Lathrop and Lalouel (Lathrop et al. 1984). Two-point linkage analyses have been performed with the

MLINK program (at Rotterdam), and multipoint analyses have been performed with the LINKMAP program using Haldane's mapping function. The genetic models used in the linkage analyses are described in table 2. Liability calculations, which are a function of sex and age, are carried out to provide appropriate weightings for individuals who are at risk of developing the disorder but who are not expressing symptoms that would result in a diagnosis of GTS.

Both of our research groups basically used the same genetic model for the inheritance of GTS. However, there are some parameter differences. The New Haven group used an age-dependent penetrance for children up to 21 years of age, while the Rotterdam group did not use an age-dependent penetrance but instead did not include in the linkage analyses children under the age of 15 years unless these children were diagnosed as affected. The second difference is that the number of possible phenocopies allowed is a somewhat higher value in the New Haven analyses. While the models employed by our two labs are not identical, the differences in the parameters used should not result in any significant differences in the linkage results obtained.

Exclusion-Map Figures

For each human autosome a stick map (fig. 1) was constructed in which the symbols of the genetic loci tested for linkage with GTS are ordered along the map. Marker symbols lacking a precise localization are placed in boxes next to the chromosome map, and arrows emerging from the boxes indicate the approximate placement of these loci along the genetic map. The scale is about 5 cM/line, the height of a locus symbol. Exclusion zones (where lod scores are ≤ -2.0) are displayed, to the left of each stick map, by darkly shaded square blocks and half-sized blocks. A square exclusion block delimits about a 5-cM region, giving a clear visual indication of eliminated areas. Within the boxes the numbers next to the marker symbols show the size of the exclusion zone, if any, around that locus.

Only genetic loci that have been tested for linkage with GTS appear on the maps. Official human gene symbols (McAlpine et al. 1989) and D-numbers assigned by the DNA committee (Kidd et al. 1989) to anonymous loci have been employed. In some instances, typically for loci unassigned to a chromosome, official symbols are not yet available. All the locus labels on the maps (except for cen, pter, and qter) which have lowercase letters are probe names rather than official locus symbols, which are always capitalized. In the space between each stick map and the exclusion-zone block can be found the estimated map distances (in centimorgans) for the adjacent loci. Haldane's mapping function was used in converting recombination fractions to map distances. The exclusion zones reflect the pooling of results from pairwise and multipoint linkage analyses. A lod score of ≤ -2 was always used in determining whether exclusionary evidence was obtained. When map distances between tested loci are very short, the locus symbols are placed together on the same line next to the stick map even when their order may be known.

We employed a wide variety of sources in constructing the genetic maps. In addition to the work done by our own labs in building up the general linkage map, we consulted summaries provided by the individual chromosome committee reports published by Human Gene Mapping 10 (1989), so that we could help scale the overall genetic length of each autosome and position various isolated loci by utilizing physical mapping data. We also utilized other published sources, including O'Connell et al. (1987, 1989), Nakamura et al. (1988*a*, 1988*b*), and Lathrop et al. (1988, 1989). Although a considerable amount of progress is being made in mapping the human genome, many uncertainties persist, including the exact genetic map length of most chromosomes but especially those of chromosomes 3, 6, 14–16, and 19–22. Note that when the map distance in a region is uncertain the continuity of the map line shown is interrupted by four dots arranged in a square (: :) and that any estimated distances displayed nearby may be especially susceptible to revision. In general, where sizeable differences occur because of uneven male and female recombination, the largest map-distance estimates have been chosen.

Results and Discussion

Table 1 reports the one-sided exclusion interval for each of the 228 marker loci tested on the basis of pairwise genetic linkage analyses and also shows which of our labs has tested each. Haldane's mapping function was used to convert recombination fractions to map distances. No strong positive evidence for linkage with GTS (i.e., lod scores >3.0) has been obtained as yet, with the exception of one false-positive finding which is discussed below. Table 1 also identifies the probes for the RFLP systems used, as well as the names of the researchers who have shared their probes with

Table 1

Group of 228 Autosomal Loci Tested for Linkage with Tourette Syndrome, and Exclusion Zones around Each Locus

Symbol	Amount Excluded[a] (cM)	Probe Name	Chromosomal Band	Lab[b]	Probe Source
ABO	1	Serological	9q34.1-q34.2	Y	R. S. Sparkes
ACADM	25	MCAD	1p31	Y	K. Tanaka
ACP1	.1	Electrophoretic	2p25	Y	R. S. Sparkes
ADA	5	pADA211	20q13.11-qter	Y	D. Wiginton
ADH2	15	pADHbeta4	4q21-q23	Y	M. Smith
AK1	0	Electrophoretic	9q34.1-q34.2	Y	R. S. Sparkes
ALB	5	B44	4q11-q13	Y	R. M. Lawn
ALPL	25	pS3-1	1p36.1-p34	Y	H. Harris
AMY@	0	pEB-8	1p21	Y	M. Meisler
APOA2	15	Mfd3	1q21-q23	E	J. Weber
APOB	25	AB1	2p24-p23	E and Y	J. Scott
APOC2	15	Mfd5	19q13.2	E	J. Weber
APRT	5	M13-APRT	16q24	Y	P. J. Stambrook
ARG1	0	Arg	6q23	Y	S. Cedarbaum
AT3	10	ATIII	1q23-q25.1	Y	S. H. Orkin
BF	10	Electrophoretic	6p21.3	Y	R.S. Sparkes
C3	5	Electrophoretic	19p13.3-p13.2	Y	R. S. Sparkes
CA2	11	H25-3.8	8q22	E and Y	R. E. Tashian
CALCA	5	pTT42	11p15.4	Y	B. D. Nelkin
CAT	1	*Sca*I-*Sna*ICAT	11p13	Y	R. A. Gravel
CD8A	35	CD8	2p12	Y	A. Bowcock and P. Kavathas
CDC2	15	pOB231	10q21.1	Y	M. Lee
CHE2	0	Electrophoretic	2q33-q35	Y	R. S. Sparkes
COL1A1	5	FG2	17q21.3-q22	Y	B. Sykes
COL1A2	5	NJ3 3.2	7q21.3-q22.1	E	P. Tsipouras
CRYB1	0	pSM 8A5	17q11.2-q12	Y	L.-C. Tsui
CRYG@	10	p5G1	2q33-q35	Y	L.-C. Tsui
CYP2E	0	mu101	10	Y	F. Gonzalez
DNTT	5	TdT	10q23-q24	Y	D. Baltimore
DRD2	10	hD2G1	11q22-q23	Y	O. Civelli
EGF	0	EGF 121	4q25	Y	J. C. Murray
EGR2	25	Zap 32, #367	10q21.1	Y	V. Sukhatme
ESD	0	pBM20-EL22	13q14.1-q14.2	Y	R. Bookstein
FGB	0	FbgB	4q28	Y	D. Chung
FNRB	10	pGem1-p32	10p11.2	Y	N. Simpson and P. Goodfellow
FNRBL	5	pGEM1-32	19p	Y	N. Simpson and P. Goodfellow
FY	10	Serological	1q21-q25	Y	R. S. Sparkes
G10P1	0	p561	10q25-q26	Y	P. Szabo
GALT	15	Electrophoretic	9p13	Y	R. S. Sparkes
GC	1	Electrophoretic	4q12-q13	Y	R. S. Sparkes
GH1	10	pchGH-800	17q22-q24	Y	H. Goodman
GLO1	10	Electrophoretic	6p21.3-p21.1	Y	R. S. Sparkes
GPT	.1	Electrophoretic	8q24.2-qter	Y	R. S. Sparkes
GRL	15	OB7	5q31-q32	Y	C. Weinberger
GRP	5	pB12	18q21	E	E. R. Spindel
GYPC	25	GPC	2q14-q21	Y	J. P. Cartron
HEXA	0	paHEX49	15q23-q24	Y	R. A. Gravel
HEXB	10	pHexX	5q13	Y	R. A. Gravel
HOX2	10	Plasmid 3(BS3)	17q21-q22	Y	F. Ruddle and T. Miki
HP	10	Hp150 a,b	16q22.1	Y	B. Bowman
HRAS	5	J841Ha6.6	11p15.5	Y	E. H. Chang

(*continued*)

Table 1 (continued)

Group of 228 Autosomal Loci Tested for Linkage with Tourette Syndrome, and Exclusion Zones around Each Locus

Symbol	Amount Excluded[a] (cM)	Probe Name	Chromosomal Band	Lab[b]	Probe Source
IGF1	5	pIGF1	12q23	E	M. Jansen
IGF2	0	pHINS-311,phigf	11p15.5	Y	G. I. Bell
IL6	0	B2INF	7p21-p14	Y	P. B. Sehgal and F. Ruddle
INS	10	pHINS-310	11p15.5	Y	G. I. Bell
INT2	10	SS6	11q13	Y	G. Casey, G. Peters, and J. Duke
JK	10	Serological	18q11-q12	Y	R. S. Sparkes
KEL	1	Serological	Unassigned	Y	R. S. Sparkes
KRAS2	10	p640	12p12.1	E	R. A. Weinberg
LDLR	0	pLDLR-2HHI	19p13.2-p13.1	E	D. W. Russel
LPL	5	LPL35	8p22	E	M. C. Scholz
MBP	1	pP535	18q22-qter	Y	C. W. Campagnoni
MET	10	pmetH	7q31	E	R. White
MNS	10	Serological	4q28-q31	Y	R. S. Sparkes
MPO	15	MPO-10A	17q21.3-q23	Y	S. C. Weil
MT2P1	10	pHM6	4p11-p21	Y	L.-C. Tsui
MYC	0	p380-8ASaSs1.8	8q24	Y	C. Croce
NGFB	1	NGF	1p13	Y	J. Darby
NGFR	15	PE51	17q21-q22	Y	M. V. Chou
OAT	10	HINC-BAM, SP35105	10q26	Y	J. Gusella, V. Ramish and R. White
P1	0	Serological	22q11.2-qter	Y	R. S. Sparkes
PAH	10	pPAH247	12q22-q24.2	E	S. L. Woo
PBGD	18	pUSE109,PBGD0.9	11q23.2-qter	E and Y	M. Goossens
PDYN	10	LHDG-1	20pter-p12	Y	M. Litt
PENK	25	Lam.ENK1;Mfd31	8q23-q24	E and Y	J. Weber and M. Litt
PGD	5	Electrophoretic	1p36.3-p36.13	Y	R. S. Sparkes
PGM1	15	Electrophoretic	1p22.1	Y	R. S. Sparkes
PGP	10	Electrophoretic	16p13	Y	R. S. Sparkes
POMC	0	pLambda26/pLp3	2p23	Y	D. Cohen and L. Cavalli-Sforza
PPY	5	PPY	17p11.1-qter	Y	T. Takeuchi
PRIP	0	pEA974	20pter-p12	Y	N. K. Robakis
RAF1	5	p627	3p25	E	B. Seizinger
RARA	0	hKIR	17q21.1	Y	C. Weinberger
RARB	0	pCOD20	3p24	E	A. Dejean
RBP3	25	cTB-IRBP-9, H4	10q11.2	Y	Y. Nakamura, C. D. Bridges, and G. I. Liou
REN	10	HRen	1q32 or 1q42	Y	Chirgwin
RH	15	Serological	1p36.2-p34	Y	R. S. Sparkes
SFTP1	1	pPSP-35k-1A-27	10q21-q24	Y	J. Floros
SPTA1	5	3021	1q21	Y	B. Forget
SST	3	pgHS7-217	3q28	Y	G. I. Bell
TCRB	15	pJ2	7q35	E	T. W. Mak
TCRG	15	Cgamma	7p15	E	J. G. Seidman
TF	5	Electrophoretic	3q21	Y	R. S. Sparkes
TH	10	TH7, J4.7-*Bam*HI	11p15.5	Y	J. Mallet
THRB	5	pBH302, pHE-A2-S	3p24.1-p22	E and Y	W. E. C. Bradley and B. Vennstrom
TK1	1	TkHC9	17q23.2-q25.3	Y	P. Lin
D1F10S1	0	DR10	1q23-qter	Y	A. J. Driesel
D1S4	0	DR78	1p21-qter	Y	A. J. Driesel
D1S16	0	p2-32	1pter-p22	Y	N. Dracopoli
D1S17	15	p3-18	1pter-p22	Y	N. Dracopoli
D1S18	10	p3-39	1pter-p22	Y	N. Dracopoli
D1S19	15	p4-03	1pter-p22	Y	N. Dracopoli

(*continued*)

Table 1 (continued)

Group of 228 Autosomal Loci Tested for Linkage with Tourette Syndrome, and Exclusion Zones around Each Locus

Symbol	Amount Excluded[a] (cM)	Probe Name	Chromosomal Band	Lab[b]	Probe Source
D1S57	15	pYNZ2	1	Y	R. White
D1S75	15	OS-6	1q22-q23	Y	T. Miki and S. Takai
D1Z1	0	p308	1q12	Y	E. Jabs and B. Migeon
D1Z3	0	p308	1cen	Y	E. Jabs and B. Migeon
D2S1	25	L 2.30	2p25	Y	E. Bakker and P. Pearson
D2S3	10	C1-5, p1-30	2q35-q37	Y	M. Litt
D2S5	10	IMR32-6	2p16-p15	Y	S. Latt
D2S6	25	pXG-18	2p23-2p15	Y	P. Szabo
D2S9	.1	Latt-3	2p25-p24	Y	S. Latt
D2S10	5	Latt-1 = IMR-1	2p25-p24	Y	S. Latt
D2S12	10	pHM20	2pter-p23	Y	R. Williamson and G. P. Bates
D2S45	10	pHHH133	2p	Y	Y. Nakamura
D2S47	15	TBA-B5-7	2p	Y	Y. Nakamura
D2S48	10	pEFD122	2pter-q32	Y	R. White
D2S49	10	pYNA15.1	2pter-p23	Y	R. White
D3S5	0	DR2	3q21-qter	Y	A. J. Driesel
D4S1	10	4c3.6/1.2	4q11-q21	Y	C. Gilliam and R. Williamson
D4S10	15	pKO82	4p16.3	Y	J. Gusella
D4S12	15	A1	4pter-q26	Y	R. Williamson
D4S35	0	pG9-20	4p11-q11	Y	C. Gilliam and J. Gusella
D4S112	0	E9P1	4q26-qter	Y	P. J. Scambler and G. I. Bell
D4S123	25	pIBS17	4pter-q21	Y	J. C. Murray
D4S171	10	Mfd22	4	E	J. Weber
D5S1	0	L1.7	5	Y	E. Bakker and P. Pearson
D5S4	10	L1.4	5pter-p15	Y	E. Bakker and P. Pearson
D5S6	1	M4	5q11.2-q13.3	Y	A. E. Retief and E. Dietzsch
D5S10	10	pD274EC	5pter-p15.3	Y	J. Wasmuth
D5S11	10	pN35E-A	5pter-p15.3	Y	J. Wasmuth
D5S12	10	pJ0209E-B	5p15.2-p15.1	Y	J. Wasmuth
D5S13	0	pJ0214H-B	5p15.3-p15.2	Y	J. Wasmuth
D5S18	1	pJ0120H-B	5p15.2	Y	J. Wasmuth
D5S19	5	pJ044E-B	5p14	Y	J. Wasmuth
D5S20	1	pJ071H-A	5p13	Y	J. Wasmuth
D5S21	10	pJ0110H-C	5p13-p11	Y	J. Wasmuth
D5S22	10	pJ0205E-D	5q34-qter	Y	J. Wasmuth
D5S36	5	pJ0157E-A	5q32-qter	Y	J. Wasmuth/ATCC[c]
D5S39	10	p105-153Ra	5q12-q14	Y	J. Wasmuth/ATCC[c]
D5S76	10	p105-599Ha	5cen-q11.2	Y	J. Wasmuth/ATCC[c]
D5S78	15	p105-798Rb	5q11.2-q13.3	Y	J. Wasmuth/ATCC[c]
D5S88	35	CARLP II 6.3	5pter-p15	Y	P. Raeymaekers
D5S106	10	pLambda3.1	5	Y	L. Cavalli-Sforza
D6S2	5	pLambda2-2	6p21-pter	Y	L. Cavalli-Sforza
D6Z1	0	p308	6cen	Y	E. Jabs and B. Migeon
D7S8	1	3.11	7q31	Y	R. Williamson
D7S13	5	pB79a	7q22.3-q31.2	E	J. Schmidtke
D7S23	15	pXV2C	7q31-q32	E	X. Estivill
D7S126	0	C33	7q31-q32	E	L.-C. Tsui
D7S135	10	pTM102L	7pter-p14	É	L.-C. Tsui
D7S144	15	TN127	7q32-q34	E	L.-C. Tsui
D7S370	10	pRMU7.4	7p	E	R. White and Y. Nakamura
D7S371	5	pTHH28	7p	E	R. White and Y. Nakamura

(*continued*)

Table 1 (continued)

Group of 228 Autosomal Loci Tested for Linkage with Tourette Syndrome, and Exclusion Zones around Each Locus

Symbol	Amount Excluded[a] (cM)	Probe Name	Chromosomal Band	Lab[b]	Probe Source
D7S372	5	pYNB3.1	7p	E	R. White and Y. Nakamura
D8S3	1	181.6b	8	Y	P. J. Scambler
D8S84	5	Mfd8	8	E	J. Weber
D8S85	5	Mfd18	8	E	J. Weber
D9S3	0	Dr6	9	E	A. J. Driesel
D9S10	3	pMCT136	9q	E	R. White and Y. Nakamura
D9S16	0	pMCOA12	9q	E	R. White and Y. Nakamura
D10S1	15	Dry5-1	10q22-q23	Y	T. Dryja and J. Gusella
D10S3	10	phage 10	10q22-q23	Y	A. Bowcock
D10S4	25	p1-101	10q22-q23	Y	M. Litt
D10S5	10	p9-12a/2dIII2.5	10q21.1	Y	H. McDermid and N. Simpson
D10S6	0	pBM1.1	10q26	Y	T. Krontiris and U. Francke
D10S15	5	pMCK2	10q11.2	Y	Y. Nakamura and R. White
D10S19	35	pTB10.171	10q21.1-q22	E and Y	Y. Nakamura and R. White
D10S20	15	OS3, OS2	10q21-q26	Y	T. Miki and S. Takai
D10S22	30	pTB10-163	10q21.1	Y	Y. Nakamura and R. White
D10S24	10	p7A9	10p13-12.2	Y	W. Cavenee
D10Z1	1	pA10RP8	10cen	Y	H. Willard
D11S12	5	pADJ762, pADJ765	11p15.5	Y	R. White
D11S16	0	pLambda32-1	11p13	Y	L. Cavalli-Sforza
D11S29	35	L7	11q23-qter	E and Y	A. E. Retief and E. Dietzsch
D11S36	5	phi2-14	11q	Y	M. Litt
D11S83	0	phi2-25	11q23-qter	Y	M. Litt
D11S84	45	p2-7-1D6	11q22	E and Y	M. Litt
D11S144	18	MCT128.1	11q22.3-q23.3	E and Y	R. White and Y. Nakamura
D11S146	5	pHBI59	11q12-q13.2	Y	Y. Nakamura
D11S147	0	pHBI-18p2	11q23-qter	E	R. White and Y. Nakamura
D11S347	0	pLambda19-2	11q13	Y	L. Cavalli-Sforza
D12S6	0	p1-7	12q14	E	M. Litt
D12S7	10	pDL32B	12q14-q24.1	E	L.-C. Tsui
D12S8	25	p7G11	12q14-qter	E	R. White
D12S17	5	pYNH15	12q	E	R. White and Y. Nakamura
D13S2	10	p9D11	13q22	E	W. Cavenee
D13S3	5	p9A7	13q21-q34	E and Y	W. Cavenee and R. White
D13S5	10	pHUB8	13q22-q34	Y	T. Dryja
D13S6	1	pHU10	13q12-q14	Y	T. Dryja
D13S7	0	pHU26	13q22	Y	T. Dryja
D13S10	0	p7D2	13q14.1-q14.2	Y	R. White
D14S1	15	pAW101	14q32.32-q32.33	E	R. White
D14S16	20	pTHH37	14q32	E	R. White and Y. Nakamura
D15S1	20	pMS1-14	15q14-q21	E	R. White
D17S5	20	pYNZ22.1	17p13.3	E	R. White and Y. Nakamura
D17S34	18	p144-D6	17p13	E and Y	M. Litt
D17S58	.1	EW301	17p11.2-cen	Y	D. F. Barker
D17S71	1	puc10-41	17p12-p11.2	Y	D. F. Barker
D17S73	15	EW207	17cen-q12	Y	D. F. Barker
D17S250	5	MFD15	17	E	J. Weber
D17Z1	0	p17H5, pDL27B	17cen	Y	H. Willard and L.-C. Tsui
D18S1	10	pHF12-62	18	E	R. White
D18S3	20	B74	18p11.3	E	J.-L. Mandel
D18S5	15	OS4	18q21.3-qter	E	T. Miki

(*continued*)

Table 1 (continued)

Group of 228 Autosomal Loci Tested for Linkage with Tourette Syndrome, and Exclusion Zones around Each Locus

Symbol	Amount Excluded[a] (cM)	Probe Name	Chromosomal Band	Lab[b]	Probe Source
D18S7	10	OLVIIA8	18q11.1-q11.2	E	G. Thomas
D18S8	15	OLVIIE10	18q21.3	E	G. Thomas
D18S11	15	pERT25	18q23	E	M. Lalande
D18S34	15	MFD26	18	E	J. Weber
D18S35	5	MFD32	18	E	J. Weber
D19S7	35	p4.1	19cen-q12	E and Y	D. J. Shaw/ATCC[c]
D19S8	18	p17.1	19q13.2	E and Y	D. J. Shaw/ATCC[c]
D19S20	10	pJC23.1	19	E	R. White and Y. Nakamura
D19S30	5	p20B18	19p13.1-q12	E	B. Wieringa
D19S41	10	MFD11	19cen-q13.2	E	J. Weber
D20S4	18	pMS1-27	20q13.2	E and Y	R. White
D20S5	15	pRI2.21	20p12	Y	D. J. Shaw
D20S6	18	pD3H12	20p12	E and Y	D. J. Shaw
D20S13	10	pPhi64	20p	Y	R. Deed
D20S14	35	p4.8	20p	Y	N. Spurr
D20S27	20	Mfd25	20	E	J. Weber
No symbol	5	Mfd23	Unassigned	E	J. Weber
No symbol	5	PHI106	Unassigned	Y	R. Deed
No symbol	.1	CL149	Unassigned	Y	D. Roses
No symbol	0	LDR92	Unassigned	Y	D. Roses
No symbol	1	LDR111	Unassigned	Y	D. Roses
No symbol	10	Phage565	Unassigned	Y	J. Wasmuth
No symbol	10	LDR93	Unassigned	Y	D. Roses
No symbol	0	BAM41	Unassigned	Y	D. Roses

[a] One-sided interval; Haldane function is used.
[b] Y = Yale University; E = Erasmus University.
[c] The individual named is the generator of the probe; ATCC is the repository.

Table 2

Genetic Model Parameters

Parameter	Results of Study at	
	Yale University	Erasmus University
Single autosomal dominant gene	Yes	Yes
Gene frequency	.003	.003
Age-at-onset correction	Yes; linear function increasing from age 2 years until maximum at 21 years	No; before age 15 years children were included only if affected
Penetrance:		
Male	.048 (minimum), .999 (maximum)	.999
Female	.027 (minimum), .561 (maximum)	.560
Phenocopies:		
Male	.0002 (minimum), .0050 (maximum)	.0002
Female	.0000 (minimum), .0001 (maximum)	.0001

our labs. The chromosomal banding interval for each locus screened can be found in this table as well.

Table 3 is a summary, by chromosome, of the computation of the total, nonoverlapping, exclusion zone. At least 59% and as much as 65% of the autosomal genetic map has been excluded by the pooled results from our two labs. Note that a simple summation of exclusion zones in table 1 will not always result in the exclusion-zone totals computed in table 3 or presented visually in the figure, because many of the markers are closely linked. When the exclusion zones overlap, we have taken into account the linkage information and have eliminated the redundant exclusionary information.

An exact calculation of the proportion of the autosomal genetic map excluded from linkage with GTS cannot be carried out, for two reasons. The first difficulty is that the total length of the autosomal map is not known very precisely. Given our earlier decision to prefer the larger sex-specific recombination map in each region so as not to overestimate the excluded region, the total length is roughly 4,860 cM, considerably in excess of the conventional estimate of approximately 3,300 cM for the female map. The second difficulty is that, in tallying the total nonoverlapping exclusion zone across chromosomes, some of the genetic markers tested for linkage have not been localized precisely. Thus, a minimum and maximum exclusion zone are calculable. The minimum assumes that all exclusions from nonlocalized markers occur in regions already excluded by loci on the map, so the contribution of nonlocalized markers is ignored. The maximum allows all the exclusions by nonlocalized markers to count fully.

The exclusion zones illustrated by chromosome in figure 1 make it easy to pick out the obvious gaps, which the search for the GTS locus can focus on next. Most of chromosomes 1, 2, 5, 7, 10, 12, 18, and 19 have been excluded, and much progress has already been made on chromosomes 4, 8, 11, 17, and 20. There are large numbers of RFLPs already documented in the literature whose physical mapping locations overlap many of these gaps, e.g., in Human Gene Mapping 10 (1989) and in the on-line Human Gene Mapping Library data base in New Haven, but the mapping relationships of these loci are equally necessary to know, so that we can assess our progress. Our ongoing efforts, as well as those of the research community at large, to establish a basic genetic map spanning the human genome will be an important element in determining how efficiently we can advance in our efforts to find the GTS locus.

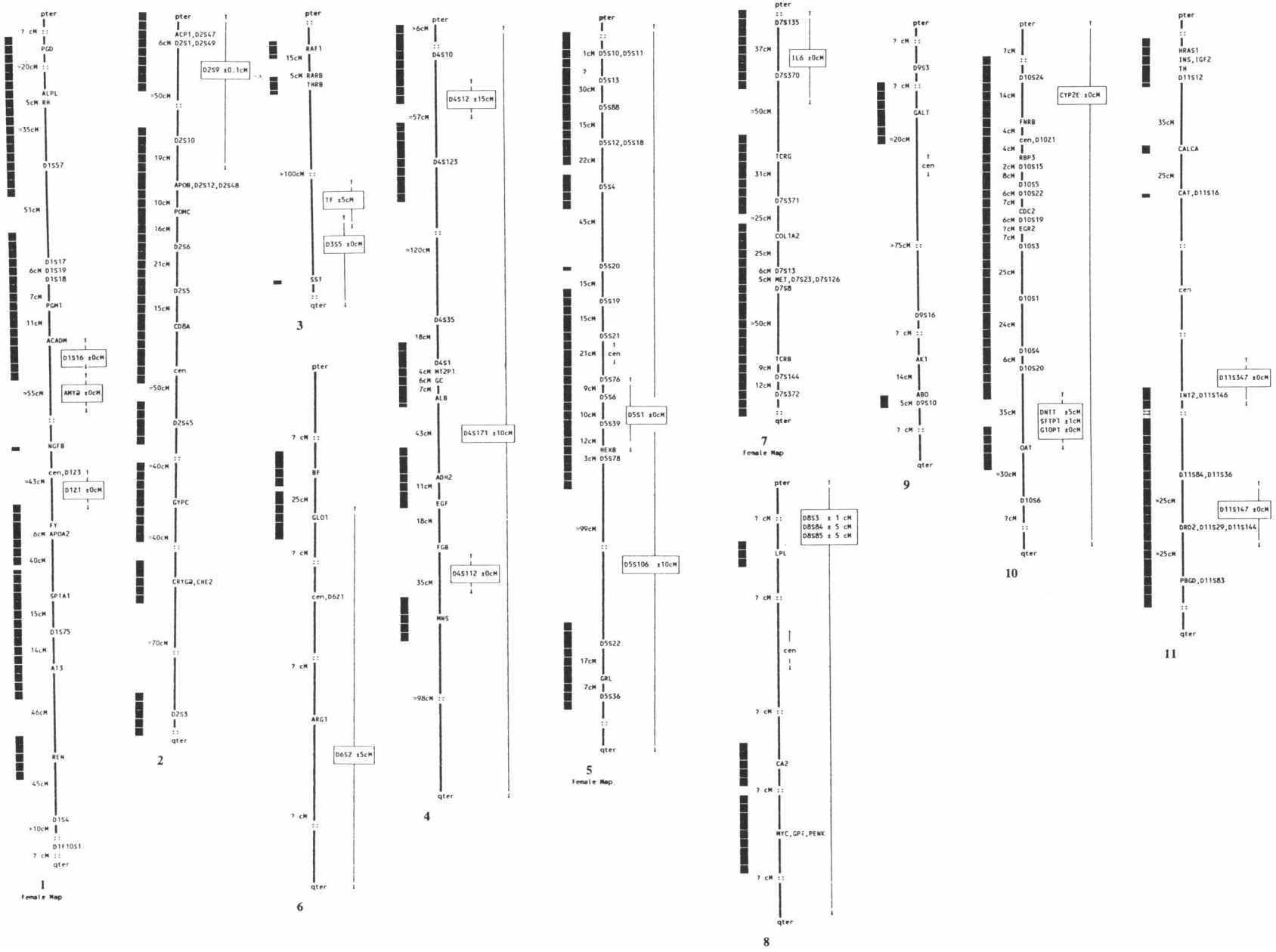
Since our concerted efforts have already excluded more than half of the relevant genome, we expect that the prize of finding the GTS locus lies in the near future. We (the New Haven group) have already had one statistical fluke to tantalize us in the course of this search. ACP1, a locus on 2p, gave a false-positive signal in one family (a peak lod just >3). Both follow-up typing of nearby flanking markers and subsequent multipoint linkage analyses allowed us to identify this very clearly as a bogus signal. We are hopeful that the next interesting signal will produce a very strong positive lod score that replicates across pedigrees and that the result will be reinforced by converging evidence from flanking markers.

Several lines of evidence (for a review, see Shapiro et al. 1988) indicate that brain neurochemical systems, in particular the dopamine system, may be involved in the etiology of GTS. The genetic markers we have tested included a number of candidate genes, such as the dopamine D2-receptor (DRD2), pro-dynorphin (PDYN), proopiomelanocortin (POMC), and the gastrin-releasing peptide (GRP). Genetic linkage to GTS was not obtained with any of these four candidate loci (Devor et al. 1990; Gelernter et al. 1990; Heutink et al. 1990). Testing candidate genes is one approach to finding a disease locus, but most such candidate loci are proposed rather casually, without a compelling case to support them. Those which have been cloned and are polymorphic are easily subsumed under the systematic screening strategy which seeks to test for linkage with a comprehensive series of polymorphic loci. Ultimately, a basic panel of polymorphisms forming a continuous map on each chromosome will exist and provide the means to test for linkage and to locate the map positions of disease-related loci.

Comings et al. (1986) postulated that the GTS gene is on chromosome 18. In the present study no evidence was found for linkage on chromosome 18. This is in agreement with the recent exclusion of chromosome 18 as a site for GTS with part of the results presented here (Heutink et al. 1990). Comings et al. (1989) suggested that the tryptophan oxygenase gene (4q25-q31.3) is a candidate gene for GTS. Several markers in this region (ADH2, EGF, FGB, MNS, and D4S112) were typed in the present study, and none of them gave evidence for linkage.

Genetic linkage analysis both for full-blown GTS only and for a broad phenotype allowing for GTS or

TS EXCLUSION MAPS



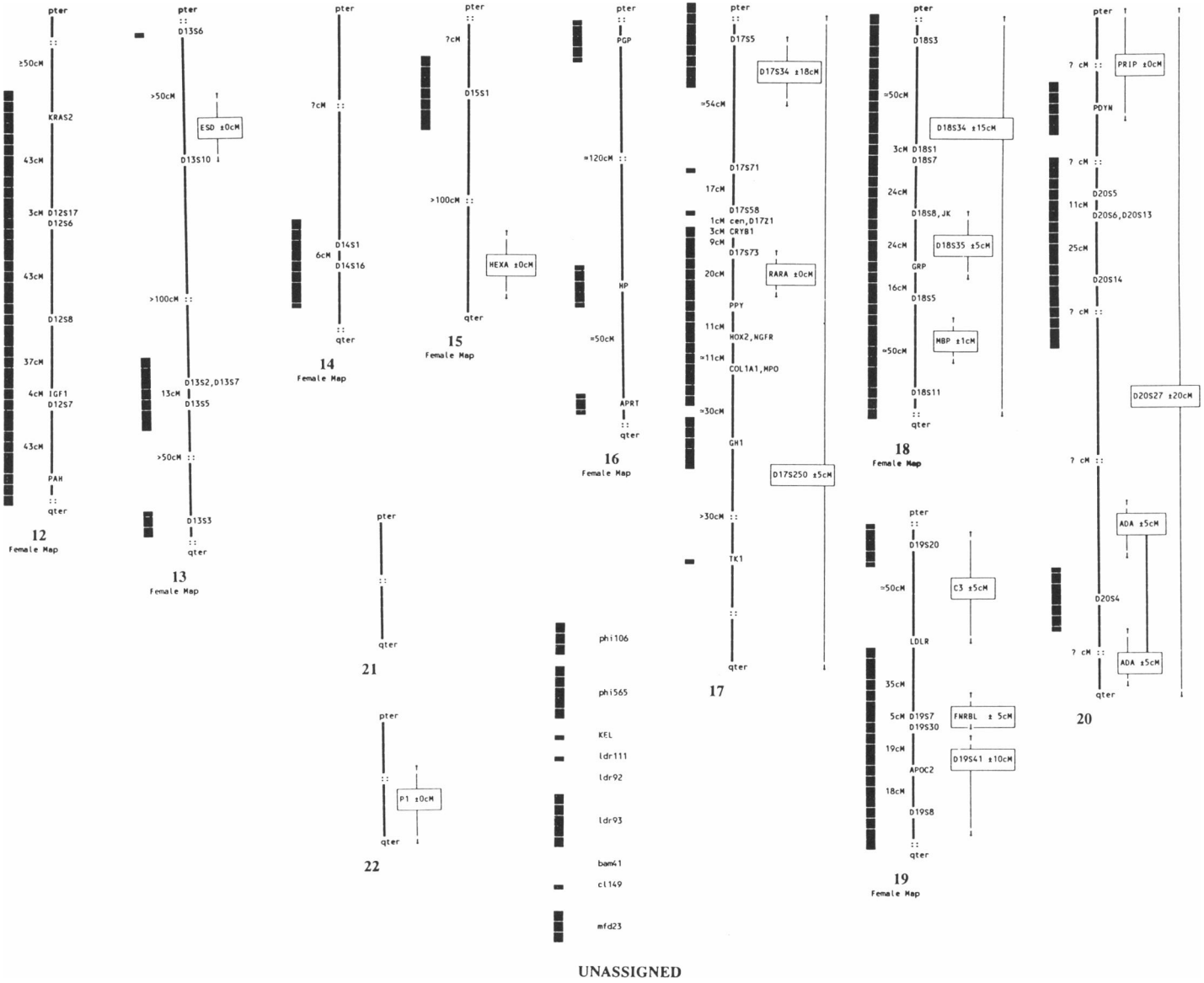


Figure 1 GTS exclusion zones along autosomal map. See text discussion of the conventions followed in constructing the exclusion maps

Table 3

Calculation of Total Nonoverlapping Exclusion Distance, by Chromosome

Chromosome	Map Distance Excluded via Linkage (cM)				Approximate Map Size (cM)
	Mapped Loci	Nonlocalized Loci	Total		
			Minimum	Maximum	
1	310	0	310	310	450
2	310	0	310	310	410
3	22	10	32	32	140
4	187	0 to 20	187	207	350
5	262	0 to 20	262	282	360
6	47	0 to 10	47	57	150
7	200	0	200	200	220
8	85	0 to 22	85	107	185
9	42	0	42	42	170
10	220	0 to 12	220	232	250
11	160	0	160	160	260
12	195	0	195	195	250
13	50	0	50	50	250
14	42	0	42	42	150
15	35	0	35	35	135
16	55	0	55	55	180
17	157	10 to 36	167	193	280
18	190	0	190	190	190
19	115	10	125	125	130
20	145	10 to 50	155	195	250
21	0	0	0	0	50
22	0	0	0	0	50
Pseudoautosomal.....	0	0	0	0	?
Unassigned.....	0	0 to 122	0	122	...
Total			2,869	3,161	4,860
% of autosome			59%	65%	100%

CMT or OCD gave results which are similar to those which we have summarized here for the affected phenotype that includes GTS or CMT. In the present study we did not include as affected those individuals presenting only OCD symptoms, since there is no general agreement on whether OCD alone can be regarded as a variant phenotype of GTS.

The possibility of heterogeneity needs to be considered. Genetic heterogeneity could mask a positive result, given the summation of lod scores across families. Both our groups routinely look at the linkage results separately for each family. Thus far no heterogeneity has been observed in the linkage results. The very small positive lod scores that have occurred so far (with the exception of the false-positive result on chromosome 2, mentioned earlier, which was clearly excluded by consideration of adjacent loci in the same family) are not very different from zero, so no formal heterogeneity test has been applied.

Conclusion

Our search for the GTS locus by means of a genetic linkage strategy has eliminated from consideration more than half—and perhaps as much as two-thirds—of the autosomal genome. This range of exclusionary values seems reasonable at this juncture. An important assumption underlying our assessment is that the major locus for GTS is the same in the different pedigrees pooled together for the present report. Thus, if there are different loci underlying GTS in some of our families, we could have missed such a linkage if, for example, the nearby marker was not segregating sufficiently (or was simply not typed) in the family with the linkage, while the marker was informative enough in the other nonlinked families to give us exclusion for the region. The search also might not be as far along as we claim, if the human autosomal map is substantially larger than present evidence suggests. Whatever the exact degree of progress, the pace of the search is gain-

ing momentum. New labs around the world either are preparing to join the hunt for the GTS locus or have recently started. Our own labs are concentrating on regions unexamined thus far, as illustrated by the exclusion maps we have presented here. Perhaps very soon we shall obtain strong, positive evidence of linkage for GTS in our pedigrees, so that the hunt for the DNA sequence of the GTS locus can begin.

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