

Evidence for a type 1 diabetes susceptibility locus (*IDDM10*) on human chromosome 10p11–q11

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A region of linkage to type 1 diabetes has been defined on human chromosome 10p11–q11 (*IDDM10*; $P = 0.0007$) using 236 UK and 76 US affected sibpairs and a 1 cM resolution microsatellite marker map. Analysis by the transmission disequilibrium test (TDT) in 1159 families with at least one diabetic child, from the UK, the US, Norway, Sardinia and Italy provided additional support for linkage at *D10S193* ($P = 0.006$, $P_c = 0.17$). Notably, 5.1 cM distal to *D10S193*, marker *D10S588* also provided positive TDT results ($P = 0.009$, $P_c = 0.25$) but the allele under analysis was also preferentially transmitted to nonaffected siblings ($P = 0.0008$, $P_c = 0.02$). This allele was positively associated in an independent UK case control study and, importantly, was neutrally transmitted in control CEPH families. These results suggest a type 1 diabetes susceptibility locus on chromosome 10p11–q11 (provisionally designated *IDDM10*) and demonstrate the necessity of analysis of non affected siblings in disease families, as well as analysis of control families.

INTRODUCTION

The cause of human type 1 or insulin-dependent diabetes mellitus (IDDM) is an undefined interaction between genetic and environmental factors. While the environmental agents are unknown, compelling evidence exists for type 1 diabetes aetiological mutations at the HLA region (chromosome 6p; *IDDM1*) (1) and the insulin gene region (chromosome 11p; *IDDM2*) (2,3). They do not, however, account for all of the risk attributable within families (4). Microsatellite marker loci based genome-wide scans of type 1 diabetic affected sibpair (ASP) families have greatly

accelerated the search for additional susceptibility mutations (5,6). By analysing affected sibpair sharing of alleles identical by descent (IBD) at each marker, >20 chromosome regions showing some positive evidence for linkage ($P < 0.05$) were identified. These regions are starting points for additional analyses in the same and independent populations. To date, three more chromosome regions have been conclusively linked to type 1 diabetes: *IDDM4* (11q13), *IDDM5* (6q25) and *IDDM8* (6q27) (7,8).

One of the regions identified by the type 1 diabetes genome scans extended from *D10S197* to *D10S220* across the centromere of chromosome 10 (5) [Généthon map distance = 24 cM (9)]. Using single point analysis, the peak evidence of linkage was at *D10S193* (MLS = 1.9, $P = 0.002$) and the proportion of sibpairs sharing neither allele IBD (Z_0) = 0.20. Furthermore, the gene which encodes the 65 kDa form of glutamic acid decarboxylase (*GAD65*), a major type 1 diabetes associated autoantigen (10), has been localized close to this region, by *in situ* hybridization (11) and linkage analysis (12). Wapelhorst *et al.* (12) investigated linkage and linkage disequilibrium of *GAD65* to type 1 diabetes in UK and US ASP families and concluded that variation in the *GAD65* gene does not play a significant role in susceptibility to type 1 diabetes. However, they did provide weak evidence ($P = 0.02$) of linkage disequilibrium to type 1 diabetes with a rare allele (frequency = 5.8%) of a microsatellite marker closely linked to *GAD65* (12). A genome scan of French and US ASP families (6) detected linkage at *D10S582* ($P < 0.05$), 11 cM distal to *D10S193* (4 cM distal to *D10S197*), possibly providing support for the linkage observed in the genome scan of UK families (5). Affected sibpair linkage and transmission disequilibrium test (TDT) (13–15) analyses of an increased density of markers in the original and additional populations should better clarify this putative region of susceptibility to type 1 diabetes on chromosome 10.

In this study, large scale, high resolution, multipoint affected sibpair linkage and single point TDT linkage analyses of chromosome 10p11–q11 has been undertaken in UK, US,

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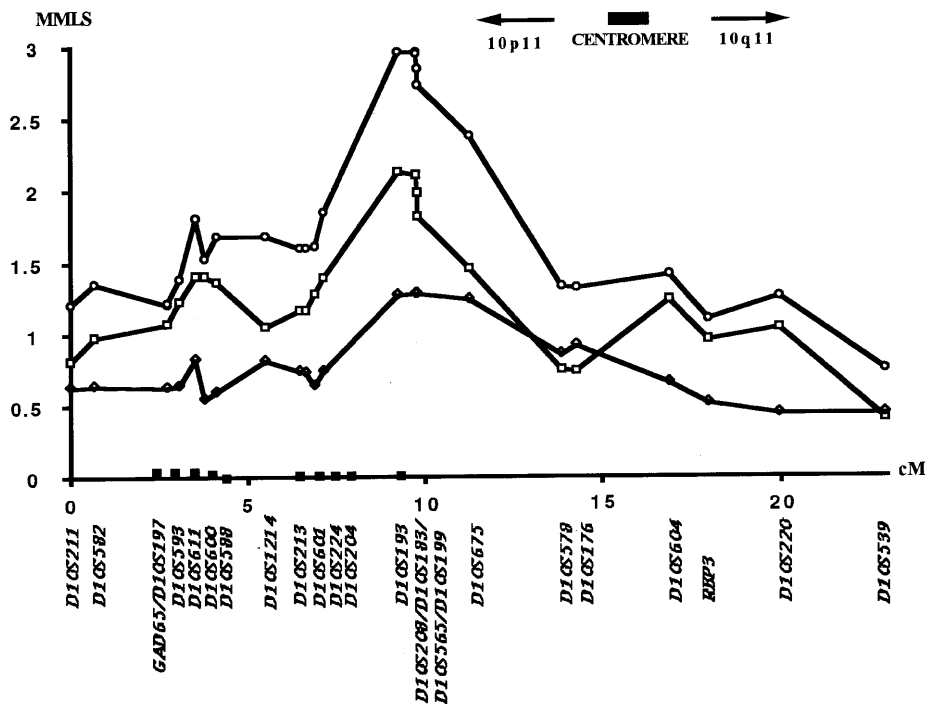


Figure 1. Multipoint maximum lod scores (MMLS) obtained in UK96 (\square), UK140 (\diamond), total 236 UK (\circ) and US76 (\blacksquare) families. The markers are, from 10p11 to 10q11, *D10S211*-0.7-*D10S582*-2.1-*GAD65/D10S197*-0.3-*D10S593*-0.4-*D10S611*-0.3-*D10S600*-0.3-*D10S588*-1.4-*D10S1214*-0.9-*D10S213*-0.2-*D10S601*-0.2-*D10S224*-0.3-*D10S204*-2.1-*D10S193*-0.5-*D10S208/D10S183/D10S565/D10S199*-1.5-*D10S675*-2.6-*D10S578*-0.4-*D10S176*-2.6-*D10S604*-1.1-*RBP3*-2.0-*D10S220*-3.0-*D10S539* (where '/' represents no observed recombination between markers). Intermarker distances were calculated from the 236 UK families and are in cM. *GAD65* has been localized to 10p11.2 (9). The average percentage of IBD status information extracted at each marker from the 236 UK families was 98% (range 89–100%). The maternal recombination frequency within this map was ~6-fold greater than the paternal recombination frequency (data not shown). An extreme sex difference in recombination frequency extends from *GAD65* to *D10S604* (maternal distance = 30.0 cM; paternal distance = 1.3 cM), in agreement with another genetic map of the region (16).

Norwegian, Sardinian and Italian type 1 diabetic Caucasian populations. We present data suggesting a type 1 diabetes susceptibility locus (*IDDM10*) in the chromosome 10p11 region.

RESULTS

Multipoint linkage mapping of type 1 diabetes susceptibility in the 10p11–10q11 region

Originally, three out of four microsatellite marker loci at the peri-centromeric region of chromosome 10 showed some positive evidence of linkage in 96 UK type 1 diabetic ASP families (UK96) ($P = 0.002$) (5). In this report analysis is extended to an additional 21 published microsatellite markers (extending 2.8 and 3.0 cM qter from the initial putative region of linkage identified by genome scanning) that had been mapped to this region of chromosome 10. A further 140 UK ASP families (UK140), in addition to the UK96, were analyzed with all 25 microsatellite markers and multipoint maximum lod score (MMLS) values calculated (Materials and Methods) (Fig. 1). Peak evidence of linkage in the UK96 was at *D10S193* (MMLS = 2.13, $P = 0.001$, $Z_0 = 0.20$). Peak evidence of linkage in the UK140 was at *D10S208* (MMLS = 1.28, $P = 0.01$, $Z_0 = 0.19$), 0.5 cM from *D10S193*, thereby extending and replicating the initial result of linkage in this region. When the UK family sets were combined the peak evidence of linkage was at *D10S208* (MMLS = 2.96, $P = 0.0002$, $Z_0 = 0.20$). These two independent observations of linkage and the overall evidence ($P = 0.0002$) in the 236 UK type 1 diabetic families provide support for the

presence of a type 1 diabetes susceptibility locus in the 10p11–q11 region. Multipoint analysis of 10 of the marker loci in 76 US type 1 diabetic ASP families provided no evidence for linkage (Fig. 1). The combined 312 UK and US families had a peak MMLS of 2.44 ($P = 0.0007$, $Z_0 = 0.22$) at *D10S208*.

TDT analysis of microsatellite marker alleles

The evidence supporting linkage for the total 312 ASP families ($P = 0.0007$) could be expected to occur by chance at least once in a genome-wide search for polygenic disease susceptibility loci (17). However, the evidence of linkage exceeds that obtained from analysis of confirmed loci *IDDM2*, *IDDM4*, *IDDM5* and *IDDM8* in similar numbers of ASPs from the same populations (4; and data not shown). Therefore, the results warranted further study by using the TDT to test for linkage in the presence of allelic association in families (13–15), which is a more sensitive approach to detecting and localizing susceptibility genes and can be done in families with only one affected child thereby extending the available data sets (4,18–20). Initially, linkage by the TDT was assessed in the 236 UK ASP families because they were linked by ASP analysis to type 1 diabetes whereas the 76 US ASP families were not. To reduce the number of independent statistical tests and the potential for false positives only the common alleles (frequency $\geq 20\%$ in parents) of those marker loci within the region of linkage with MMLS > 1.5 ($P < 0.006$) were analyzed. Because the candidate gene marker locus *GAD65* lay < 1 cM distal of this region our analysis was extended to that marker. Table 1 shows TDT analysis in the total 236 UK families of the 17 marker

loci in the region of linkage (a total of 28 alleles were tested). Allele 7 (298 bp) of *D10S193* and allele 4 (142 bp) of *D10S588* provided some evidence ($P < 0.05$) by the TDT for linkage with type 1 diabetes.

TDT and case control analysis of *D10S193*

Analysis of transmission from parents to type 1 diabetics of allele 7 of *D10S193* was extended to an additional 142 UK ASP and simplex families, 185 US ASP families (including the 76 US ASP families previously tested by MMLS), 375 Norwegian, 167 Sardinian and 54 Italian simplex families. Non-diabetic siblings from these families, for which DNA was collected, were also analyzed. Table 2a shows transmission of allele 7 of *D10S193* from parents to diabetic and non-diabetic children. In all populations, transmission of allele 7 to diabetic children was $>50\%$. However, only in the UK was the P value <0.05 (55% transmission; $P = 0.01$). When the data for all populations were combined the transmission was 54% [$P = 0.006$, and when

corrected for the number of independent tests (Materials and Methods) $P_c = 0.17$]. To determine that transmission of the allele to diabetic children is dependent upon type 1 diabetes, and not owing to segregation distortion, we investigated transmission of allele 7 to non-diabetic children from the same type 1 diabetic families (Table 2a) and to all available members of the 61 CEPH reference families. When the data for transmission to non affected children of all type 1 diabetic families were combined the transmission was 47%. A 2×2 test of heterogeneity provides evidence ($P = 0.006$, $P_c = 0.17$) that transmission of allele 7 to diabetic children is increased in comparison with non-diabetic children in type 1 diabetic families. Transmission of allele 7 to all available members of the 61 CEPH reference families exceeded non-transmission, but not significantly [274 transmissions (T) versus 235 non-transmissions (NT); 54%, $P > 0.05$]. A population based test for association of allele 7 of *D10S193* to type 1 diabetes gave no independent evidence of linkage disequilibrium with type 1 diabetes (32, 100 and 98 patients with two, one or zero 298 bp alleles versus 52, 168 and 197 controls).

Table 1. Transmission to diabetic children in 236 UK ASP families of the common alleles of microsatellite marker loci on chromosome 10p11

Marker	Allele	Frequency	Transmitted	Not transmitted	% transmitted	P
<i>GAD65</i>	5	22	115	119	49	
	8	25	125	112	53	
<i>D10S197</i>	5	20	124	146	46	
	6	43	251	216	54	
<i>D10S593</i>	12	55	182	154	54	
<i>D10S611</i>	1	20	137	145	49	
	2	30	184	204	47	
	3	45	231	199	54	
<i>D10S600</i>	10	26	182	175	51	
	12	22	151	157	49	
<i>D10S588</i>	4	68	233	189	55	0.03
<i>D10S1214</i>	NA	NA	NA	NA	NA	
<i>D10S213</i>	14	31	202	164	55	
<i>D10S601</i>	4	21	157	149	51	
<i>D10S224</i>	5	32	187	181	51	
	6	44	216	235	48	
<i>D10S204</i>	3	35	227	198	53	
<i>D10S193</i>	5	30	188	192	49	
	7	32	229	187	55	0.04
<i>D10S208</i>	6	22	120	137	47	
	7	35	153	176	47	
	8	23	141	118	54	
<i>D10S183</i>	14	22	149	129	54	
<i>D10S565</i>	10	86	92	114	45	
<i>D10S199</i>	4	23	146	168	46	
	6	22	132	162	45	
	7	26	185	154	55	
<i>D10S675</i>	5	27	183	194	49	
	9	47	233	227	51	

Alleles are designated a rank by the PCR product size (1 = smallest). Only alleles $\geq 20\%$ frequency in the parental population were included for analysis. A χ^2 test against the hypothesis of 50% transmission was used, only P values <0.05 are given. NA, no alleles were present at a frequency $\geq 20\%$.

Table 2. (a) Percentage transmission of allele 7 of *D10S193* to diabetic and non-diabetic children in UK, US, Norwegian, Sardinian and Italian families. Novel PCR primers were designed for this study, allele 7 (298 bp) is identical to the 225 bp allele amplified using the published Généthon primers (7). (b) Percentage transmission of allele 4 of *D10S588* to diabetic and non-diabetic children in UK, US, Norwegian, Sardinian and Italian families

	UK (n = 378)	US (n = 185)	Norwegian (n = 375)	Sardinian (n = 167)	Italian (n = 54)	Total (n = 1159)
(a) <i>D10S193</i> (allele 7)						
Diabetic	55 (365:299) ^a	54 (174:151)	51 (134:127)	52 (51:48)	55 (12:10)	54 (736:635) ^b
Non-diabetic	48 (58:64)	51 (24:23)	47 (155:175)	45 (38:47)	44 (12:15)	47 (287:324)
(b) <i>D10S588</i> (allele 4)						
Diabetic	55 (344:286) ^a	57 (163:121) ^a	51 (137:130)	49 (59:62)	38 (12:20)	54 (715:619) ^b
Non-diabetic	57 (74:56)	66 (46:24) ^b	55 (190:154)	52 (51:48)	61 (22:14)	56 (383:296) ^c

Number of transmissions versus number of non-transmissions are in brackets. A χ^2 test against the hypothesis of 50% transmission was used.

^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$.

Table 3. Association of allele 4 of *D10S588* in UK type 1 diabetic patients and controls

Allele/phenotype/genotype	Diabetics (n = 221)		Controls (n = 404)	
Allele frequencies ^a				
4	312	71%	532	66%
X	130	29%	276	34%
Phenotype frequencies ^a				
4	199	90%	369	91%
X	22	10%	35	9%
Genotype frequencies ^b				
4/4	113	51%	163	40%
4/X	86	39%	206	51%
X/X	22	10%	35	9%

^a χ^2 test of heterogeneity $P > 0.05$; ^b χ^2 test of heterogeneity $P = 0.01$, odds ratio for 4/4 genotype = 1.5 (95% C.I. = 1.1–2.2; $P = 0.009$).

TDT and case control analysis of *D10S588*

We extended our analysis of the transmission of allele 4 of *D10S588* to the same families used for the TDT study of *D10S193*. Table 2b shows transmission from parents to diabetic and non-diabetic children, of allele 4 of *D10S588* in the UK, US, Norwegian, Sardinian and Italian type 1 diabetic families. When the data for all populations were combined the transmission to diabetic children was increased (54%; $P = 0.009$, $P_c = 0.25$). However, transmission was only increased in the UK (55%; $P = 0.02$) and US (57%; $P = 0.01$) populations at $P < 0.05$. Surprisingly, transmission of allele 4 to non-diabetic children in type 1 diabetic families was >50% in all five populations. When the data for all populations were combined the transmission to non-diabetic children was 56% ($P = 0.0008$, $P_c = 0.02$). We tested transmission of allele 4 to all available members of the 61 CEPH

reference families and found little deviation from the expected 50:50 (239T versus 242NT).

Association of allele 4 of *D10S588* to type 1 diabetes was tested in an independent collection of UK Caucasian diabetics and controls. Table 3 shows some evidence that the allele 4 homozygous genotype confers increased risk to type 1 diabetes (odds ratio = 1.5; 95% C.I. = 1.1–2.2; $P = 0.009$).

DISCUSSION

Linkage to type 1 diabetes on human chromosome 10p11–q11 provides suggestive evidence for a susceptibility locus (*IDDM10*) in this region. Evidence of linkage in the presence of association at *D10S193* and *D10S588* extends this evidence. The type 1 diabetes locus on chromosome 10p11–q11 has provisionally been designated *IDDM10*.

The TDT is valid as a test of association but only when transmission is analysed to probands in multiplex families (15). Support for association in the 1159 families is not significant for allele 7 of *D10S193* in the 1159 families [P_{proband} (Materials and Methods) = 0.057] but significant for allele 4 of *D10S588* ($P_{\text{proband}} = 0.021$). Possible linkage disequilibrium of allele 4 of *D10S588* with type 1 diabetes is also supported by the independent evidence of association of this allele in a case-control study. However, it has to be stressed that owing to population stratification effects arising from incorrectly matched controls, case-control results must be viewed with caution (21). The evidence is, however, complicated by the increased transmission of allele 4 to non-diabetic siblings of type 1 diabetics, whereas transmission in the CEPH reference families is at the expected level of 50%. Islet cell autoantibodies (ICA) are a predictor of type 1 diabetes in first-degree relatives of type 1 diabetic patients (22). It is possible that the observed positive transmission of allele 4 of *D10S588* to non affected siblings in the diabetic families reflects transmission to those siblings that are positive for ICA or for other autoantibodies and insulinitis, and that allele 4 is associated with a disease allele that predisposes to ICA development/insulinitis and overt disease. This would be consistent

with both the neutral transmission in the CEPH families and the evidence of association in the case control study. The ICA status of non affected siblings used in this study is unknown. Evidence for linkage of chromosome 2q34 with both type 1 diabetes and ICA positivity has been reported (23).

Given that the *GAD*₆₅ microsatellite and the very closely linked *D10S197* microsatellite give no evidence of linkage by the TDT to type 1 diabetes a role for *GAD*₆₅ in genetic susceptibility is less likely, at least in the populations studied here. Furthermore, whilst transmission of the rare allele 8 of the *GAD*₆₅ microsatellite to diabetic children was increased in the study of Wapelhorst *et al.* (12) (54T versus 24NT), we found no significant increase in transmission of allele 8 to diabetic children (34T versus 30NT). Our results are in agreement with their conclusion that the *GAD*₆₅ gene does not play a significant role in genetic susceptibility to type 1 diabetes. Nevertheless, only a very high resolution linkage disequilibrium map of single nucleotide mutations including and surrounding the gene and regulatory region, in multiple populations, can comprehensively exclude a minor role for *GAD*₆₅ in susceptibility to type 1 diabetes.

Experience with type 1 diabetes indicates that identification of regions of linkage and/or linkage disequilibrium to polygenic disease is difficult. Investigations in one or two small populations are prone to false positives and are not sufficient to define a disease locus. In this study the chance of false positives was reduced by analysing a large number (1159) of type 1 diabetic families and minimizing the number of statistical tests [for example, by analyzing only frequent ($\geq 20\%$) alleles]. Even so, >1159 families or families from more genetically isolated populations, and haplotypes of very closely linked markers from regions showing evidence of increased transmission of microsatellite alleles to affected children will have to be analysed to provide further evidence for the existence of a type 1 diabetes gene in the chromosome 10p11–q11 region.

MATERIALS AND METHODS

The 1159 type 1 diabetic families in this study are Caucasian with at least one diabetic child and both parents included. Where available DNA from non-diabetic siblings was obtained. The 378 UK families consisted of 24 simplex families collected from the Yorkshire region incorporating one diabetic diagnosed under age 17 years and one unaffected sibling, and 354 ASP families recruited as part of the British Diabetic Association–Warren repository. The total 185 US families were obtained from the Human Biological Database Interchange (HBDI). The UK96, UK140 and 76 US portions of the total ASP families, used for MMLS and TDT analyses, consisted of one diabetic diagnosed under age 17 years and the other under age 29. The additional 118 UK and 109 US ASP families, analyzed by TDT only, were not restricted by number of diabetic children nor age of diagnosis. The 375 Norwegian simplex families were comprised of one diabetic diagnosed under age 15 years and available non-diabetic siblings. The 167 Sardinian simplex families incorporated one diabetic diagnosed under age 17 years and at most one non-diabetic sibling. The 54 Italian type 1 diabetic families consisted of one diabetic child and available non-diabetic siblings. Unrelated UK diabetics and controls were as defined by Bain *et al.* (24). DNA from all available members of the total 61 CEPH reference families were used and the genotypes for marker loci *D10S193* and *D10S588* submitted to CEPH.

Microsatellite markers were identified from the public databases except for the *GAD*₆₅ locus microsatellite identified from Wapelhorst *et al.* (12). Fluorescence-based genotyping was undertaken using ABI 373A automated sequencers and associated genotyping software (Applied Biosystems) as previously described (25). Modifications were as follows. (i) PCRs were performed in 96-well microtitre plates (Costar) in a 10 μ l volume containing 0.2 U of AmpliTaq polymerase (Perkin-Elmer). (ii) Twenty-eight cycles (5 s at 95°C, 30 s at 50–60°C, 1 s at 72°C) were performed in PTC-200 thermocyclers (MJ Research). (iii) Co-precipitation of PCR products was omitted and ~0.1 μ l per marker was combined with the internal size standard and formamide. (iv) Single strand size markers GS-350 or GS-500 (Applied Biosystems) were used and three separate electrophoresis runs made with each gel. Raw allele sizes were allocated into bins and designated as population-specific alleles using the global adaptive binning facility of the Genome Analysis System software [GAS version 2.0: (c) A. Young, University of Oxford, 1993–1995] available on the internet at <http://users.ox.ac.uk/~ayoung/gas.html>.

The order of markers with the least total number of apparent recombinants was identified by the BESTORDER option of the SIBMAP analysis routine in GAS and used in conjunction with the Whitehead institute/MIT human physical mapping project database (<http://www-genome.wi.mit.edu>) to produce the order of markers shown in Figure 1. The inter-marker recombination fractions were calculated by GAS. Distances <0.5 cM are based on one or zero recombinants and are therefore not accurate. This rapid method of linkage mapping provides a rudimentary map of very closely linked markers for which only a detailed physical map will identify the exact marker order and inter-marker distance. Novel primers were designed for marker loci *D10S213*, *D10S193*, *D10S183*, *D10S199*, *D10S578*, *D10S604*, *D10S220* and *D10S539*. Primer sequences and allele frequencies for all markers are available on the internet at <http://www.well.ox.ac.uk>.

Multipoint maximum lod scores (MMLS), Z_0 values and informativity across the region were calculated with the MAP-MAKER/SIBS program (26). The *P* values assigned to MMLS scores are theoretical (27).

The transmission disequilibrium test (TDT) (13–15) was undertaken using the ASSTDT analysis routine in GAS. Allelic transmissions from heterozygous parents to all children in each family are included. The test statistic (the 'TDT') is a χ^2 (1df) statistic, which tests deviation of transmission from the expected 50% transmission of an allele from heterozygous parents to offspring. TDT *P* values are reported uncorrected (*P*) and corrected (*P*_c) for the multiple tests done initially (*P* values were multiplied by 28, to account for the 28 microsatellite marker alleles initially tested by the TDT). Extent of linkage disequilibrium of a marker allele with disease was quantitated by percent transmission which is the number of times an allele is transmitted from heterozygous parents to affected children divided by the total number of transmissions, expressed as a percentage. The TDT was also used as a test of association by testing transmission to probands only (15) (*P*_{proband}).

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