Exclusion of familial dysautonomia from more than 60% of the genome

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Abstract

Familial dysautonomia (FD) is a recessive neurological disorder that affects the development of the sensory and autonomic nervous system. The gene defect appears to be limited to the Ashkenazi Jewish population, where the carrier frequency is 1 in 30. One hundred and ninety-one marker loci representing all autosomes were tested for linkage with the FD genetic defect in 23 families. A combination of pairwise and multipoint analyses excluded the FD gene from at least 60% of the autosomal genome. The program EXCLUDE predicted regions of chromosomes 2, 4, 5q, 9, or 10 as the most promising locations for future analyses.

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Familial dysautonomia (FD), or the Riley-Day syndrome, is a rare inherited neurological disease affecting the development and survival of sensory, sympathetic, and some parasympathetic neurones.[1–3] It is the most common and best known of a group of rare disorders, termed congenital sensory neuropathies, that are characterised by widespread sensory and variable autonomic dysfunction. Patients with FD are affected from birth with a variety of symptoms such as decreased sensitivity to pain and temperature, vomiting crises, and cardiovascular instability, all of which might result from a deficiency in a neuronal growth factor pathway.[4,5] Diagnosis of FD is based on the following cardinal criteria: absence of fungiform papillae on the tongue, absence of flare after injection of intradermal histamine, decreased or absent deep tendon reflexes, and absence of overflow of emotional tears.[2,3] Neuropathological findings have clearly differentiated FD from other congenital neuropathies.[2] The disorder has autosomal recessive inheritance with complete penetrance and currently appears to be confined to persons of Ashkenazi Jewish descent.[6] In this population, the estimated carrier frequency is 1 in 30 with a disease incidence of 1 in 3600 live births.[7] The clear cut pattern of transmission, apparent restriction to one ethnic population, straightforward diagnosis, and lack of confounding phenocopies suggest that all cases of FD might have descended from a single founder mutation.[2]

Chromosomal localisation of the gene causing FD would facilitate genetic counselling and prenatal diagnosis in affected families. Subsequent delineation of closely linked markers which may show strong linkage disequilibrium with the disorder and, ultimately, identification of the defective gene could allow screening of the entire at risk population to identify carriers, and potentially reduce the incidence of new cases. Consequently, we have sought to identify the position of the FD gene using genetic linkage analysis. While the disease gene has not yet been located, the exclusion of large regions of the genome will serve to guide future analyses to accelerate discovery of the FD locus.

Subjects and methods

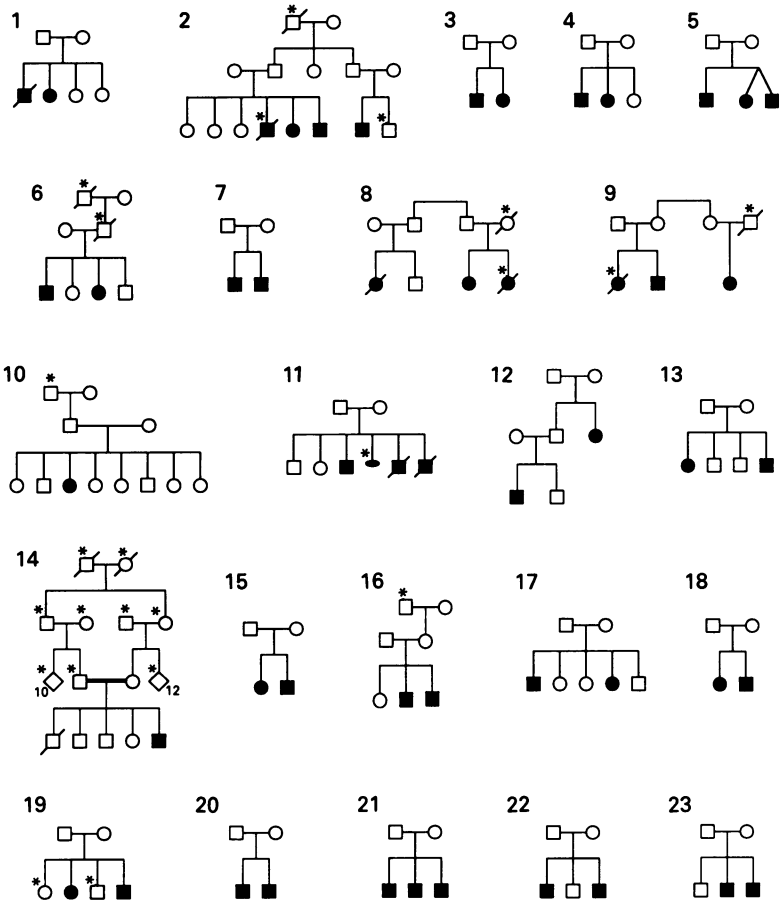
FD FAMILIES

Patients' files from the Dysautonomia Center at the New York University Medical Center were used to obtain families with more than one dysautonomic child. Twenty-one families were identified as having two or three surviving affected subjects (fig 1). Two additional families were chosen for linkage analysis based on a large number of unaffected sibs (family 10, fig 1) and parents who are first cousins (family 14, fig 1). All families were from North America or Israel. The diagnosis of FD was confirmed in all cases based on standard criteria.[2,3] Cell lines (130 lymphoblast, two fibroblast) were established from family members shown in fig 1 (except for those marked with *).

DNA ANALYSIS

For typing restriction fragment length polymorphisms (RFLPs), genomic DNA was isolated from cell lines, digested with restriction endonuclease, resolved by electrophoresis on 0·8% agarose gels, and transferred to Hybond N^+ membranes.[8] Blots were hybridised with probe DNA radioactively labelled by random priming and visualised by autoradiography as previously described.[8]

For typing simple sequence repeat polymorphisms, the method described by Weber and May[9] was used with the following modifications: PCR reaction volume was reduced to 10 µl using 5 to 10 ng genomic DNA, 40 ng of each primer, and about 0·25 U *Taq* polymerase (Boehringer). In most cases, α-^{32}P-dGTP (3000 Ci/mmol, Amersham) was used as the labelled nucleotide. PCR conditions varied as described for the specific markers. Dried gels were subjected to autoradiography for four to 16 hours using Kodak X-OMAT AR film.



*Blood not collected.

Figure 1 Twenty-three families with familial dysautonomia used for linkage studies.

LINKAGE ANALYSIS

The LIPIN (v 2·1) data management program[10] was used for entry of marker phenotypes into a VAX8700 computer. Pairwise lod scores were calculated using MLINK (v 3·5).[11] Autosomal recessive inheritance, complete penetrance, no rate of new mutations, and a gene frequency of 1/60 were assumed for FD. Multipoint analyses used LINKMAP (v 4·9)[11] to analyse groups of three to five markers at a time. Exclusion maps of entire chromosomes were generated from a set of overlapping three to five point analyses (for example, analysis 1: A-B-C-D; analysis 2: C-D-E-F, etc). No significant differences were found for any interval calculated in more than one analysis (for example, C-D above). Likelihood estimates from non-overlapping intervals (for example, A-B-C and D-E-F) flanking the overlapping interval were concatenated to construct the overall multipoint curve. Lod scores < -2 were used to define exclusion limits in both pairwise and multipoint analyses. When several polymorphisms were tested at the same marker locus, haplotyping was performed. Potential locations of the FD gene were estimated using the program EXCLUDE.[12] The input to the EXCLUDE program consists of chromosomal position, locus name, data type, and the linkage data in the form of a standard lod table (table). The location of each marker on a chromosome is expressed as a fraction corresponding to the proportion of the chromosome's physical length between the locus and the p-terminus. When the potential location of a marker extends over a large physical segment, that marker is arbitrarily assumed to be in the centre of the segment.

Results and discussion

In total, 191 marker loci were typed in the FD pedigrees. Thirty-three of these were essentially uninformative, yielding lod scores between $-0{\cdot}5$ and 0·5 at all recombination fractions. Lod scores for these markers are not presented. The lod scores for linkage to *FD* of the other 158 marker loci are shown in the table. None of the markers provided significant evidence of linkage, although several yielded weak positive lod scores with the highest being $+1{\cdot}02$ for *D5S22*. Most markers allowed some degree of exclusion of the respective flanking regions. The largest ranges of exclusion, up to $\theta = 0{\cdot}24$ on either side of the marker (corresponding to 26 cM based on the Kosambi mapping function or 33 cM based on the Haldane mapping function), came from the highly informative simple sequence repeat polymorphisms. The region of exclusion (expressed in the recombination fraction corresponding to $\theta = -2$) for each marker is also given in the table.

To maximise the information derived from specific chromosomes with well established genetic maps, we performed multipoint analyses assuming equal male and female recombination rates, with distances fixed from the sex averaged genetic maps.[13 20 23] Fig 2 presents examples of multipoint exclusion of the FD gene from chromosomes 1q, 17, 21q, and 22q. Two candidate genes for FD have been mapped to chromosome 1: NFGB, encoding the nerve growth factor β subunit (previously excluded as the site of the FD defect[4]), and NTRK1, encoding a tropomyosin receptor kinase (a protein that serves as a component of the high affinity NGF receptor[25]). Since NTRK1 maps to chromosome 1q32–q41[26] (a region covered by *REN*, *D1S81*, and *D1S103*), we decided to perform multipoint analysis of chromosome 1q. The probes used span almost the entire length of 1q, covering 167 cM from *NGFB* in 1p13 to *D1S102* in 1q32–44.[14] *FD* was excluded from most intermarker intervals as well from 4 cM distal to *NGFB* and from 16 cM distal to *D1S102*. Only two regions of chromosome 1q were not formally excluded (score < -2): a segment of the 45 cM interval between *D1S61* and *REN*, and a segment between *CRP* and *NGFB*.

Chromosome 17 was analysed because one Ashkenazi Jewish subject is afflicted with both FD and Charcot-Marie-Tooth disease type 1a (unpublished data). *CMT1A* has been assigned to chromosome 17p12–p11.2 between *D17S1* in 17p13.2–p13.3 and *D17S71* in 17p12–p11.2.[27] The low affinity NGF receptor (*NGFR*), another candidate gene for FD that was excluded as the gene causing the disorder,[5] also maps to chromosome 17. The marker

Results of pairwise linkage analysis of familial dysautonomia with various polymorphic DNA loci.

Chromosomal location*	Locus	Lod score at θ						Excluded region (θ)†	Ref
		0·00	0·05	0·10	0·20	0·30	0·40		
1p32	*D1S57*	−∞	−3·58	−2·05	−0·77	−0·26	−0·05	±10	13
1p31.2	*D1S116*	−∞	−7·57	−3·91	−1·30	−0·43	−0·09	±16	14
1p13	*NGFB*	−∞	−1·65	−0·86	−0·24	−0·04	+0·00	± 4	13
1(q21.1)	*CRP*	−∞	−2·84	−1·15	−0·08	+0·10	+0·05	± 7	13
1(q21.3)	*D1S104*	−∞	−13·16	−7·55	−2·96	−1·10	−0·32	±24	13
1(q22)	*D1S61*	−∞	−2·95	−1·01	−0·14	+0·25	+0·09	± 7	13
1(q31)	*REN*	−∞	−5·51	−2·73	−0·73	−0·13	−0·00	±13	13
1(q31)	*D1S58*	−∞	−2·88	−1·53	−0·54	−0·19	−0·04	± 8	13
1(q32)	*D1S81*	−∞	−10·03	−5·62	−2·09	−0·71	−0·14	±20	13
1(q32)	*D1S103*	−∞	−12·83	−6·93	−2·42	−0·77	−0·15	±22	13
1(q42)	*D1S02*	−∞	−6·73	−3·73	−1·31	−0·41	−0·08	±16	13
2p25	*POMC*	−∞	−2·63	−1·38	−0·41	−0·09	−0·00	± 7	14
2p24	*APOB*	−∞	−2·16	−0·87	−0·06	+0·07	+0·03	± 6	14,15
2(p23)	*D2S48*	−∞	−0·30	+0·37	+0·59	+0·39	+0·12	± 0	15
2p12	*CD8A*	−∞	−10·02	−5·01	−1·29	−0·11	+0·13	±17	14
2q12–q21	*IL1*	−∞	−4·92	−1·87	−0·06	+0·35	+0·18	±10	14
2(q21.1)	*D2S44*	−∞	−2·96	−1·35	−0·23	+0·04	+0·04	± 7	15
2(q34)	*D2S50*	−∞	−0·97	−0·51	−0·17	−0·05	−0·01	± 0	15
2q36–q37	*PGLU*	−∞	−8·87	−4·77	−1·52	−0·37	−0·01	±17	16
2(q36)	*D2S61*	−∞	−2·74	−1·41	−0·41	−0·09	−0·01	± 7	15
2q37	*D2S3*	−∞	−1·71	−0·82	−0·20	−0·03	−0·00	± 4	14
2	*D2S71*	−∞	−4·26	−1·49	−0·19	+0·36	+0·13	± 9	14
2	*D2S72*	−∞	−5·99	−2·49	−0·33	+0·06	+0·01	±11	14
3p24.1–p22	*THRB*	−∞	−1·68	−0·79	−0·19	−0·03	−0·01	± 4	14
3q21–q24	*RHO*	−∞	−12·70	−7·49	−3·06	−1·14	−0·27	±24	14
3q27–q28	*D3S196*	−∞	−8·41	−3·85	−0·86	−0·09	+0·04	±15	14
3q28	*SST*	−∞	−3·87	−1·47	+0·02	+0·22	+0·09	± 9	14
4p16.3	*D4S10*	−∞	−1·87	−1·11	−0·47	−0·18	−0·04	± 5	14
4p16.1	*RAF1P1*	−∞	−1·93	−1·14	−0·46	−0·17	−0·04	± 5	14
4p13–p12	*GABRB1*	−∞	−8·07	−3·66	−0·66	+0·06	+0·08	±14	14
4p	*D4S124*	−∞	−2·24	−1·07	−0·27	−0·05	−0·00	± 6	14
4q25	*EGF*	−∞	−0·98	−0·49	−0·13	−0·03	−0·00	± 0	14
5(p15.3)	*D5S117*	−∞	−4·95	−1·58	+0·42	+0·57	+0·22	± 9	17
5p14	*D5S19*	−∞	−2·86	−1·38	−0·30	−0·01	+0·02	± 7	14
5(p14)	*D5S111*	−∞	−4·67	−2·15	−0·33	+0·10	+0·07	±11	17
5(p13)	*D5S108*	−∞	−3·52	−1·48	−0·14	+0·11	+0·05	± 8	17
5(q11.2)	*D5S118*	−∞	−6·68	−3·31	−0·86	−0·14	+0·00	±14	17
5(q13)	*D5S107*	−∞	−7·70	−3·48	−0·63	+0·05	+0·07	±14	17
5(q13)	*D5S39*	−∞	−13·11	−7·22	−2·56	−0·80	−0·14	±21	17
5q33.3–q34	*CFS1*	−∞	−7·53	−3·51	−0·74	−0·04	+0·03	±14	17
5q34–qter	*D5S22*	−∞	−3·61	−0·57	+1·02	+0·87	+0·30	± 7	17
6p25–p24	*F13A1*	−∞	−9·22	−4·58	−1·10	−0·04	+0·14	±16	14
6p23–q12	*TRM1*	−∞	−1·84	−0·29	+0·51	+0·43	+0·14	± 5	14
6p	*D6S105*	−∞	−6·57	−3·27	−0·96	−0·30	−0·13	±14	14
6q27	*D6S37*	−∞	−6·40	−3·59	−1·31	−0·43	−0·09	±17	14
6	*TCF2D*	−∞	−11·60	−6·18	−1·98	−0·54	−0·11	±20	18
7pter–p21	*PDGFA*	−∞	−1·37	−0·65	−0·16	−0·04	−0·00	± 2	14
7q35–qter	*D7S396*	−∞	−3·79	−1·94	−0·58	−0·15	−0·02	±10	14
7	*D7S435*	−∞	−9·00	−4·84	−1·66	−0·53	−0·11	±18	14
7	*D7S440*	−∞	−6·25	−2·59	−0·21	+0·27	+0·20	±11	14
8p21.1–p11.2	*ANK1*	−∞	−3·38	−1·84	−0·78	−0·39	−0·20	±10	14
8p12	*D8S87*	−∞	−5·54	−2·62	−0·66	−0·17	−0·05	±12	14
8q13–q21.2	*D8S84*	−∞	−6·92	−3·56	−1·25	−0·53	−0·19	±15	14
8q21–q22	*D8S85*	−∞	−6·55	−3·04	−0·64	−0·04	+0·02	±13	14
8q23–q24	*PENK*	−∞	−5·15	−2·69	−0·82	−0·21	−0·03	±13	14
8q24	*MYC*	−∞	−1·27	−0·43	+0·10	+0·15	+0·06	± 2	14
8	*D8S17*	−∞	−1·57	−0·48	+0·18	+0·22	+0·08	± 3	14
8	*D8S39*	−∞	−2·46	−1·41	−0·54	−0·18	−0·04	± 7	14
8	*D8S88*	−∞	−9·70	−5·14	−1·64	−0·45	−0·07	±18	14
9pter–q11	*D9S1*	−∞	−6·75	−3·87	−1·46	−0·49	−0·10	±16	14
9q34	*D9S7*	−∞	−2·78	−1·38	−0·36	−0·06	−0·00	± 8	14
9q34.1	*ASS*	−∞	−5·07	−1·64	+0·41	+0·54	+0·20	± 9	14
9q34–qter	*D9S66*	−∞	−6·34	−2·40	+0·08	+0·44	+0·20	± 6	14
10q22–q23	*D10S4*	−∞	−0·46	−0·06	+0·16	+0·14	+0·05	± 0	14
11p15.5	*HRAS*	−∞	−1·42	−0·59	−0·06	+0·03	+0·15	± 3	14
11p15.5	*INS*	−∞	−5·33	−2·38	−0·40	−0·06	+0·07	±11	14
11q13	*INT2*	−∞	−6·43	−2·99	−0·61	−0·02	+0·03	±13	14
11q21–q22	*D11S35*	−∞	−2·85	−0·50	+0·67	+0·54	+0·17	± 6	14
11q32	*D11S351*	−∞	−2·20	−0·53	+0·34	+0·31	+0·10	± 6	14
11q22.3–q23.3	*D11S144*	−∞	+0·09	+0·57	+0·61	+0·36	+0·11	± 0	14
11q23–qter	*D11S29*	−∞	−0·42	−0·06	+0·09	+0·06	+0·02	± 0	14
11q23.3–q24	*D11S420*	−∞	−8·99	−4·62	−1·32	−0·27	−0·01	±17	14
11	*D11S145*	−∞	−3·04	−1·58	−0·47	−0·11	−0·01	± 9	14
12p12.1	*KRAS2*	−∞	−0·58	−0·29	−0·08	−0·02	−0·00	± 0	14
12q12	*KRT18*	−∞	−2·99	−1·42	−0·33	−0·03	+0·02	± 8	AB, unpublished
12q14	*D12S6*	−∞	−3·93	−2·34	−0·96	−0·35	−0·08	±11	14
12q23–qter	*PLA2*	−∞	−3·27	−0·91	+0·36	+0·38	+0·13	± 7	14
12q	*D12S14*	−∞	−0·69	−0·12	+0·17	+0·14	+0·05	± 0	14
12q	*D12S17*	−∞	−0·86	−0·26	+0·08	+0·09	+0·03	± 0	14
12	*D12S43*	−∞	−9·88	−5·22	−1·66	−0·41	−0·01	±19	14
13q12	*FLT1*	−∞	−4·61	−2·42	−0·74	−0·18	−0·01	±12	14
13q14	*D13S37*	−∞	−2·93	−1·67	−0·63	−0·21	−0·04	± 7	14
13q14.2	*RB1*	−∞	−2·71	−1·30	−0·27	+0·01	+0·02	± 7	14
13q21	*D13S12*	−∞	−1·38	−0·84	−0·36	−0·14	−0·03	± 2	14
13q32.2	*D13S71*	−∞	−7·85	−4·03	−1·20	−0·29	−0·03	±16	14
13	*D13S17*	−∞	−4·00	−2·27	−0·84	−0·28	−0·06	±11	14
14q24.3	*D14S43*	−∞	−7·03	−3·52	−0·96	−0·16	+0·02	±15	14
14q23.33	*D14S1*	−∞	−2·72	−1·15	−0·10	+0·10	+0·05	± 7	14
14	*D14S13*	−∞	−2·23	−1·23	−0·43	−0·14	−0·03	± 6	14
14	*D14S34*	−∞	−4·39	−1·62	+0·07	+0·28	+0·12	± 9	14
15q15	*THBS1*	−∞	−1·33	−0·36	+0·15	+0·15	+0·05	± 3	14
15(q31)	*D15S30*	−∞	−3·51	−2·07	−0·90	−0·36	−0·09	±10	14,19
15(q22.1)	*D15S28*	−∞	−1·45	−0·75	−0·42	−0·06	−0·01	± 2	14,19
15(q22.3)	*D15S26*	−∞	−1·18	−0·69	−0·27	−0·10	−0·02	± 2	14,19
15(q24)	*D15S37*	−∞	−2·43	−1·03	−0·13	+0·05	+0·03	± 6	14,19
15(q26.1)	*D15S3*	−∞	−4·73	−2·61	−0·94	−0·31	−0·07	±12	14,19

Table—continued

Chromosomal location*	Locus	Lod score at θ						Excluded region (θ)†	Ref
		0·00	0·05	0·10	0·20	0·30	0·40		
16p	*D16S83*	−∞	−1·62	−0·39	+0·29	+0·27	+0·09	± 4	14
16q	*D16S261*	−∞	−9·35	−5·20	−1·91	−0·61	−0·09	±20	14
16q	*D16S265*	−∞	−8·90	−4·50	−1·18	−0·15	+0·05	±16	14
16q	*D16S266*	−∞	−9·77	−5·26	−1·75	−0·49	−0·07	±19	14
16	*D16S260*	−∞	−7·06	−3·88	−1·32	−0·37	−0·04	±17	14
17(p13.3)	*D17S5*	−∞	−4·63	−2·17	−0·49	−0·67	+0·00	±11	20
17p13.3	*D17S28*	−∞	−1·68	−0·65	−0·04	+0·05	+0·02	± 4	14
17(p13.1)	*TP53*	−∞	+0·11	+0·19	+0·13	+0·04	+0·01	± 0	20
17(p13.1)	*D17S1*	−∞	−5·05	−2·76	−0·93	−0·25	−0·01	±13	20
17p12–p11.2	*D17S261*	−∞	−3·45	−1·79	−0·57	−0·16	−0·02	± 9	14
17p11.2	*D17S71*	−∞	−5·20	−2·81	−1·02	−0·35	−0·07	±13	20
17(q12)	*D17S58*	−∞	−5·95	−3·42	−1·31	−0·45	−0·09	±16	20
17(q21)	*MTBT*	−∞	−1·99	−1·25	−0·57	−0·23	−0·05	± 5	20
17(q21.3)	*NGFR*	−∞	−3·26	−1·95	−0·81	−0·30	−0·07	±10	20
17(q23)	*GH*	−∞	−4·11	−2·35	−0·90	−0·32	−0·07	±12	20
17(q25)	*TK1*	−∞	−2·62	−1·28	−0·35	−0·07	−0·00	± 7	20
18p11.3	*D18S3*	−∞	−0·74	−0·31	−0·02	+0·04	+0·02	± 0	14
18p11	*D18S6*	−∞	−1·28	−0·59	−0·11	+0·00	+0·01	± 2	14,19
18(q11.1)	(19D)‡	−∞	−1·87	−1·01	−0·32	−0·00	−0·01	± 5	LJO, unpublished
18(q11.2)	(LDR111)§	−∞	−0·45	+0·22	+0·42	+0·25	+0·07	± 0	LJO, unpublished
18(q12.1)	*D18S19*	−∞	−0·95	−0·25	+0·11	+0·10	+0·03	± 0	14,19
18q21.3	*BCL2*	−∞	−1·29	−0·68	−0·23	−0·07	−0·01	± 3	14
18q21.3–qter	*D18S5*	−∞	−2·62	−1·39	−0·43	−0·10	−0·01	± 7	14
18q22–qter	*ERV1*	−∞	−1·74	−1·02	−0·45	−0·19	−0·05	± 4	14
18q22–qter	*MBP*	−∞	−2·88	−1·41	−0·32	−0·01	+0·02	± 8	14
18q23	*D18S11*	−∞	−8·47	−4·79	−1·83	−0·65	−0·15	±19	14
18(q23)	*D18S17*	−∞	−3·81	−2·24	−0·90	−0·32	−0·07	±11	14,19
18	(27F)‡	−∞	−2·06	−1·27	−0·57	−0·23	−0·06	± 5	LJO, unpublished
19q12–q13.1	*D19S75*	−∞	−4·70	−1·78	+0·04	+0·25	+0·05	±10	14
19q13.1	*D19S47*	−∞	−7·65	−3·94	−1·15	−0·30	−0·10	±16	14
19q13.4	*D19S22*	−∞	−1·20	−0·57	−0·18	−0·07	−0·01	± 1	14
20p12	*D20S27*	−∞	−9·95	−5·16	−1·60	−0·44	−0·09	±17	14
20q	*D20S19*	−∞	−7·32	−4·21	−1·61	−0·56	−0·12	±17	14
20	*D20S32*	−∞	−3·18	−2·16	−1·16	−0·60	−0·24	±11	21
21q11.1	*D21S13*	−∞	−6·15	−3·36	−1·13	−0·32	−0·05	±15	22,23
21q11.1	*D21S52*	−∞	−5·13	−2·74	−0·95	−0·32	−0·07	±13	23
21(q11.2)	*D21S1*	−∞	−5·60	−3·10	−1·12	−0·36	−0·07	±14	22,23
21(q11.2)	*D21S11*	−∞	−2·51	−1·32	−0·42	−0·11	−0·02	± 7	22,23
21(q21)	*D21S8*	−∞	−3·56	−1·89	−0·63	−0·19	−0·03	±10	22,23
21(q21)	*APP*	−∞	−3·46	−1·90	−0·62	−0·17	−0·02	±10	22,23
21(q21)	*D21S58*	−∞	−3·08	−1·52	−0·41	−0·08	−0·01	± 8	22,23
21(q21)	*D21S17*	−∞	−1·74	−0·64	−0·04	+0·13	+0·05	± 4	22,23
21(q21)	*ETS2*	−∞	−0·93	−0·46	−0·11	−0·01	+0·00	± 0	23
21(q22.1)	*D21S15*	−∞	−3·89	−2·15	−0·77	−0·25	−0·05	±11	22,23
21(q22.2)	*D21S19*	−∞	−2·39	−1·25	−0·42	−0·13	−0·03	± 7	22,23
21(q22.3)	*CD18*	−∞	−4·22	−2·27	−0·77	−0·23	−0·04	±11	23
22(q11.1)	*D22S9*	−∞	−2·55	−1·23	−0·30	−0·05	+0·00	± 7	24
22(q11.2)	*IGLV*	−∞	−1·66	−0·00	+0·73	+0·53	+0·17	± 4	24
22(q11.2)	*D22S10*	−∞	−1·56	−0·43	+0·16	+0·16	+0·05	± 4	24
22(q11.2)	*BCR*	−∞	−2·79	−1·35	−0·30	−0·02	+0·01	± 7	24
22(q11.2)	*CRYB2*	−∞	−2·85	−1·46	−0·38	−0·05	+0·01	± 7	JLH, unpublished
22(q11.2)	*D22S15*	−∞	+0·10	+0·45	+0·51	+0·32	+0·10	± 0	24
22(q11.2)	*D22S1*	−∞	−1·68	−0·75	−0·10	+0·05	+0·03	± 4	24
22(q12.1)	*D22S28*	−∞	−1·12	−0·03	+0·43	+0·29	+0·09	± 1	24
22(q12.1)	*D22S29*	−∞	−0·40	+0·43	+0·68	+0·42	+0·13	± 0	24
22(q12.2)	*D22S20*	−∞	−1·15	−0·45	−0·04	+0·03	+0·01	± 1	24
22(q12.3)	*MB*	−∞	−1·71	−0·78	−0·16	−0·01	+0·00	± 4	JLH, unpublished
22(q13.1)	*PDGFB*	−∞	−6·06	−3·19	−0·98	−0·30	−0·12	±14	24
22(q13.1)	*D22S64*	−∞	−0·25	+0·26	+0·40	+0·25	+0·08	± 0	JLH, unpublished
22(q13.2)	*D22S22*	−∞	−3·21	−1·61	−0·42	−0·07	−0·00	± 8	24
22(q13.3)	*D22S21*	−∞	−1·71	−0·80	−0·20	−0·03	+0·00	± 4	24
22(q13.3)	*D22S45*	−∞	−1·12	−0·50	−0·05	+0·04	+0·02	± 0	JLH, unpublished

* Based on physical mapping (without brackets) or genetic mapping (with brackets). The chromosomal location for each marker locus was based on the physical location cited in the reference, or on updated information from the Genome Data Base.[14] When genetic mapping information was used to estimate physical map locations, the location is given in brackets. When both were available, the genetic mapping was preferred over the physical mapping, since the chromosomal location listed in the table was used to determine relative chromosomal location for the EXCLUDE program (see Methods).
† Exclusion was calculated as the recombination fraction θ at $Z = -2$.
‡ Probes without locus names.
§ LDR111 is a probe previously assigned to chromosome 17. Linkage data maps it to chromosome 18.

loci tested on chromosome 17 span almost the entire chromosome, 147 cM, from *D17S5* in 17p13 to *TK1* in 17q23.2–q25.3.[14] *FD* was excluded from all intermarker intervals, including the region of *CMT1A*, and from 10 cM beyond each end of the genetic map (fig 2). Thus, all of chromosome 17, except for the most telomeric regions, was excluded as a potential site for the FD locus.

Chromosomes 21 and 22 are small acrocentric chromosomes with well established genetic maps.[22–24] For chromosome 21, the overall negativity of the pairwise scores (table) was reflected in the multipoint analysis (fig 2). The entire 95 cM map, along with 15 cM beyond either end, was excluded, essentially eliminating this autosome in FD. For chromosome 22, several markers gave weak positive lod scores (table). The most informative markers on the chromosome were used in the multipoint analysis (fig 2) which excluded *FD* from the entire chromosome 22 genetic map, except for the 20 cM interval between *D22S21* and *D22S45*, near the telomere of 22q.

Other chromosomal regions that showed weak positive scores in pairwise analyses, chromosome 11q and chromosome 9q34, were also analysed using multipoint methods. Most of 11q was excluded (data not shown). The intervals between *ASS*, *D9S66*, and *D9S7* on 9q were both excluded, but a non-significant positive lod score of 1·45 remained at $\theta = 0{\cdot}2$ centromeric to *ASS*. Similar but more limited multipoint analyses have excluded a number of

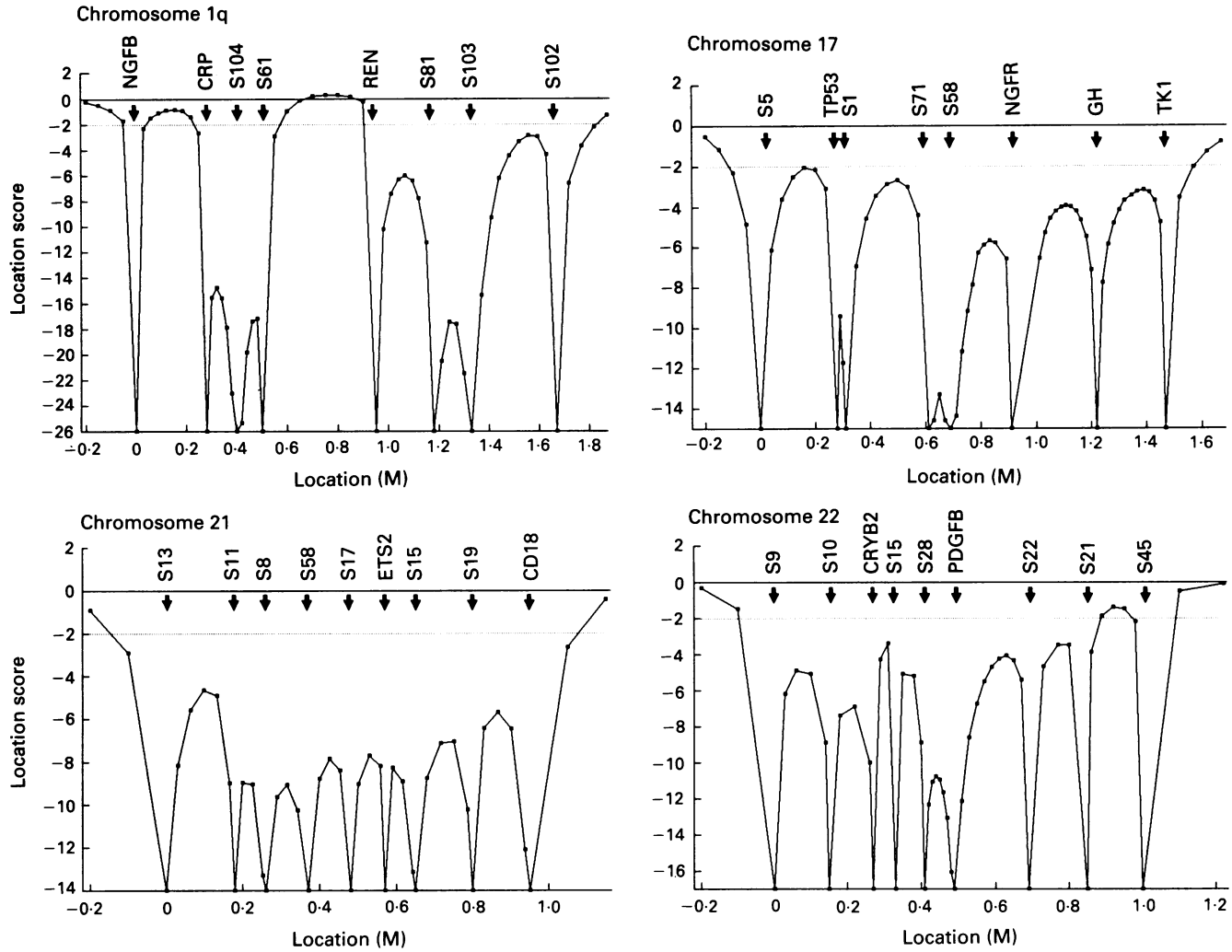


Figure 2 Multipoint exclusion of FD from chromosomes 1q, 17, 21, and 22. Multipoint exclusion was performed in all cases as described in Subjects and methods. The markers were positioned according to their genetic map locations, with the locus closest to the p telomere arbitrarily assigned the map location 0·0 (LJH, unpublished results).[13 20 23 24] *Arrows denote the locations of each named marker locus. Map positions are given in Morgans. Location score of −2 (the conventional value for exclusion) is marked.*

intermarker intervals on other chromosomes. In no case did multipoint analysis yield a significant positive lod score (> +3).

It is not possible to provide an exact calculation of the percentage of the genome excluded using the pairwise and multipoint methods, owing to uncertainties concerning the exact length of the human genetic map and the precise degree of overlap between the excluded regions for adjacent markers. However, given the number of markers tested and their dispersion throughout the genome, we conservatively estimate that our data exclude *FD* from at least 60% of the autosomal genome.

Since the major goal of this research is to define the position of the FD gene, we have used the program EXCLUDE to predict promising locations for future analysis. Fig 3 presents the results of this analysis. The program estimates the probability that the FD gene is located on a given chromosome relative to all other chromosomal regions and expresses this relative probability as a shaded area, adjacent to a line drawing that is proportional to the chromosome's length. The most likely site of the disease gene on that chromosome can then be judged from the distribution of the shaded region. This approach is valid under the assumption of genetic homogeneity, a reasonable likelihood in this case since FD is restricted to Ashkenazi Jews, suggesting a founder effect in this population. The most likely locations are: chromosome 5q distal to *D5S22*; the entire chromosome 10, where just one marker locus was tested; the centromeric part of chromosome 9q; and chromosome 4q.

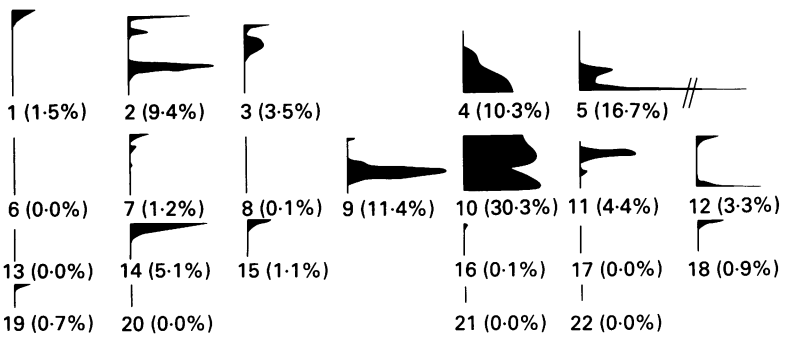


Figure 3 Exclusion map for FD using the program EXCLUDE. All marker loci from the table were analysed as described in Subjects and methods. Vertical lines represent the proportional length of each chromosome. Below each chromosome is the autosome number, accompanied by the relative probability that the FD gene is located on that autosome. The shaded regions along the chromosome show the possible locations of the disease gene with the areas of the shaded regions corresponding to the relative probability referred to above.

Other locations are less favourable and probes mapped to these regions will be examined only after testing all of the regions indicated above. Although the EXCLUDE program appears to eliminate *FD* from at least 85% of the genome, it should be noted that such calculations involve numerous assumptions that overestimate the extent of the excluded regions. However, given the number of FD families available for linkage analysis, the conservative exclusion of at least 60% of the genome, and the prediction by EXCLUDE of the most probable locations, a linked marker for familial dysautonomia should be found soon.

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1 Riley CM, Day RL, Greely DMcL, Langford WS. Central autonomic dysfunction with defective lacrimation. *Pediatrics* 1949;**3**:468–77.
2 Axelrod FB, Pearson J. Congenital sensory neuropathies. Diagnostic distinction from familial dysautonomia. *Am J Dis Child* 1984;**138**:947–54.
3 Axelrod FB. Familial dysautonomia and other congenital sensory and autonomic neuropathies. In Black IB, ed. *Cell and molecular biology of neuronal development*. New York: Plenum Press, 1984:331–40.
4 Breakefield XO, Orloff G, Castiglione C, Coussens L, Axelrod FB, Ullrich A. Structural gene for β-nerve growth factor not defective in familial dysautonomia. *Proc Natl Acad Sci USA* 1984;**81**:4213–6.
5 Breakefield XO, Ozelius L, Bothwell MA, *et al*. DNA polymorphisms for the nerve growth factor receptor gene exclude its role in familial dysautonomia. *Mol Biol Med* 1986;**3**:483–94.
6 Brunt PW, McKusick VA. Familial dysautonomia, a report of genetic and clinical studies, with a review of the literature. *Medicine (Baltimore)* 1970;**49**:343–74.
7 Maayan C, Kaplan E, Shachar S, Peleg O, Godfrey S. Incidence of familial dysautonomia in Israel 1977–1981. *Clin Genet* 1987;**32**:106–8.
8 Ozelius L, Kramer PL, Moskowitz CB, *et al*. Human gene for torsion dystonia located on chromosome 9q32–34. *Neuron* 1989;**2**:1427–34.
9 Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 1989;**44**:388–96.
10 Trofatter JA, Haines JL, Conneally PM. LIPIN: an interactive data entry and management program for LIPED. *Am J Hum Genet* 1986;**39**:147–8.
11 Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multipoint linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;**81**:3443–6.
12 Edwards JH. Exclusion mapping. *J Med Genet* 1987; **24**:539–43.
13 Dracopoli NC, O'Connell P, Elsner TI, *et al*. The CEPH consortium map of human chromosome 1. *Genomics* 1991;**9**:686–700.
14 Genome Data Base (GDB) version 4.1. Welch WH Medical Library, Baltimore, MD 21205.
15 O'Connell P, Lathrop GM, Nakamura Y, Lepert ML, Lalouel JM, White R. Twenty loci form a continuous linkage map of markers for human chromosome 2. *Genomics* 1989;**5**:738–45.
16 Polymeropoulos MH, Rath DS, Xiao H, Merril CR. Dinucleotide repeat polymorphism at the human preproglucagon gene. *Nucleic Acids Res* 1991;**19**:688.
17 Weber JL, Polymeropoulos MH, May PE, *et al*. Mapping of human chromosome 5 microsatellite DNA polymorphisms. *Genomics* 1991;**11**:695–700.
18 Polymeropoulos MH, Rath DS, Xiao H, Merril CR. Dinucleotide repeat polymorphism at the human transcription factor IID gene. *Nucleic Acids Res* 1991;**19**:4307.
19 Keats B, Ott J, Conneally M. Report of the committee on linkage and gene order. HGM10. *Cytogenet Cell Genet* 1989; **51**:459–502.
20 Haines JL, Ozelius LJ, McFarlane H, *et al*. A genetic map of chromosome 17. *Genomics* 1990;**8**:1–6.
21 Yamada Y, Xiang K, Bell GI, Seino S, Nishi M. Dinucleotide repeat polymorphism in a gene on chromosome 20 encoding a G-protein coupled receptor (D20S32e). *Nucleic Acids Res* 1991;**19**:2519.
22 Tanzi RE, Haines JL, Watkins PC, *et al*. Genetic linkage map of human chromosome 21. *Genomics* 1988;**3**:129–36.
23 Tanzi RE, Watkins PC, Stewart GD, *et al*. A genetic linkage map of human chromosome 21, analysis of recombination as a function of sex and age. *Am J Hum Genet* 1992;**50**:551–8.
24 Rouleau GA, Haines JL, Bazanowski A, *et al*. A genetic linkage map of the long arm of human chromosome 22. *Genomics* 1989;**4**:1–6.
25 Hempstead BL, Martin-Zanca D, Kaplan DR, Parada LF, Chao MV. High-affinity NGF binding requires coexpression of the *trk* proto-oncogene and the low-affinity NGF receptor. *Nature* 1991;**350**:678–82.
26 Miozzo M, Pierotti MA, Sozzi G, *et al*. Human *trk* proto-oncogene maps to chromosome 1q32–q34. *Oncogene* 1990;**5**:1411–4.
27 Lupski JR, Montes de Oca-Luna R, Slaugenhaupt S, *et al*. DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell* 1991;**66**:219–32.