

Precise Genetic Mapping and Haplotype Analysis of the Familial Dysautonomia Gene on Human Chromosome 9q31

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Summary

Familial dysautonomia (FD) is an autosomal recessive disorder characterized by developmental arrest in the sensory and autonomic nervous systems and by Ashkenazi Jewish ancestry. We previously had mapped the defective gene (*DYS*) to an 11-cM segment of chromosome 9q31-33, flanked by *D9S53* and *D9S105*. By using 11 new polymorphic loci, we now have narrowed the location of *DYS* to <0.5 cM between the markers 43B1GAGT and 157A3. Two markers in this interval, 164D1 and *D9S1677*, show no recombination with the disease. Haplotype analysis confirmed this candidate region and revealed a major haplotype shared by 435 of 441 FD chromosomes, indicating a striking founder effect. Three other haplotypes, found on the remaining 6 FD chromosomes, might represent independent mutations. The frequency of the major FD haplotype in the Ashkenazim (5 in 324 control chromosomes) was consistent with the estimated *DYS* carrier frequency of 1 in 32, and none of the four haplotypes associated with FD was observed on 492 non-FD chromosomes from obligatory carriers. It is now possible to provide accurate genetic testing both for families with FD and for carriers, on the basis of close flanking markers and the capacity to identify >98% of FD chromosomes by their haplotype.

Introduction

Familial dysautonomia (FD; also known as Riley day syndrome or hereditary sensory neuropathy type III; MIM 223900), with its extensive sensory dysfunction and variable autonomic dysfunction, is the most common and widely recognized of the congenital sensory neuropathies (Axelrod et al. 1974; Axelrod 1984; Axelrod and Pearson 1984). FD affects the development and survival of sensory, sympathetic, and parasympathetic neurons. It is a devastating and debilitating disease, present from birth, with a variety of symptoms, including gastrointestinal dysfunction, vomiting crisis, recurrent pneumonias, altered sensitivity to pain and temperature, and cardiovascular instability (Riley et al. 1949; Axelrod et al. 1974; Axelrod 1996). There is progressive neuronal degeneration throughout life, and, despite recent advances in the management of FD, survival statistics indicate that the probability of reaching 30 years of age is only 50% (Axelrod and Abularrage 1982). The diagnosis of FD is based on the following cardinal criteria: absence of fungiform papillae on the tongue; absence of axon flare after injection of intradermal histamine; decreased or absent deep tendon reflexes; absence of overflow emotional tears; and, because of its high prevalence in this population, Ashkenazi Jewish ancestry (Brunt and McKusick 1970; Axelrod 1984; Axelrod and Pearson 1984). There have been rare reports of non-Jewish FD patients (Suzuki et al. 1976; Levine et al. 1977; Orbeck and Oftedal 1977; Metha 1978; Harris et al. 1980; Klebanoff and Neff 1980). We have examined two patients from these families (Levine et al. 1977; Orbeck and Oftedal 1977) and have determined that they were affected with congenital sensory neuropathies other than FD. Therefore, non-Jewish FD probably is exceedingly rare, and Ashkenazi ancestry remains an important criterion of the disease. FD is inherited in an autosomal recessive fashion, with an incidence of 1 in 3,700 live births, which corresponds to

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a carrier frequency of 1 in 32 among the Ashkenazim (Maayan et al. 1987).

The clinical features of FD are due to a striking progressive depletion of unmyelinated sensory and autonomic neurons (Pearson and Pytel 1978*a*, 1978*b*; Pearson et al. 1978; Axelrod 1995). These pathological findings have led to the suggestion that the FD gene, named "DYS," may encode a member of the growing family of neurotrophic factors or their receptors that are crucial for the embryonic development and postnatal survival of neurons (Schwartz and Breakefield 1980; Wrathall 1986). All such candidate genes identified to date have been excluded either on the basis of their chromosomal localization or by direct examination, but it is conceivable that the FD gene may be an as yet undiscovered neurotrophin or receptor or a downstream participant in a related signal-transduction pathway (Breakefield et al. 1984, 1986; Blumenfeld et al. 1993*b*; Slangenaupt et al. 1995).

By studying 26 families with multiple affected members, we previously mapped *DYS* to chromosome 9q31-q33, in an 11-cM region between *D9S53* and *D9S105*. The genetic defect was completely linked to *D9S58*, with a LOD score of 21, and showed strong disequilibrium with the 18 allele at this marker (Blumenfeld et al. 1993*b*). To precisely localize the *DYS* gene and to examine the evident founder effect in the Ashkenazim, we analyzed 11 additional DNA markers, 7 of which were generated in this study. These markers enabled us to locate *DYS* centromeric to *D9S58*, in a 0.5-cM interval between markers 157A3 and 43B1GAGT. Haplotype analysis revealed a major core haplotype on >98% of FD chromosomes, indicating that almost all FD carriers share a common ancestor.

Families and Methods

Families with FD

For this work we used patient samples from two major sources, the Dysautonomia Diagnostic and Treatment Center at the New York University Medical Center and the Israeli Center for Familial Dysautonomia at Hadassah University Hospital, with approval from the institutional review boards at these institutions and at Massachusetts General Hospital. For all patients included in this study, FD was diagnosed either by F.B.A. or by C.M., on the basis of the standard criteria (Axelrod 1984; Axelrod and Pearson 1984). We studied 212 Ashkenazi families, including 41 families with more than one affected member (siblings, first cousins, and affected uncles or aunts) and 2 families with consanguinity and a single affected child. Together, 271 individuals with FD (441 distinct FD chromosomes) were studied. Unaffected parents in all 212 families with FD were studied;

in 102 of the families, siblings of the affected members, grandparents, and siblings of the parents also were studied (492 non-FD chromosomes from obligatory carriers). Control chromosomes (total of 324) were obtained from unaffected individuals marrying into the families with FD.

Identification of New Markers

Seven new polymorphic markers were generated from cosmids in the FD critical region, by hybridization with synthetic dioligonucleotides, trioligonucleotides, tetraoligonucleotides, and pentaoligonucleotides. Positive cosmids were shotgun subcloned, and the positive subclones were sequenced. Four of these markers, 157A3, *D9S310*, *D9S309*, and *D9S311*, are (GT)_n repeats; 88B2GA is a (GA)_n repeat; 43B1GAGT is a (GA)_n(GT)_n repeat; and 164D1 is an (AAAAC)_n repeat (table 1).

DNA Analysis

Genomic DNA was prepared either from lymphoblast cell lines (Anderson and Gusella 1984), with the SDS-proteinase K method followed by phenol extraction, or directly from blood, with the Chelex-100 method (Walsh et al. 1991). PCR analysis was performed on genomic DNA, by use of published oligonucleotide primer pairs and annealing temperatures (Kwiatkowski et al. 1992; Weissenbach et al. 1992; Gyapay et al. 1994; Dib et al. 1996; also see Genome Database) or in accordance with table 1. Typing of simple-sequence-repeat polymorphisms was performed as described by Blumenfeld et al. (1993*a*).

Results

*Order of Markers in the *DYS* Region*

We previously had localized *DYS* close to *D9S58*, in an 11-cM region between *D9S53* and *D9S105*. For the present study, 13 single-sequence-repeat polymorphisms from the *DYS* region were used, including both *D9S58* and *D9S105*. On the proximal side, we substituted the closer marker *D9S172* (6 cM from *D9S58*) for the more distant marker *D9S53* (8 cM from *D9S58*). The order of the 10 additional markers (table 1), with respect to the aforementioned three anchoring loci, is as follows: cen-*D9S172*-*D9S261*-88B2GA-43B1GAGT-164D1-*D9S1677*-157A3-*D9S310*-*D9S309*-*D9S58*-*D9S160*-*D9S311*-*D9S105*-tel. This map order was determined sequentially from recombination events in reference pedigrees (Povey et al. 1997) and from recombination events in our families with FD. No crossovers were observed between 164D1 and *D9S1677*, but their relative order was established by isolation of a bacterial artificial chromosome clone containing *D9S1677* and 157A3 but not 164D1.

Table 1**Polymorphic Markers in the FD Haplotype**

| Marker | PCR Primers (5'→3') ^a or Reference | Allele (Size [in bp] ^b) | Allele Frequency ^c | Heterozygosity | CEPH Standards |
|----------|--|--|----------------------------------|----------------|-------------------|
| 88B2GA | GCCTGGGTGACAAGAGC CTCATTGTATCCTTACATGGTG | 1 (116) | .38 | .72 | 133101 1,4 |
| | | 2 (118) | .17 | | |
| | | 3 (120) | .01 | | |
| | | 4 (122) | .31 | | |
| | | 5 (124) | .01 | | |
| 43B1GAGT | GATACACCATGCATTTGC GAAAATACAACCTGTTCCAAG | 11 (136) | .12 | .59 | 133101 5,8 |
| | | 3 (80) | .01 | | |
| | | 4 (82) | .03 | | |
| | | 5 (84) | .09 | | |
| | | 6 (86) | .10 | | |
| | | 8 (90) | .61 | | |
| 164D1 | CACCAGTATACTCCAGC TTAGATAGAAGTTATATTGC | 9 (92) | .01 | .61 | 133101 4,5 |
| | | 10 (94) | .15 | | |
| | | 2 (149) | .01 | | |
| | | 3 (154) | .15 | | |
| 157A3 | CTGCTGTAATAGAAGGGAAAGG TCAACACCTAAGTCTAATCACC | 4 (159) | .35 | .29 | 133101 12,15 |
| | | 5 (164) | .49 | | |
| | | 12 (140) | .03 | | |
| | | 13 (142) | .84 | | |
| | | 14 (144) | .08 | | |
| D9S310 | Slaugenhaupt et al. (1994) | 15 (146) | .02 | .75 | 133101 3,5 |
| | | 16 (148) | .01 | | |
| | | 17 (150) | .02 | | |
| | | 1 | .02 | | |
| | | 2 | .01 | | |
| | | 3 | .20 | | |
| | | 4 | .07 | | |
| D9S309 | Slaugenhaupt et al. (1994) | 5 | .24 | .78 | 133101 9,11 |
| | | 6 | .38 | | |
| | | 7 | .08 | | |
| | | 1 | .01 | | |
| | | 2 | .02 | | |
| | | 3 | .02 | | |
| | | 4 | .07 | | |
| | | 5 | .03 | | |
| | | 6 | .08 | | |
| | | 7 | .08 | | |
| | | 8 | .06 | | |
| D9S311 | Slaugenhaupt et al. (1994) | 9 | .37 | .40 | 133101 6,9 |
| | | 10 | .24 | | |
| | | 11 | .01 | | |
| | | 15 | .01 | | |
| | | -1 | .01 | | |
| | | 1 | .08 | | |
| | | 3 | .01 | | |
| | | 4 | .01 | | |
| | | 5 | .01 | | |
| | | 6 | .01 | | |
| 7 | .10 | | | | |
| D9S172 | Weissenbach et al. (1992) | 8 | .03 | .67 | 133101 2,4 |
| | | 9 | .70 | | |
| | | 10 | .01 | | |
| | | 13 | .04 | | |
| | | 1 | .07 | | |
| 2 | .48 | | | | |
| 3 | .05 | | | | |
| 4 | .24 | | | | |
| 5 | .10 | | | | |

(continued)

Table 1 (continued)

| Marker | PCR Primers (5'→3') ^a or Reference | Allele (Size [in bp] ^b) | Allele Frequency ^c | Heterozygosity | CEPH Standards |
|---------|---|--|----------------------------------|----------------|---------------------------|
| D9S261 | Gyapay et al. (1994) | 6 | .02 | .67 | 133101 8,8 133102 2,4 |
| | | 7 | .03 | | |
| | | 8 | .01 | | |
| | | 0 | .01 | | |
| | | 1 | .07 | | |
| | | 2 | .09 | | |
| | | 3 | .01 | | |
| | | 4 | .17 | | |
| | | 5 | .01 | | |
| | | 6 | .01 | | |
| | | 7 | .05 | | |
| | | 8 | .53 | | |
| | | 10 | .01 | | |
| | | 15 | .04 | | |
| D9S1677 | Dib et al. (1996) | 1 | .01 | .71 | 133101 9,9 133102 8,10 |
| | | 3 | .02 | | |
| | | 4 | .02 | | |
| | | 5 | .02 | | |
| | | 6 | .23 | | |
| | | 7 | .08 | | |
| | | 8 | .14 | | |
| | | 9 | .31 | | |
| | | 10 | .08 | | |
| | | 11 | .05 | | |
| | | 12 | .02 | | |
| | | 13 | .01 | | |
| | | 14 | .01 | | |
| | | D9S58 | Kwiatkowski et al. (1992) | | |
| 2 | .01 | | | | |
| 3 | .02 | | | | |
| 4 | .02 | | | | |
| 5 | .03 | | | | |
| 6 | .02 | | | | |
| 7 | .02 | | | | |
| 8 | .06 | | | | |
| 9 | .08 | | | | |
| 10 | .04 | | | | |
| 11 | .05 | | | | |
| 12 | .05 | | | | |
| 13 | .11 | | | | |
| 14 | .11 | | | | |
| 15 | .07 | | | | |
| 16 | .06 | | | | |
| 17 | .01 | | | | |
| 18 | .05 | | | | |
| 19 | .03 | | | | |
| 20 | .10 | | | | |
| D9S160 | Weissenbach et al. (1992) | 23 | .01 | .72 | 133101 6,6 133102 6,7 |
| | | 24 | .01 | | |
| | | 26 | .03 | | |
| | | -1 | .01 | | |
| | | 0 | .01 | | |
| | | 2 | .03 | | |
| | | 3 | .06 | | |
| | | 4 | .10 | | |
| 5 | .06 | | | | |
| 6 | .34 | | | | |
| 7 | .38 | | | | |

(continued)

Table 1 (continued)

| Marker | PCR Primers (5'→3') ^a or Reference | Allele (Size [in bp] ^b) | Allele Frequency ^c | Heterozygosity | CEPH Standards |
|---------------|---|-------------------------------------|-------------------------------|----------------|----------------|
| <i>D9S105</i> | Genome Database | 1 | .02 | .83 | 133101 8,8 |
| | | 2 | .07 | | 133102 4,8 |
| | | 3 | .14 | | |
| | | 4 | .14 | | |
| | | 5 | .08 | | |
| | | 6 | .06 | | |
| | | 7 | .11 | | |
| | | 8 | .31 | | |
| | | 9 | .05 | | |
| | | 10 | .01 | | |
| | | 11 | .01 | | |

^a For new polymorphisms.

^b For new markers.

^c On the basis of the analysis of 497 non-FD Ashkenazi Jewish chromosomes.

Fine Localization of DYS and Definition of New Flanking Markers

To refine the minimum FD candidate region, we analyzed 102 families with FD (41 with multiple affected members). On the proximal side, the recombination event depicted in figure 1A set 43B1GAGT as the closest centromeric flanking marker. No additional crossovers were detected by 88B2GA or *D9S261*, although the more distant *D9S172* (~6 cM away) detected 14 recombinations with *DYS*. On the distal side, the closest flanking marker is 157A3, on the basis of the crossover shown in figure 1B. One additional crossover was found in each of the following intervals: 157A3–*D9S309*, *D9S309*–*D9S310*, and *D9S309*–*D9S58*. No recombinants were observed between *DYS* and 164D1–*D9S1677*. Thus, the FD candidate region has been reduced to the interval 43B1GAGT–164D1–*D9S1677*–157A3, which, from our analysis, was estimated to span <0.5 cM.

A Major FD Haplotype

We next undertook the haplotype analysis of FD, in an attempt to further refine the candidate region and to estimate the number of independent mutations represented in the FD population. A major founder haplotype

was observed for 435 (98.6%) of the 441 FD chromosomes examined, with a core of alleles 8-4-12 at 43B1GAGT–164D1–*D9S1677* (table 2) and with a consensus set of alleles, for markers on either side, that decayed because of historic recombination events. The major founder haplotype in FD is recognizable across the interval *D9S261*–*D9S105*, which is ~3 cM. The chromosomes that support historic recombinations across the *D9S261*–*D9S58* interval are depicted in figure 2. No events that would have narrowed the candidate region were detected, although we observed one ancestral recombination event with 157A3 that confirms 157A3 as the closest telomeric flanking marker (fig. 2). The next distal flanking markers, *D9S310* and *D9S309*, yielded evidence for an additional four and six ancestral recombinations, respectively. On the centromeric side, three apparent ancestral recombinations were observed with 88B2GA, and six additional events were seen with *D9S261*.

D9S1677 forms part of the conserved haplotype but displays some allelic variation because of “slippage” events that create new alleles (table 3). On most “major haplotype” FD chromosomes, *D9S1677* is represented by a 12 allele (83.5%), but, on the remainder of FD chromosomes, it is represented by a 10 allele (0.5%), an

Table 2

Haplotypes Associated with FD

| Haplotype | <i>D9S</i> -172 | <i>D9S</i> -261 | 88B2GA | 43B1-GAGT | 164D1 | <i>D9S</i> -1677 | 157A3 | <i>D9S</i> -310 | <i>D9S</i> -309 | <i>D9S</i> -58 | <i>D9S</i> -160 | <i>D9S</i> -311 | <i>D9S</i> -105 | No. of Chromosomes |
|-----------|-----------------|-----------------|--------|-----------|-------|------------------|-------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|--------------------|
| Major | 2 | 8 | 4 | 8 | 4 | 12 ^a | 13 | 5 | 10 | 18 | 7 | 9 | 8 | 435 ^b |
| 1 | 2 | 8 | 4 | 8 | 5 | 9 | 13 | 3 | 9 | 13 | 6 | 9 | 8 | 2 |
| 2 | 2 | 3 | 11 | 5 | 5 | 5 | 12 | 7 | 11 | 11 | 6 | 4 | 1 | 3 |
| 3 | 2 | 8 | 11 | 5 | 5 | 10 | 13 | 5 | 9 | 15 | 7 | 6 | 9 | 1 |

^a 10-11-12-13-14 (see table 3).

^b See figure 2 for details.

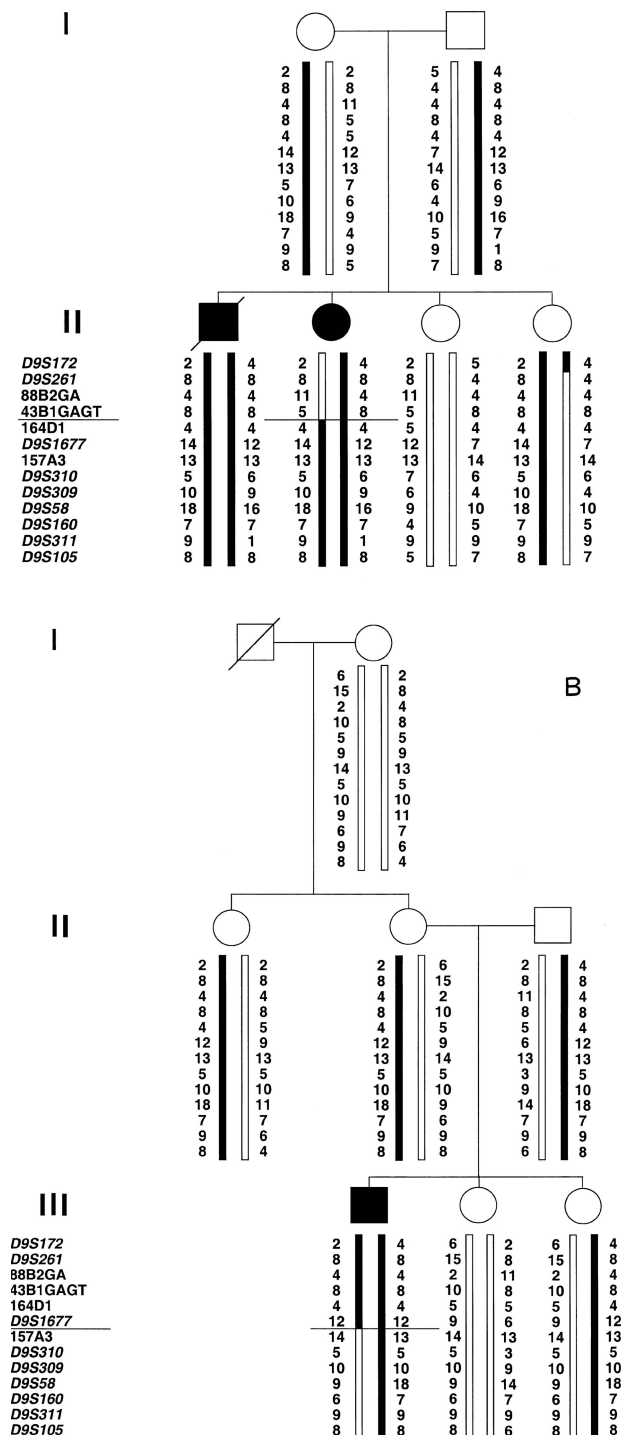


Figure 1 FD pedigrees showing recombination events that define the candidate interval. *A*, Centromeric cross. *B*, Telomeric cross. Blackened bars represent FD chromosomes, and unblackened bars represent non-FD chromosomes. The horizontal lines show the locations of the recombination events.

11 allele (3%), a 13 allele (3%), or a 14 allele (10%), even though adjacent markers remain unchanged. By contrast, the extreme *D9S1677* alleles—12, 13, and 14—are present on only 2.4%, 0.6%, and 0.6% of non-FD chromosomes, respectively. The instability of *D9S1677* is further supported by our observation of two allele changes from the 12 allele to the 13 allele, during parent-child transmissions, in our families with FD.

Other FD Haplotypes

Six of 441 FD chromosomes revealed three different haplotypes across the candidate region (table 2). All three of these other haplotypes were observed in compound heterozygotes with the major haplotype. We observed minor haplotype 1 in two unrelated families, haplotype 2 in three families, and haplotype 3 in one family. The third rare haplotype was inherited from a woman who claimed not to be of Jewish descent; she was of Irish-German/Sicilian origin. Other than the unusual family history, this child exhibited all the diagnostic criteria for FD and had classic symptoms.

Discussion

In an extensive study of families with FD, undertaken to refine the location of *DYS* in 9q31, we used 11 new polymorphic markers, including 7 specifically developed as part of this research study. Within the 11-cM candidate region that we had reported previously (Blumenfeld et al. 1993a), we observed recombination events, in families with FD, that define a *DYS* candidate region of <0.5 cM, between the new markers 43B1GAGT and 157A3.

One major haplotype for the *DYS* region was detected on >98% of FD chromosomes. Indeed, all the patients with FD studied have at least one copy of the major haplotype. This dramatic linkage disequilibrium indicates that one major founder mutation is responsible for virtually all FD cases among the Ashkenazim. For several other recessive hereditary diseases, a major founder mutation has been observed among Ashkenazi Jews, but none is a single founder mutation that is as predominant as the haplotype found for FD. For example, the major mutations found in Tay Sachs disease, Gaucher disease, and cystic fibrosis are observed on 78%, 76%, and 48% of disease chromosomes, respectively (Triggs-Raine et al. 1990; Abeliovich et al. 1992; Beutler et al. 1993).

Of our 271 patients with FD, only 9, from six families, were compound heterozygotes with one atypical haplotype. These three rare haplotypes may reflect independent FD mutations. In particular, the inheritance of haplotype 3 from a non-Ashkenazi parent suggests that at least one of the rare FD haplotypes may have been introduced from a non-Ashkenazi population. However,

~1.8 Mb to ~150 kb (Ozelius et al. 1997). Similarly, in Huntington disease, haplotype studies that revealed ancestral crossovers progressively narrowed a 2-Mb candidate region to ~200 kb (Gusella and MacDonald 1993). We observed significant linkage disequilibrium on FD chromosomes across a region of ~3 cM, from *D9S261* to *D9S105*. Despite the fact that we genotyped a very large number of FD chromosomes, the candidate region could not be narrowed further by use of ancestral recombination events. Only one additional historic recombination event was observed, with 157A3, and none were seen with 43B1GAGT (fig. 2). Thus, the extent of linkage disequilibrium on FD chromosomes and the comparison of historic and actual recombination events in FD suggests that the major FD mutation probably occurred relatively recently in the Ashkenazi population, certainly within a few hundred years. The high incidence of FD in the Ashkenazim suggests that the mutation likely was present during a period of rapid population expansion from a small number of founders (Risch et al. 1995).

The incidence of FD is 1 in 3,700 live births among Ashkenazi Jews, and the calculated carrier frequency is 1 in 32 individuals (Maayan et al. 1987). We genotyped 324 control chromosomes from spouses of FD carriers, and the major FD haplotype was observed on 1.54% (expected frequency 1.56%). The fact that none of the FD-associated haplotypes was observed in non-FD chromosomes, together with the ability to identify the major FD haplotype in the general Ashkenazi Jewish population, indicates that accurate and sensitive genetic testing can be provided for family members with FD and their spouses (Blumenfeld et al. 1995; Eng et al. 1995; Odoux et al. 1995).

The definition of a precise candidate region for *DYS* has set the stage for the identification of the FD defect, through location cloning. FD belongs to a family of hereditary sensory neuropathies for which accurate diagnosis challenges clinicians. The observation of at least one non-Jewish FD chromosome in our data indicates that other non-Jewish patients might have escaped diagnosis for FD. Cloning of the *DYS* gene, on the basis of its chromosomal location, will provide the means for direct comparison both of "atypical" cases and of cases of other sensory neuropathies, to FD, allowing classification based on the primary genetic cause rather than on subtle symptomatic differences.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, <http://www.gdb.org> (for oligonucleotide primer pairs used in PCR analysis)
Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for FD [MIM 223900])

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