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Autosomal Dominant Familial Spastic Paraplegia: Tight Linkage to Chromosome 15q

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Summary

Autosomal dominant, uncomplicated familial spastic paraplegia (FSP) is a genetically heterogeneous disorder characterized by insidiously progressive lower-extremity spasticity. Recently, a locus on chromosome 14q was shown to be tightly linked with the disorder in one of three families. We performed linkage analysis in a kindred with autosomal dominant uncomplicated FSP. After excluding the chromosome 14q locus, we observed tight linkage of the disorder to a group of markers on chromosome 15q (maximum two-point lod score 9.70; $\theta = .05$). Our results clearly establish the existence of a locus for autosomal dominant FSP in the centromeric region of chromosome 15q. Comparing clinical and genetic features in FSP families linked to chromosome 14q with those linked to chromosome 15q may provide insight into the pathophysiology of this disorder.

Introduction

Familial spastic paraplegia (FSP) (MIM 18260; McKusick 1988*b*) constitutes a group of clinically and genetically diverse disorders that share the primary feature of progressive, severe lower-extremity spasticity (Rhein 1914; Philipp 1949; Schwarz and Liu 1956; Roe 1963; Cartlidge and Bone 1973; Sutherland 1975; Holmes and Shaywitz 1977; Kenwrick et al. 1986; Boustany et al. 1987; Keppen et al. 1987; McKusick 1988*a;* Baraitser 1990; Scheltens et al. 1990; Behan and Maia 1993; Harding 1993; Polo et al. 1993; Skre 1993; Durr et al 1994; Fink et al., in press) (for review, see Harding 1993). FSP is classified according to the mode of inheritance and to whether progressive spasticity occurs in isolation (uncomplicated FSP) or with other neurological abnormalities (complicated FSP), including optic neuropathy, retinopathy, extrapyramidal disturbance, dementia, ataxia, ichthyosis, mental retardation, or deafness. Autosomal dominant, uncomplicated FSP has been classified (Harding 1981) further as type I (spasticity exceeds weakness, onset occurs at $\leq$35 years of age, and progression is slow) and type II (onset occurs $\geq$35 years of age, and patients exhibit weakness in addition to spasticity, distal sensory loss, urinary bladder disturbance, and faster progression) (Holmes and Shaywitz 1977; Opjordsmoen and Nyberg-Hansen 1980; Harding 1981). The major neuropathologic feature of autosomal dominant, uncomplicated FSP is axonal degeneration that is maximal in terminal portions of the longest descending and ascending tracts within the central (not peripheral) nervous system (corticospinal tracts to the legs and fasciculus gracilis fibers from the legs, respectively) (Schwarz and Liu 1956; Behan and Maia 1993; Harding 1993).

Genetic linkage analysis and positional cloning are important approaches to finding the molecular cause(s) of FSP, since there are no known biochemical markers of the disease that can be monitored. In view of FSP's clinical heterogeneity, it is not unexpected that autosomal recessive (Hentati et al. 1993), X-linked (Keppen et al. 1987), and autosomal dominant (Hazan et al. 1993) types of FSP have been shown to be genetically heterogeneous. Recently, Hazan et al. (1993) showed that autosomal dominant, uncomplicated FSP was genetically heterogeneous and was tightly linked to a group of microsatellite markers on chromosome 14q (the FSP1 locus) in one large kindred. We examined a large kindred with autosomal dominant, uncomplicated FSP (Fink et al., in press) and performed genetic linkage analysis using microsatellite DNA polymorphisms. We showed that the disorder was excluded from the FSP1 locus on chromosome 14q. We now report both the results of a genomewide search for the disease locus in this family and the observation of tight linkage (Fink et al. 1994) of the disorder to loci on chromosome 15q.

Subjects, Material, and Methods

Subjects

We examined 126 members of a nonconsanguinous, North American kindred of Irish descent (fig. 1). Clinical

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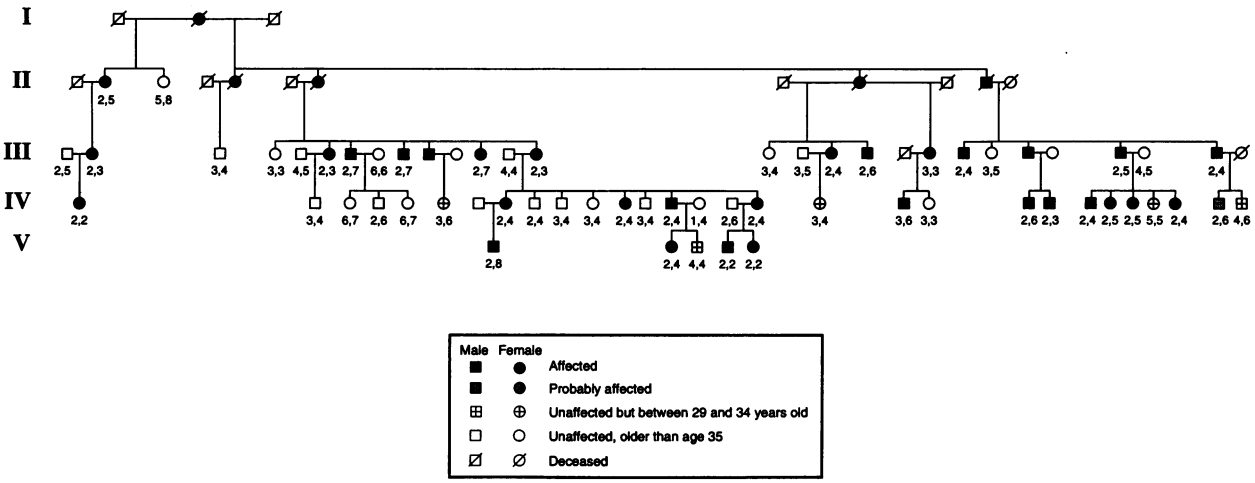

Figure 1 Familial spastic paraplegia, D15S128 genotypes

features of this family have been described elsewhere (Fink et al., in press). Pedigree information was obtained from many relatives. Informed consent was obtained from each subject, as specified by the University of Michigan Institutional Review Board. Subjects were diagnosed at the time of blood collection and prior to genotyping. Subjects were diagnosed as *unaffected* (neurologically asymptomatic, normal neurological examination, and >30.5 years of age), *possibly affected* (neurologically asymptomatic and normal gait; and examination suggested possible corticospinal tract deficits—i.e., mildly hyperactive deep tendon reflexes [DTRs] and three or four beats of ankle clonus—but plantar responses were flexor), *probably affected* (subjects denied gait disturbance, but examination showed mild corticospinal tract deficits in lower extremities [grade 3–4+ lower-extremity DTRs]), and *affected* (subject reported progressive gait disturbance, and examination showed frank corticospinal tract deficits in the lower extremities, including grade 4 hyperreflexia and extensor plantar responses). Deceased subjects were diagnosed as "affected" or "unaffected" on the basis of descriptions by at least two relatives. Age at disease onset was obtained by interviewing 30 living affected subjects. Estimated age at disease onset in deceased subjects was not used in this calculation.

FSP was diagnosed in 31 living subjects who developed insidiously progressive gait disturbance at age 12–35 years. The unimodal distribution of age at symptom onset (mean 22.0 ± SD 5.3 years) was similar to that of FSP type I families reported by Harding (1981) (mean age at onset 20.5 ± 17.9 years). Neurological examination of affected subjects revealed hyperreflexia and spasticity in the lower extremities, weakness of hip flexion and ankle dorsiflexion, extensor plantar response, diminished vibratory sense in the feet, and pes cavus. We did not observe wasting of distal muscles, a feature of Harding's type II patients (Harding 1981). Muscle atrophy, when present, was noted only in the shins. Bladder disturbance, a frequent feature of FSP type II patients reported by Harding (1981) and Boustany et al. (1987), was present in three affected subjects.

We performed genetic linkage analysis on 31 affected subjects and 31 unaffected subjects. Unaffected subjects were neurologically asymptomatic, had normal neurological examinations, and were >35 years of age (N = 26) or 30.5–34 years of age (N = 3), except two subjects who were 29 and 30.3 years old. According to the distribution of age at disease onset (Fink et al., in press) asymptomatic at-risk subjects 30.5–35.5 years of age had ~3% chance of developing FSP. Nine spouses of descendants were included in genetic linkage analysis. These subjects were asymptomatic, had normal neurological examinations, and had no evidence of similar neurological disorders in their families. One "probably affected" subject (an asymptomatic individual with lower extremity spasticity, hyperreflexia, and extensor plantar responses) was included in our analysis. "Possibly affected" (an asymptomatic individual with mild hyperreflexia) and "at-risk" subjects (asymptomatic individuals with normal neurological examinations who were <29 years of age) were not included in genetic linkage analysis.

Genotyping and Linkage Analysis

DNA was extracted from peripheral blood leukocytes, as described elsewhere (Bell et al. 1981). Microsatellite DNA polymorphisms, used to genotype subjects, were amplified by PCR, according to standard procedures. Amplifications were performed in 25-μl volumes in 96-well trays, using Coy and Techne thermocyclers for 35 cycles. One primer was labeled with ^{32}P-dATP, using T4 polynucleotide kinase. Amplified DNA was electrophoresed on 7% polyacrylamide/6-M urea-formamide gels, and alleles were scored from autoradiographs.

Table 1

Results of Two-Point Linkage Analysis for Microsatelite Polymorphisms on Chromosome 15q

		Two-Point Lod Scores at θ =						
Marker Locus	Penetrance	.001	.01	.05	.1	.2	.3	.4
D15S128	.90	8.72	9.57	9.70	9.18	7.53	5.40	2.85
D15S122	.90	−.37	.56	1.11	1.25	1.19	.91	.48
D15S156	.90	1.98	1.95	1.81	1.63	1.23	0.81	.40
D15S165	.90	−.27	1.63	2.94	3.30	3.11	2.36	1.27
UT511	.90	−4.87	−.14	2.97	3.86	3.79	2.70	1.08
D15S118	.90	−9.24	−5.27	−1.60	.21	1.52	1.62	1.06

Two-point linkage analyses were performed with the MLINK subroutine of the LINKAGE program (Lathrop et al. 1985), using an autosomal dominant model of disease inheritance and disease-allele frequency of .001. We did not observe instances of incomplete genetic penetrance (Fink et al., in press). Nonetheless, to calculate maximum lod scores conservatively, we assigned genetic penetrance .90. Marker-allele frequencies were calculated from the FSP family. The LINKMAP program of LINKAGE, used for multipoint linkage analysis, utilized published (Weissenbach 1992) locations of D15S122, D15S128, D15S156, and D15S165.

Results

Exclusion Analysis

Elsewhere, Fink et al. (in press) examined microsatellite markers (D14S75, D14S69, and D14S66) for which Hazan et al. (1993) found significant linkage to autosomal dominant FSP in one of three kindreds. AFM267zd5 is of particular importance, because Hazan et al. observed that this marker cosegregated completely with the disorder (Z_{max} = 9.58; θ = .00). In contrast, our results (lod score −13.70 [θ= .001] and −2.61 [θ = .20]) exclude the disease locus in our family from being within 20 cM of the FSP1 locus.

We then analyzed 47 microsatellite polymorphisms on chromosomes 1, 3, 4, 8, and 12–18 for linkage with the disorder in our FSP kindred. Included among these loci were markers that had shown tight linkage to other inherited neurological disorders, including Machado-Joseph disease (D14S55) (Takiyama et al. 1993), Friedreich ataxia (D9S15) (Chamberlain et al. 1993), spinocerebellar ataxia I (D6S89) (Ranum et al. 1991), spinocerebellar ataxia II (D12S58) (Roa et al. 1993), Charcot-Marie-Tooth type 1A (TCF2 and D17S83) (Lupski et al. 1991), and Charcot-Marie-Tooth 1B (APOA2 MFD3). Our results (data not shown) strongly excluded the disorder from being within 10–30 cM of any of these 47 microsatellite polymorphisms.

Linkage to Chromosome 15q

We observed linkage to a group of marker loci (table 1) mapped to the centromeric region of chromosome 15q. When we assumed that genetic penetrance was .90, the two-point lod score was 9.70 (θ = .05; lod score = 8.72 for θ = .0) for D15S128. Genotypes for D15S128 are shown (fig. 1). We repeated two-point analyses using marker allele frequencies calculated from the family (instead of assuming equal frequencies of marker alleles) and found no significant change (data not shown), except for D15S165, whose lod score reduced from 3.3 (θ = .10) to 1.75 (θ = .20). We then performed multipoint linkage analysis, assigning genetic penetrance of .90 and using published distances (Weissenbach 1992) between the following loci: D15S122–(.01)–D15S128–(.07)–D15S156–(.07)–D15S165. Multipoint linkage analysis of these markers reached a maximum lod score (Z_{max}) of 10.16 between markers D15S128 and D15S156. From the multipoint analysis, the most likely location of the FSP locus is D15S128–(.04)–FSP–(.03)–D15S156.

Discussion

Our data clearly establish the existence of a locus for autosomal dominant, uncomplicated FSP type I in the centromeric region of chromosome 15q. Previous physical mapping (NIH/CEPH Collaborative Mapping Group 1992) of the microsatellite markers allows us to assign the FSP locus to chromosome 15q11.1 Presently, our data localize this region to an ~7.3-cM interval. Analysis of additional flanking markers will increase the precision of this localization.

Defining the extent of clinical heterogeneity between different genetic types of FSP will advance our understanding of the relationships between abnormal gene products and the clinical disorder. With identification of FSP loci on chromosomes 14q and 15q, it is possible to compare phenotypes in families where the disorder is linked to each of these loci and in those where each of these loci are excluded. The most important difference between our patients (linked to chromosome 15q) and those that Hazan et al. (1993) reported (linked to chromosome 14q) is the severity of the illness. The disorder in the kindreds studied by Hazan et al. may be more mild, insofar as only one patient (aged 69 years) required a wheelchair. At least 9 of

our 31 affected subjects required a wheelchair (beginning for some as early as their late 40s). Despite this difference, our patients are quite similar to those reported by Hazan et al. This observation raises the possibility that the different abnormal gene products responsible for these disorders participate in a common biochemical cascade that results in a similar pattern of CNS degeneration.

Axonal degeneration in uncomplicated FSP is maximal in the terminal portions of the longest CNS axons. Pathological changes are confined to central, not peripheral, axons. The distribution of pathological changes raises the possibility that genes involved in neurotrophic regulation, those involved in maintenance of axonal cytoskeleton (within the central, not peripheral, nervous sytem), or those involved in axoplasmic flow could be involved. Identifying genetic mutations responsible for FSP will greatly advance our understanding of this condition and, one hopes, other inherited and degenerative brain and spinal cord disorders characterized by axonal degeneration.

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