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A New Locus for Autosomal Recessive Hereditary Spastic Paraplegia Maps to Chromosome 16q24.3

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Summary

Hereditary spastic paraplegia is a genetically and phenotypically heterogeneous disorder. Both pure and complicated forms have been described, with autosomal dominant, autosomal recessive, and X-linked inheritance. Various loci (SPG1–SPG6) associated with this disorder have been mapped. Here, we report linkage analysis of a large consanguineous family affected with autosomal recessive spastic paraplegia with age at onset of 25–42 years. Linkage analysis of this family excluded all previously described spastic paraplegia loci. A genomewide linkage analysis showed evidence of linkage to chromosome 16q24.3, with markers D16S413 (maximum LOD score 3.37 at recombination fraction [θ] of .00) and D16S303 (maximum LOD score 3.74 at θ = .00). Multipoint analysis localized the disease gene in the most telomeric region, with a LOD score of 4.2. These data indicate the presence of a new locus linked to pure recessive spastic paraplegia, on chromosome 16q24.3, within a candidate region of 6 cM.

Introduction

Hereditary spastic paraplegias (HSPs) are a group of inherited disorders characterized by progressive spasticity and weakness of lower limbs (Fink et al. 1996). The most relevant pathological feature is degeneration of the corticospinal tracts and of the posterior columns. Epidemiological studies on HSP reported a prevalence ratio of 9.6×10^{-5} in Cantabria, Spain (Polo et al. 1993), and 2.7×10^{-5} in Molise, Italy (Filla et al. 1992). HSP has been divided into pure and complicated forms. Pure HSP is defined clinically by spastic gait, hyperreflexia, and Babinski sign. Minimal clumsiness of upper limbs, urinary symptoms, lower-limb deep sensory loss, slight distal amyotrophy, and pes cavus may be present. Complicated forms of HSP have additional features, such as mental retardation, pigmentary retinal degeneration, optic atrophy, extrapyramidal features, cerebellar signs, severe muscular atrophy, severe sensory neuropathy, ichthyosis, and skin depigmentation.

Autosomal dominant (AD), autosomal recessive (AR), and X-linked recessive varieties of HSP have been recognized. Pure AD-HSP is genetically heterogeneous. Loci for AD-HSP have been identified on chromosomes 14q (SPG3, MIM 182600; Hazan et al. 1993), 2p (SPG4, MIM 182601; Hazan et al. 1994; Hentati et al. 1994*b*), and 15q (SPG6, MIM 600363; Fink et al. 1995). A locus for pure AR-HSP has been found on chromosome 8q (SPG5A, MIM 270800; Hentati et al. 1994*a*). The existence of one AR-HSP family for whom this locus was excluded (Hentati et al. 1994*a*) indicated the existence of additional, as yet unidentified AR-HSP loci (SPG5B, MIM 600146). Mutations have been found for X-linked uncomplicated HSP (Xp22; SPG2, MIM 312920) in the proteolipoprotein gene (Kobayashi et al. 1994; Saugier-Veber et al. 1994), allelic to the Pelizaeus-Merzbacher disease locus (Gencic et al. 1989). Jouet et al. (1994) described mutation of the L1CAM gene in a complicated form of X-linked HSP (SPG1, MIM 312900) in X-linked hydrocephalus patients and in cases of mental retardation, aphasia, shuffling gait, and adducted thumbs syndrome, defining these three syndromes as allelic disorders.

Here we report the identification of a large pedigree affected by AR-HSP. We demonstrate that, in this family, the disease does not segregate with the previously reported loci. We performed a genomewide search and found that, in this pedigree, the disease locus is linked to marker loci on chromosome 16q24.3. Mutation at

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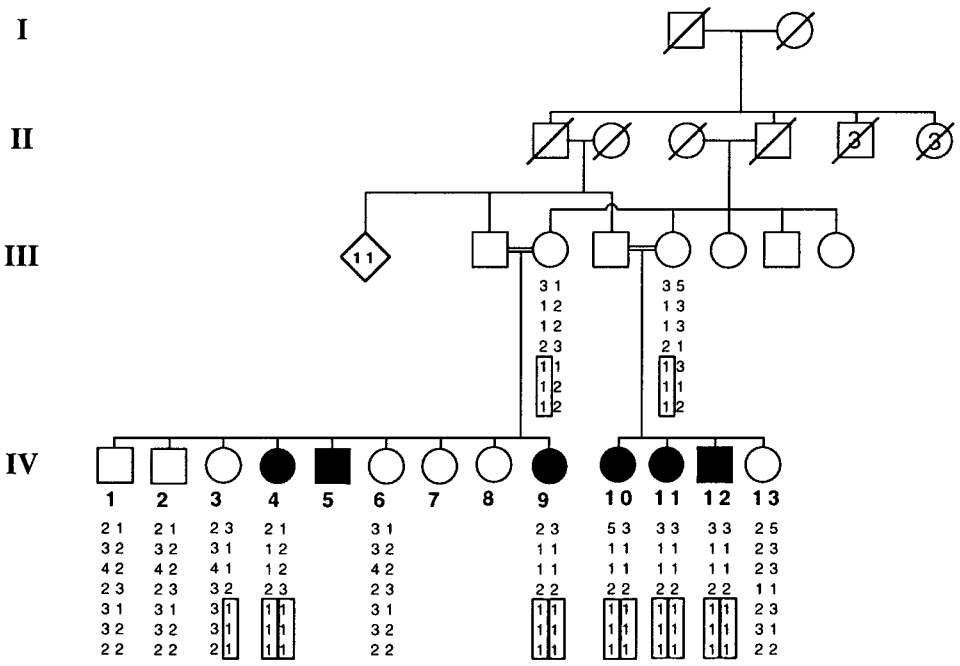

Figure 1 Pedigree of the AR-HSP family and haplotype analysis of markers (listed from top to bottom) D16S511, D16S3037, D16S520, D16S3048, D16S413, D16S3023, and D16S303.

this newly identified locus could account both for families in which HSP is not linked to previously described loci and for sporadic HSP patients.

Subjects and Methods

Pedigree Assessment

The family was identified during an epidemiological survey performed in Molise, a region in southern Italy (Filla et al. 1992). The family is highly consanguineous, since two brothers married two sisters, all being first cousins. All living members of the pedigree were personally examined by the authors (A.F., G.D.M., and R.M.), except individual IV-5 (fig. 1). He was reported as being affected but was unavailable for examination because he had moved to Australia. Individuals were considered affected if they presented lower-limb hyperreflexia associated with at least one of the following: increased tone, pyramidal weakness, or extensor plantar response.

Clinical Features

The main clinical findings in our patients are shown in table 1. Mean age at onset ± SD was 30 ± 8 years (range 25–42 years). A reliable age at onset was not obtained for patient IV-12. Abnormal gait was the presenting symptom in all cases, and it was associated with leg pains in patient IV-10. Weakness and extensor plantar response were absent in the patient (IV-9) with the shortest disease duration. Vibration sense was frequently decreased in lower limbs. Hypernasal and slowed speech were present in two patients and dysphagia in one. Urinary urgency was present in three patients (IV-4, IV-9, and IV-10), scoliosis and pes cavus in two patients (IV-10 and IV-11), and pale optic disk in two patients (IV-4 and IV-11). Cerebellar and extrapyramidal signs were absent in all cases. Magnetic resonance imaging of the brain and spinal cord, performed on three patients, was normal in two (patients IV-9 and IV-10) and showed vascular lesions in the right frontal cortex of patient IV-12. Peripheral nerve conduction studies performed on patient IV-9 showed slight axonal neuropathy. Disease progression was slow. All patients were able to walk independently, with the exception of the oldest patient (IV-4), who needs constant assistance.

Linkage Analysis

A 20-ml venous blood sample was obtained from five patients and seven healthy relatives who gave their informed consent. DNA was extracted from peripheral blood lymphocytes, in accordance with procedures described elsewhere (Miller et al. 1988). Linkage to chromosome 8 (SPG5A) was tested by use of D8S260 and D8S166 markers. Linkage to AD-HSP loci was tested by use of D2S352 (SPG4), D14S269 (SPG3), and D15S128 (SPG6) markers. Oligonucleotide sequences and PCR conditions were as described elsewhere. PCR products were separated by electrophoresis on 7% de-

Table 1

Clinical Findings

Clinical Feature	IV-4	IV-9	IV-10	IV-11	IV-12
Age (years)	60	46	49	46	43
Age at onset (years)	25	42	27	26	?
Spastic gait	+++	+	++	++	++
Dysarthria	+	−	−	−	+
Dysphagia	−	−	−	−	+
Upper limbs:					
Increased tone	−	−	−	−	−
Tendon reflexes	Brisk	Brisk	Brisk	Brisk	Brisk
Weakness	−	−	+	+	−
Wasting	−	−	−	−	−
Decreased vibration sense	−	−	−	+	−
Lower limbs:					
Increased tone	++	+	+	+	−
Tendon reflexes	Brisk	Brisk	Clonus	Brisk	Brisk
Weakness	+	−	+	+	+
Wasting	−	−	−	−	−
Decreased vibration sense	+	−	−	+	+
Plantar response	Ext	Normal	Ext	Ext	Ext

NOTE.—A minus sign (−) indicates absence; a plus sign (+) indicates presence, to various degrees (+, mild; ++, moderate; and +++, severe); and a question mark (?) indicates unknown. Ext = extensor.

naturant polyacrylamide gels. After electrophoresis, gels were stained by means of silver staining. The size of alleles was determined by comparison to M13 DNA sequence.

The fluorescence-labeled set of markers used in the genomewide scan consists of 358 highly polymorphic dinucleotide markers (Gyapay et al. 1994; Dib et al. 1996) that are evenly distributed throughout the genome at an average distance of 10 cM. All of the available pedigree members have been genotyped by use of the whole marker set. Two-point linkage analysis was performed on a SUN Ultra-SPARC workstation with use of the MLINK feature of the LINKAGE package (Lathrop et al. 1985). Because of computing constraints deriving from the consanguinity loops present in this family, multipoint linkage analysis was performed with use of the GENEHUNTER software (Kruglyak et al. 1996). For linkage calculations, AR-HSP was modeled as an AR trait with complete penetrance, and the mutation rate was assumed to be 10^{-5}. Recombination frequencies were assumed to be equal between males and females. Published marker-allele frequencies were used for these calculations.

Results

Exclusion of Linkage with Previously Described Loci

We performed linkage analysis with the previously described AR form (SPG5A) locus on chromosome 8 by using D8S260 and D8S166 markers. LOD score values were negative (D8S260: maximum LOD score −19.85 at a recombination fraction [θ] of .00; D8S166: maximum LOD score −13.85 at $\theta = .00$). These results indicated that our pedigree was not linked to chromosome 8 and that a different gene must be responsible for the observed phenotype.

Therefore, we concluded that this family can be considered to have a new form of AR-HSP not linked to chromosome 8. As the next step, we tested this pedigree for linkage with markers of the AD-HSP forms (SPG3, SPG4, and SPG6). Negative LOD scores were obtained with D2S352 (SPG4), D14S269 (SPG3), and D15S128 (SPG6) (data not shown).

Genomewide Scanning for the New AR-HSP

On the basis of our exclusion mapping data, we considered this pedigree for genomewide linkage mapping. The probability of detecting significant linkage was tested, by means of simulation analysis on 400 simulated pedigrees, by SLINK 2.5v software (Ott 1989), on a SUN Ultra-SPARC workstation. The analysis was performed under the assumptions of AR inheritance, complete penetrance, marker heterozygosity .75, and disease frequency 1×10^{-5}. The maximum LOD score at $\theta = .02$ was 4.01, with 48% conditional probability of detecting significant LOD score values.

A genomewide search was performed on all of the available pedigree members. Two loci suggestive of linkage (LOD score >1.0) were identified on chromosomes 16 and 17, and the marker density was locally increased by testing all pedigree members with two additional markers, ~5 cM telomeric and ~5 cM centromeric, for each candidate locus. The candidate locus on chromosome 17 was clearly excluded from linkage (data not shown). The chromosome 16 locus gave a LOD score value of 2.42 at $\theta = .05$ for marker D16S520, which maps on the telomeric region of the long arm of chromosome 16, and was confirmed by flanking markers (maximum LOD score at $\theta = .00$: D16S413, 3.37; D16S3023, 3.26; and D16S303, 3.74).

As reported in the two-point LOD score table (table 2) and in the multipoint linkage graph (fig. 2) showing a multipoint LOD score value of 4.2, we have localized a new AR-HSP locus in the telomeric region of chro-

Table 2

LOD Scores of Selected 16q Dinucleotide Markers

	LOD SCORE AT θ =						
MARKER	.00	.01	.05	.10	.20	.30	.40
D16S511	−∞	−3.87	−1.31	−.40	.18	.25	.15
D16S3037	−∞	1.61	2.05	2.01	1.59	.99	.35
D16S520	−∞	1.98	2.42	2.39	1.97	1.38	.69
D16S3048	−∞	.88	1.33	1.32	1.04	.68	.31
D16S413	3.37	3.30	3.03	2.69	1.96	1.20	.47
D16S3023	3.26	3.20	2.96	2.65	1.99	1.29	.61
D16S303	3.74	3.68	3.41	3.06	2.30	1.48	.61

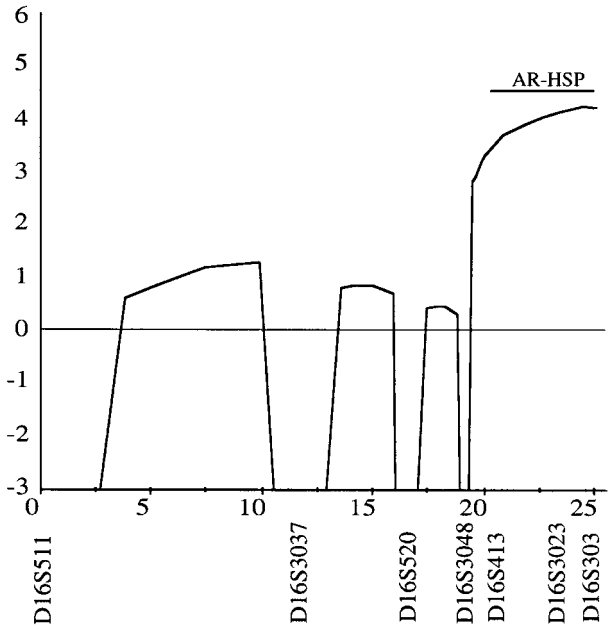

Figure 2 Multipoint LOD score analysis of the distal 16q region. Distances are in centimorgans$_{Hald}$ from D16S511, which was taken arbitrarily as 0.

mosome 16 (16q24.3), in an area of ~6 cM. The 13 members of the IV generation are the offspring of two consanguineous first-cousin marriages (fig. 1); among them, all affected patients participating in the study share a common homozygous haplotype for markers D16S413, D16S3023, and D16S303 (1-1-1). Patient IV-4 shows a recombination between loci D16S3048 and D16S413, allowing us to narrow the candidate area to the telomeric region of 16q.

Two additional uncomplicated AR-HSP families, both excluded from linkage to the chromosome 8 locus, were tested for linkage to chromosome 16q24.3 markers. One of these pedigrees is compatible with linkage to this new locus, even though the LOD score value is not significant, whereas in the other family the linkage with 16q telomeric markers is excluded (data not shown).

Discussion

We mapped a new locus responsible for AR-HSP to chromosome 16. AR forms of pure spastic paraplegia are less frequent than AD forms (Harding 1981; Polo et al. 1993). The phenotype does not differ clearly from that of AD-HSP. Besides spastic paraplegia, the following have been reported: urinary disturbances, sensory loss, pes cavus, and upper-limb clumsiness. Harding (1981) suggested genetic heterogeneity within AR-HSP on the basis of a different age at onset, reporting two families with childhood onset and one with adult onset. Polo et al. (1993) reported onset within 20 years in six patients from two families. In the five families reported by Hentati et al. (1994*a*), the ages at disease onset were 1–20 years. Molecular analysis has been performed only in the latter families, and, in four of them, the disease locus was mapped to the pericentric region of chromosome 8. The highest LOD score value was 8.77, with no recombination with the marker D8S260. Nonetheless, linkage to this region was excluded in one family.

In the present paper, we describe a large inbred family with a pure form of AR-HSP. Linkage to the previously described locus for AR-HSP on chromosome 8 was excluded. Since there are several examples of disorders in which AD and AR forms are allelic (OMIM 1996; see disorders and MIM numbers listed in the Introduction section), we have also excluded linkage with markers of the AD-HSP forms (SPG3, SPG4, and SPG6). These results suggest that the genetic locus involved in HSP in our family is different from all of those previously described in other forms of HSP.

The genetic heterogeneity of AR-HSP, which resulted from previously reported data and from our exclusion mapping data, induced us to consider this pedigree for a genomewide linkage mapping study. A new AR-HSP locus was identified on a region spanning ~6 cM on the chromosome 16q terminus (16q24.3).

The family we report here is characterized by age at onset >25 years; this is different from the majority of the previously reported families, in which age at onset is <20 years, as usually occurs in AR disorders. There are no other clinical peculiarities that clearly distinguish our family. However, the most severely affected patients showed dysarthria and dysphagia, symptoms that are not usually found in HSP. Dürr et al (1994) reported dysarthria in 2 of 70 patients with AD-HSP. The small number of patients does not allow us to establish whether these features characterize this particular form of AR-HSP. Other families need to be tested for this new HSP locus to establish whether this pedigree carries a private mutation or whether our data establish a new genetic class of AR-HSP. However, further genetic heterogeneity of pure AR-HSP is expected, since we have excluded chromosome 8 and chromosome 16 loci from linkage to HSP in one pedigree.

Several partial and complete transcripts have been mapped to the telomeric region of chromosome 16q, where the gene involved in this new form of HSP must lie. These include the gene responsible for Fanconi anemia (Fanconi Anaemia/Breast Cancer Consortium 1996), the cellular adhesion regulator molecule gene, which is involved in the suppression of tumor invasion (Pullman and Bodmer 1992), and the melanocyte-stimulating hormone receptor (Mountjoy et al. 1992). Detailed screening of the partial transcript sequences (expressed sequence tag, National Center for Biotechnology

Information) mapped in the critical region is being performed to identify the gene possibly involved in the neuronal degeneration of HSP.

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Electronic-Database Information

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

National Center for Biotechnology Information, Research Tools, http://www.ncbi.nlm.nih.gov/SCIENCE96/ResTools.html (for expressed sequence tags in 16q24.3)

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for SPG1 [MIM 312900], SPG2 [MIM 312920], SPG3 [MIM 182600], SPG4 [MIM 182601], SPG5A [MIM 270800], and SPG6 [MIM 600363])

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