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Prenatal diagnosis of hereditary spastic paraplegia†

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Hereditary spastic paraplegia (HSP) is a degenerative neurologic disorder that causes progressive, often severe, spastic weakness in the legs. Autosomal dominant HSP is a highly penetrant, genetically heterogeneous disorder with loci present on chromosomes 2p21-24, 2q24-34, 8q23-24, 10q23.3-24, 12q13, 14q12-23, 15q11-14 and 19q13.1. We identified a large HSP kindred in which the disorder was tightly linked to chromosome 14q12-23. We tested chorionic villus DNA samples of two at-risk fetuses for inheritance of microsatellite polymorphisms flanking and within this locus that segregated with the disease in this family. Whereas samples from the first fetus showed inheritance of a haplotype segregating with the disease allele (indicating high risk of developing HSP), samples from the second fetus showed inheritance of a haplotype segregating with the normal allele (indicating low risk of developing HSP). This is the first report of prenatal testing for HSP. Published in 2001 by John Wiley & Sons, Ltd.

KEY WORDS: paraplegia; spasticity; Strumpell-Lorrain

INTRODUCTION

Hereditary spastic paraplegia (HSP) is a group of clinically similar disorders characterized by insidiously progressive lower extremity spastic weakness (for reviews see Fink *et al*., 1996; Fink, 1997a,b; Fink and Hedera, 1999). Progressive gait disturbance begins in childhood, adolescence, or adulthood. Wheelchairs and other assistive devices are often necessary. Depending on the clinical subtype, urinary bladder disturbance and other neurologic deficits may occur. HSP is classified as 'uncomplicated' when the predominant disturbance is progressive lower extremity spastic weakness which may be accompanied by urinary bladder disturbance and impaired vibratatory sensation in the lower extremities. In uncomplicated or 'pure' HSP, upper extremity strength and dexterity remain normal. HSP is classified as 'complicated' when progressive spastic paraplegia is accompanied by additional neurologic deficits such as optic neuropathy, ataxia, amyotrophy or mental retardation. Specific treatments are not available to prevent, arrest, or reverse this progressively disabling disorder.

HSP may be transmitted as an autosomal recessive, autosomal dominant and X-linked disorder. Each of these HSP forms is genetically heterogeneous. Loci for autosomal dominant HSP exist on chromosome 2p21-24, 2q24-34, 8q23-24, 10q23.3-24, 12q13, 14q12-23, 15q11-14 and 19q13.1 (Hazan *et al*., 1993; Hentati *et al*., 1994; Gispert *et al*., 1995; Hedera *et al*., 1999a,b; Reid *et al*., 1999; Seri *et al*., 1999; Fontaine *et al*., 2000; Reid *et al*., 2000). Uncomplicated HSP usually shows nearly complete, age-dependent penetrance. Nonetheless, disease severity, rate of progression, age of symptom onset, and extent of functional disability may be variable within a given kindred; between families linked to the same locus; as well as between kindreds linked to different HSP loci (Fink and Hedera, 1999).

Among the 13 different genetic types of HSP (eight autosomal dominant, three autosomal recessive, and two X-linked loci), four HSP disease genes have been identified. Mutations in the proteolipidprotein gene (PLP) have been shown to cause both Pelizeaus-Merzbacher disease and X-linked HSP (Kobayashi *et al*., 1994; Saugier-Veber *et al*., 1994; Cambi *et al*., 1995). Mutations in the L1CAM gene have been shown to cause a complicated form of X-linked spastic paraplegia (Jouet *et al*., 1994). Casari *et al*. (1998) showed that autosomal recessive HSP linked to chromosome 16 was due to mutations in a novel gene designated 'paraplegin' which encodes a mitochondrial metalloprotease. Recently, mutations in a novel gene ('spastin') were identified as causing the most common form of autosomal dominant HSP (linked to the SPG4 locus on chromosome 2p). Little is known about spastin's function although homology analysis predicts that spastin is a member of the AAA (ATPase Associated with diverse cellular Activities) class of of peptides and may be involved in assembly or function of nuclear protein complexes (Hazan *et al*., 1999). Genes for the remaining types of HSP, including autosomal dominant HSP linked to chromosome 14q, are unknown.

Two couples in a large Turkish kindred with autosomal dominant HSP sought prenatal genetic testing. We evaluated the kindred, demonstrated that the disorder was tightly linked to chromosome 14q12-23, showed that the consultands were informative for linked microsatellite polymorphisms, and performed prenatal testing for two at-risk fetuses.

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METHODS

Human subjects

Informed consent was obtained and subjects participated as specified in the University of Michigan Institutional Review Board approved protocol. The portion of the kindred analyzed in this study (Figure 1) included 17 living affected subjects, six living unaffected subjects and four married-in spouses. Age-of-symptom onset was determined by interviewing the 17 affected subjects. Subjects were diagnosed as definitely affected with HSP or definitely unaffected according to published criteria (Fink *et al.*, 1996).

Genetic linkage to chromosome 14q

Leukocyte DNA was extracted from peripheral blood samples from the 17 affected and eight unaffected subjects and the four married-in spouses. After ^{32}P kinase labeling one oligonucleotide primer, polymorphic microsatellite markers D14S75, D14S306, D14S600, D14S269, D14S746, D14S1031, D14S584 and D14S281 were amplified by the polymerase chain reaction (PCR) according to standard protocols: 100 μg DNA, 1.5 mM $MgCl_2$, 35 cycles of 94°C 30 s, 55°C 30 s, 72°C 30 s. PCR products were separated by electrophoresis on 6% polyacrylamide gels and visualized by autoradiography. Two-point lod scores were calculated by MLINK using a disease allele frequency of 0.001 and a genetic penetrance of 0.9.

Analysis of chorionic villus DNA

After obtaining informed consent, chorionic villus sampling was performed at 12 weeks in a 25-year-old gravida two, para zero woman (subject III-6, Figure 1) and a 21-year-old primigravida (subject IV-3, Figure 1). DNA was extracted according to standard procedures. Microsatellite polymorphisms D14S75,

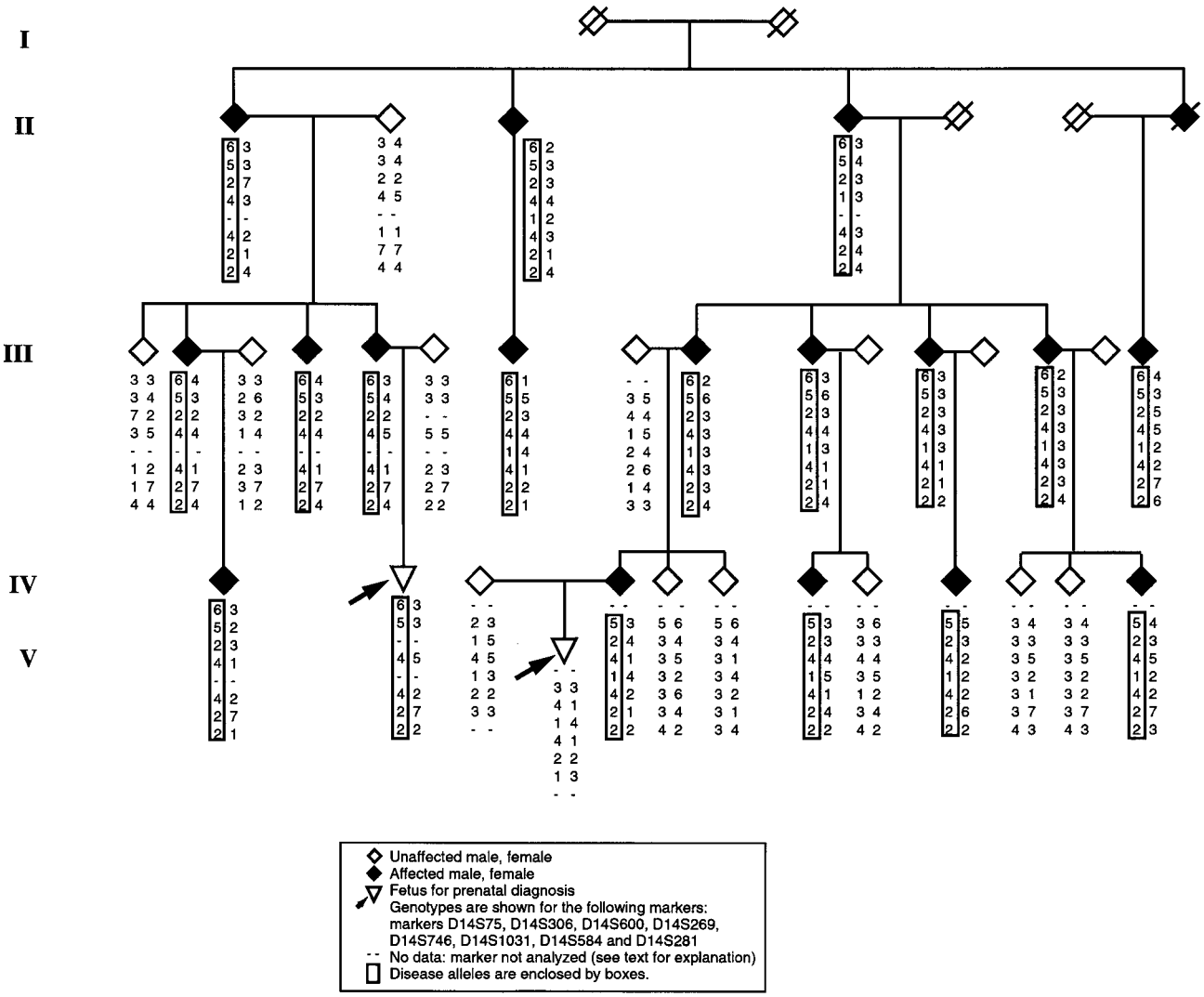

Figure 1—HSP kindred linked to chromosome 14q (SPG3). Gender identity is disguised to preserve anonymity. Genotypes are shown (in descending order) for microsatelite markers D14S75, D14S306, D14S600, D14S269, D14S746, D14S1031, D14S584 and D14S281. Haplotype segregating with the disease is boxed

D14S306, D14S600, D14S269, D14 S 746, D14 S 1031, D14 S 584 and D14 S 281 were PCR amplified and analyzed as described above.

RESULTS

Clinical features

Each affected subject was the product of uneventful gestation, labor and delivery and attained developmental milestones normally. Each affected subject experienced onset of gait disturbance before age 3 years. Although many subjects experienced very little worsening, others reported that gait disturbance slowly increased, particularly in adolescence and adulthood. Neurologic examination of affected subjects showed relatively symmetrical, lower extremity spasticity, hyperreflexia, and weakness and extensor plantar responses. Disease severity was variable. While the majority of subjects experienced marked gait disturbance, several elderly affected subjects had only subtle gait impairment. Males and females were affected with equal frequency and severity. There was no evidence of genetic anticipation. Every affected subject had one affected parent. Observed genetic penetrance therefore was complete.

Genetic analysis

Two point lod scores (Table 1) reached a maximum of +5.59 for D14S584 at $\theta = 0$. Two point lod scores for the other markesr (D14S75, D14S306, D14S600, D14S746, D14S269, D14S1031 and D14S281) were between +3.0 and +4.91 at $\theta = 0$. These findings established conclusive linkage between the disorder and the HSP locus (SPG3) on chromosome 14q.

We analyzed haplotypes of 17 affected subjects and six unaffected subjects including the nuclear families seeking prenatal testing (parents III-5 and III-6 and their at-risk fetus IV-2; and parents IV-3 and IV-4 and their at-risk fetus V-1; Figure 1). The two couples seeking prenatal assessment were shown to be informative for markers (Figure 2) D14S75, D14S306, D14S269, D14S1031 and D14S281 (parents III-5 and III-6; Figure 1), and D14S306, D14S600, D14S269, D14S746, D14S1031 and D14S584 (parents IV-3 and IV-4; Figure 1) that segregated with the disorder in their family. The marker D14S746 was not mapped to

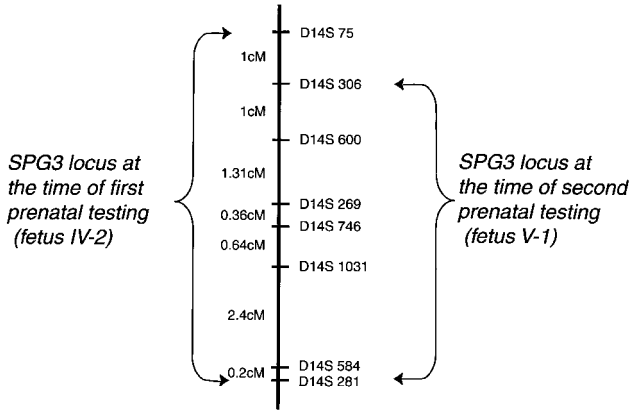

Figure 2—HSP locus (SPG3) on chromosome 14q12-23 illustrating location of microsatellite polymorphisms used for prenatal testing. Distances between markers are obtained from the Genome Database (http://www.gdb.org)

the SPG3 locus at the time of the first prenatal testing (at-risk fetus IV-2; Figure 1). We did not include marker D14S75 in the second prenatal testing (at-risk fetus V-1; Figure 1) because the locus had been reduced and this was no longer a flanking marker. Marker D14S600 was not analyzed in the first prenatal testing (at-risk fetus IV-2; Figure 1) and marker D14S281 was not analyzed in the second prenatal testing (at-risk fetus V-1; Figure 1) because one parent was uninformative for each of these markers (Figure 1).

At-risk fetus IV-2 inherited haplotypes that segregated with the disease for markers D14S75, D14S306, D14S269, D14S1031 and D14S281; markers D14S600 and D14S584 were uninformative. At-risk fetus V-1 inherited haplotypes for markers D14S306, D14S600, D14S269, D14S746, D14S1031 and D14S584 that segregated with the normal allele; marker D14S281 was uninformative.

DISCUSSION

In the first case (parents III-5 and III-6, fetus IV-2; Figure 1), analysis of chorionic villus DNA showed that the fetus inherited the disease allele for each microsatellite marker tested. Fetal inheritance of the *normal* allele would have required double simultaneous recombination between closely spaced markers, for which the combined probability is 0.0021 (see Figure 2

Table 1—Results of two-point linkage analysis for microsatellite polymorphisms on chromosome 14q12-23

Marker	Penetrance	$\theta = 0.001$	$\theta = 0.05$	$\theta = 0.1$	$\theta = 0.2$	$\theta = 0.3$	$\theta = 0.4$
D14S75	0.90	3.06	2.75	2.44	1.78	1.10	0.42
D14S306	0.90	4.94	4.68	4.21	3.28	2.21	1.02
D14600	0.90	3.47	3.16	2.83	2.10	1.27	0.43
D14S269	0.90	3.21	2.92	2.63	1.97	1.22	0.45
D14S746	0.90	3.07	2.74	2.41	1.71	0.97	0.29
D14S1031	0.90	4.21	3.80	3.37	2.47	1.51	0.55
D14S584	0.90	5.59	5.10	4.60	3.52	2.34	1.06
D14S281	0.90	3.20	2.83	2.43	1.60	0.72	0.03

for inter-marker distances). Conversely, the probability that the fetus inherited the HSP disease allele is 0.9979.

In order to calculate disease risk conservatively, we assumed genetic penetrance to be 0.90 (even though observed genetic penetrance was 1.0). Given the chance that the fetus inherited the disease gene (0.9979) and assuming penetrance to be 0.9, we calculated the probability that the fetus would be affected with HSP to be at least 0.898. The family was informed and elected to terminate the pregnancy. Fetal tissues were not examined.

In the second case (parents IV-3 and IV-4, fetus at risk V-1; Figure 1), analysis of chorionic villus DNA showed that the fetus inherited the normal allele for each microsatellite marker tested. Fetal inheritance of the *disease* allele would have required double simultaneous recombination between closely spaced markers, for which the combined probability is 0.079 (see Figure 2 for inter-marker distances). Conversely, the probability that the fetus inherited the HSP normal allele is 0.921.

The family was informed that the fetus would most likely inherit the normal allele and was thus at extremely low risk of inheriting HSP. They elected to continue the pregnancy. Follow-up information regarding the clinical status of the child is not yet available.

This first report of prenatal assessment of HSP risk illustrates important concerns regarding genetic counseling of HSP. First, although genetic penetrance in this family (and other autosomal dominant HSP kindreds) is very high, disease severity varies significantly. Although the majority of affected subjects in this kindred had moderate to severe spastic displegia, several adult subjects had only subtle gait disturbance that was not functionally disabling. Although the risk of inheriting the disease gene and genetic penetrance could be estimated, there were too few subjects to permit reliable estimates of the probability that the disease would be mild and not disabling instead of severe and functionally limiting. Second, although uncomplicated HSP (in this family and in general) is a lifelong, often disabling disorder, it does not shorten life span. Nonetheless, some HSP patients wish to conceive only if prenatal diagnosis is possible. Analysis of haplotypes of microsatellite polymorphisms that segregate with the disorder permits, for informative couples, prenatal risk assessment for dominantly inherited HSP linked to loci on chromosomes 8q, 10q, 12q, 14q, 15q and 19q; recessively inherited HSP on chromosomes 8p and 15q. Prenatal testing based on spastin gene analysis is applicable to approximately 45% of autosomal dominant HSP (Fink *et al*., 1996). Although quite rare, prenatal testing is also possible for autosomal recessive HSP due to paraplegin gene mutations (Casari *et al*., 1998), for X-linked HSP due to proteolipoprotein gene mutations (Cambi *et al*., 1995; Dube *et al*., 1997) and for X-linked HSP due to L1CAM gene mutations (Jouet *et al*., 1994). It is important to recognize, however, that each form (autosomal recessive, autosomal dominant and X-linked) of uncomplicated HSP is genetically heterogeneous and that currently known loci do not account for all autosomal dominant HSP kindreds (Fink and Hedera, unpublished observations). Identifying these other loci will extend our ability to offer genetic counseling and prenatal diagnosis for this disorder and may reveal potential HSP candidate genes.

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