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Etiological Heterogeneity in X-linked Spastic Paraplegia

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SUMMARY

We describe a large family (K313) having 12 males affected with X chromosome–linked recessive hereditary spastic paraplegia (HSP). The disease phenotype in K313 is characterized by hyperreflexia and a spastic gait, but intelligence is normal. Carrier females have normal gait and unremarkable neurologic profiles. Eight widely spaced X-linked DNA markers were used to genotype 43 family members. In contrast to a published study of another family, in whom complete linkage of X-linked recessive HSP to distal chromosome Xq markers DXS15 and DXS52 was reported, we observed complete linkage with two DNA markers, pYNH3 and DXS17, located on the middle of the long arm of the X chromosome. These data have been combined with linkage data from a large reference panel of normal families to localize the new X-chromosome marker, pYNH3, and to provide evidence of significant locus heterogeneity between phenotypically distinct forms of X-linked recessive HSP.

INTRODUCTION

Hereditary spastic paraplegia (HSP), first described by Seeligmueller (1876) and Strumpell (1880), is a disorder characterized by a slow, upwardly progressive spasticity of the lower extremities. HSP has been divided by some investigators into pure and complicated forms (Harding 1983). In pure HSP, spasticity

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and hyperreflexia are the only signs (Baraitser 1982). Complicated HSP has additional manifestations, such as mental retardation, dysarthria, epilepsy, optic atrophy, pigmentary retinal degeneration, and extrapyramidal symptoms (Sutherland 1975; Harding 1981).

Inheritance is most commonly autosomal dominant and constitutes ~70% of the cases of pure HSP. Autosomal recessive inheritance occurs 30% of the time (Holmes and Shaywitz 1977). X chromosome–linked inheritance is very rare, and, in the absence of adequate clinical criteria for diagnosis of carrier females, several reviews have questioned whether X-linked recessive pure HSP exists at all (Harding 1981, 1983).

Kenwrick et al. (1986) recently reported a family with X-linked HSP complicated by mental retardation and optic atrophy. The mutation in this family was linked to the DNA markers DXS52 and DXS15. In the present paper we report the analysis of linkage between several X-linked DNA markers and the disease locus in a family (K313) with 12 normally intelligent males affected with X-linked recessive HSP (fig. 1). The hypothesis of genetic heterogeneity between the clinical entities represented by Kenwrick et al.'s family and K313 is examined.

CLINICAL PRESENTATION

All but four cases of HSP in K313 were personally examined by one of the authors (L.D.K.). Medical records and information from relatives substantiated the diagnosis of X-linked HSP in the four individuals not examined.

This family's disorder is usually characterized by delayed motor milestones. In early childhood most affected males have been able to walk and run but have had either a tendency to trip or a gait described as unsteady or clumsy. The typical spastic gait with scissoring and in-toeing generally develops during adolescence in this family. Affected individuals experience a gradual deterioration of the lower extremities that stabilizes after 20–30 years. Intelligence, speech, vision, and function of the upper extremities are unaffected. Fertility in males is difficult to assess, since few of the males in K313 have married. However, three affected males in this kindred have reproduced, and no male-to-male transmission of HSP has occurred in the small number of sons of affected males.

Case Studies

H.C. (II-6; not examined by L.D.K.), born September 20, 1927, was the first affected male in this pedigree. He started walking at age 2 years and had a normal walk in grade school. He enlisted in the military service at age 25 years and served 2 years. Gait was significantly impaired in his early 30s. Speech and intelligence are normal. He has one son and two daughters. His physician reports that he has spastic scissors gait, hyperreflexia, atrophy of the lower limbs, and urinary frequency.

C.W. (III-1), born August 15, 1928, is the most severely affected. His gait has never been normal, and he has required a wheelchair since age 47 years. He completed the eighth grade and has normal intelligence. He has had urinary

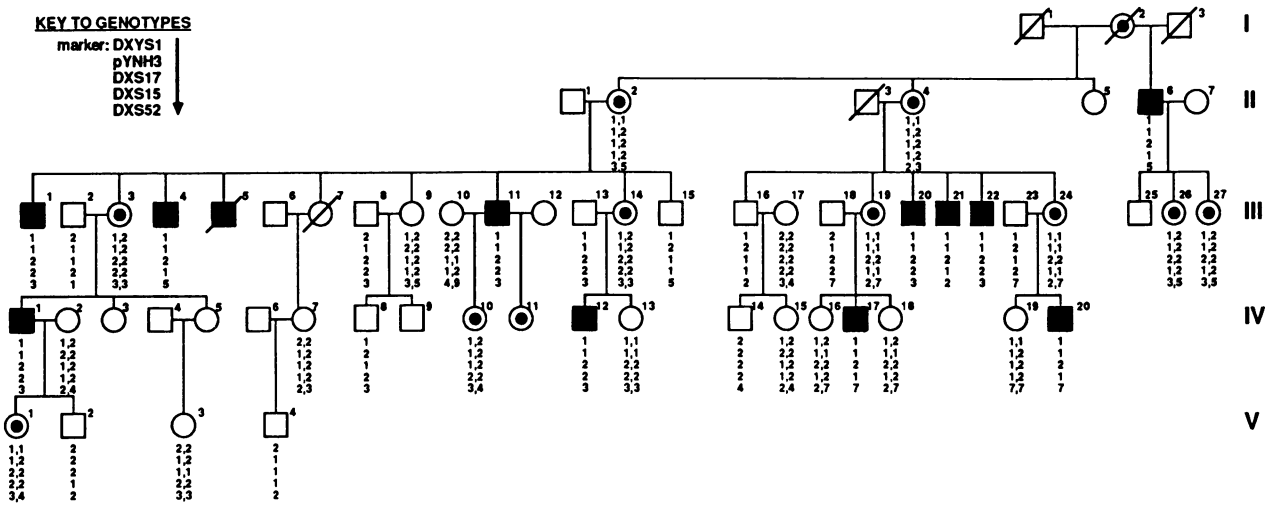

FIG. 1.—Pedigree of K313. Affected individuals are designated by black squares; symbols for obligate carrier females have black center circles. Numbers under symbols represent genotypes for tested individuals at five loci, reading down in the order indicated by the key.

frequency and urgency since age 52 years. He has normal use of the upper extremities. He has hyperreflexia of the upper and lower extremities, ankle clonus, and left equinovarus.

F.W. (III-4), born November 23, 1933, started walking at age 3 years and has never had a normal gait. He reports that walking down steps has always been especially difficult. He completed the ninth grade and has normal intelligence. He required a cane at age 22 years and a wheelchair at age 46 years. He has urinary frequency and urgency. He has hyperreflexia of the upper and lower extremities and ankle clonus.

W.W. (III-5; not examined by L.D.K.), born June 11, 1939, died in a tractor accident at age 18 years. He walked at age 2 years and developed a spastic gait during adolescence. An autopsy was not done.

J.W. (III-11), born March 25, 1947, had a normal gait in grade school but developed a scissors walk during early adolescence. He has normal intelligence and received good grades in school but quit in the tenth grade because of teasing about his unusual walk. Heel-cord lengthening was done at age 28 years. He has required two crutches for ambulation since age 23 years. He worked as a mechanic in the past but is now disabled. He reports no bladder problems and has normal use of upper extremities. His two daughters are normal. He has hyperreflexia of the lower extremities and hammer toes but no ankle clonus.

T.B. (III-20; not examined by L.D.K.), born October 19, 1940, was born with a cleft lip and palate. He started walking at age ~20 mo and developed a spastic gait at age 19 years. Currently he walks with crutches. He has been evaluated at a neuromuscular clinic. The neurologist who examined him reported a normal mental status, hyperreflexia, sustained ankle clonus, upgoing plantar reflexes, a scissors gait, and normal sensation. He has dysarthria secondary to his unrepaired cleft palate.

C.B. (III-21), born December 31, 1941, developed a spastic gait at age 12 years. He attended vocational technical school and has worked as an electronic technician and bookkeeper. He has required two crutches for ambulation since age 35 years. He has hyperreflexia of the lower extremities, ankle clonus, a normal position sense, muscle atrophy of the right leg, and flexion contractures of the knee.

L.B. (III-22), born June 16, 1946, walked at age 12 mo. He was able to walk and run normally in school and played football. He developed a spastic gait in his 20s but was able to work as a truck driver until age 36 years. He had a normal lumbar myelogram at age 30 years, and heel cords were lengthened at age 37 years. He now requires crutches for ambulation. He has a high school diploma and has completed 6 credit hours in college. He has been married for 1 year. He has hyperreflexia of the upper and lower extremities, sustained ankle clonus, and toe contractures.

M.W. (IV-1), born December 18, 1950, has had a slower progression of symptoms. He ran track in school and enlisted in the service at age 17 years. Spasticity of the lower extremities developed during late adolescence, but he can walk unassisted at age 35 years. He has had increased urinary frequency

since age 34 years. He has a clinically normal daughter and son. He has hyperreflexia of the lower extremities and unsustained ankle clonus.

W.D. (IV-12; not examined by L.D.K.), born May 23, 1968, walked at age 2 years and developed a spastic gait at age 14 years. He had a hamstring tenotomy for flexion contractures at age 15 years. He was an honor student in high school and walks unassisted.

R.H. (IV-17), born November 23, 1960, walked at age 16 mo and was able to participate in physical education and football in school. He was evaluated for leg spasticity at age 14 years; heel cords were lengthened at age 15 years; and he has required crutches for ambulation since his early 20s. He worked as a truck driver until age 22 years. He has hyperreflexia of the upper and lower extremities, ankle clonus, and upgoing plantar reflexes.

T.H. (IV-20), born July 22, 1978, reportedly had a traumatic birth and required resuscitation. His birth weight was 8 lbs. 6 oz. He sat at age 9 mo, crawled at age 1½ years, and walked at age 2½ years. Heel cords were lengthened at age 3 years. Gait has never been normal. He walks unassisted but frequently stumbles. He attends regular classes in school.

Seven obligate carrier females and four females at risk to be carriers were also examined. All carrier females have normal gait, and neurological examinations have been unremarkable. None of the carrier females has hyperreflexia or any evident spasticity of the lower extremities.

MATERIAL AND METHODS

The present study was performed using kindred-K313 blood samples, from which high-molecular-weight DNA was prepared from peripheral lymphocytes or lymphoblastoid cell lines. Methods for the preparation of genomic DNA, DNA probes, DNA transfers, and hybridizations were as documented in earlier reports (Barker et al. 1984; Cavenee et al. 1984), with the exception that DNA transfers were performed using 0.4 M NaOH (Reed and Mann 1985). With one probe, DXS52, exposures were carried out without intensifying screens, in order to better resolve closely spaced autoradiographic bands.

DNA Markers

The DNA markers used in the present study were selected on the basis of adequate heterozygosity and coverage of the genetic linkage map of the X chromosome. Markers St14 (DXS52), DX13 (DXS15), p52A (DXS51), pDP34 (DXYS1), p19-2 (DXS3), S21 (DXS17), and pL1.28 (DXS7), positioned on the X-chromosome map of Drayna and White (1985), were initially run through the family, as was a new X-chromosome DNA probe, pYNH3. Subsequent to detection of linkage with pYNH3 and DXS17, markers p22-33 (DXS11) and p43-15 (DXS42) were run to better resolve this segment of the X chromosome.

The DNA markers listed above have been genotyped in the majority of the Utah linkage-reference families in previous published (Drayna and White 1985) and unpublished studies. We have combined the K313 data with the normal linkage map of chromosome X to strengthen the linkage analysis.

Data Analysis

Genotypic data generated in the family study were analyzed with the two-point and multipoint options of the LINKAGE program (Lathrop and Lalouel 1984; Lathrop et al. 1984). Genotypes read from autoradiographs were entered into the LINKAGE format, printed, and compared with the original autoradiograms to verify the absence of errors in data entry or interpretation.

RESULTS

The linkage analysis assumed an X-linked recessive mode of inheritance for HSP, with complete penetrance and a gene frequency of .001 for the recessive allele; the results remained unaltered when the gene frequency was varied over a wide range. The lod scores obtained for each marker, reported in table 1A, indicate that linkage is significant for two markers on the long arm of the X chromosome, pYNH3 and DXS17. In neither instance is there evidence of recombination. The corresponding confidence upper bounds on recombination, computed as those values for which the maximum lod is decreased by one unit (Conneally et al. 1985), are .14 and .15, respectively. There also is no evidence of recombination for the locus DXYS1, but the lod score of 0.81 is too low to assess support for the observation of linkage with this marker. Linkage is also suggested for DXS3. By contrast, there is no evidence of linkage either with DXS7, on the short arm, or with DXS15 or DXS52, both located on the distal part of the long arm.

Kenwrick et al. (1986) reported significant linkage between HSP and the two distal markers DXS15 and DXS52, with no evidence for recombination; close linkage to DXYS1 was rejected. A published genetic map of the X chromosome

TABLE 1

PAIRWISE LINKAGE TESTS IN HSP: LOD SCORES

A. K313

	Recombination Fraction					
Locus	.00	.05	.10	.20	.30	.40
DXYS1	0.81	0.74	0.66	0.50	0.34	0.17
DXS3	...	1.53	1.83	1.75	1.34	0.73
pYNH3	4.48	4.14	3.77	2.99	2.10	1.09
DXS17	4.00	3.67	3.32	2.60	1.80	0.92
DXS11	0.20	0.17	0.14	0.08	0.04	0.01
DXS42	...	−1.22	−0.34	0.15	0.08	−0.10
DXS15	...	−3.03	−1.76	−0.75	−0.39	−0.22
DXS52	...	−9.64	−6.45	−3.39	−1.73	−0.68

B. Kenwrick et al. Family

DXYS1	...	−4.19	−2.74	−1.38	−0.66	−0.24
DXS42	...	−1.33	−0.80	−0.33	−0.12	−0.03
DXS15	3.21	2.93	2.64	2.02	1.34	0.59
DXS52	3.43	3.13	2.82	2.14	1.40	0.61

TABLE 2

TESTS OF HETEROGENEITY IN RECOMBINATION FREQUENCIES FOR THREE MARKERS IN TWO SEPARATE HSP PEDIGREES

Locus and Data Set	Recombination Estimate	χ^2_1
DXS15:		
K313	.692	
Kenwrick et al. family	.001	
K313 + Kenwrick et al. family	.188	10.26
DXYS1:		
K313	.001	
Kenwrick et al. family	.616	
K313 + Kenwrick et al. family	.494	4.18
DXS52:		
K313	.691	
Kenwrick et al. family	.001	
K313 + Kenwrick et al. family	.277	14.34

(Drayna and White 1985) indicates that DXYS1, DXS3, and DXS17 are apparently unlinked to the two distal markers, DXS15 and DXS52. In view of these conflicting results, we performed tests of heterogeneity by comparing the sum of the lod scores maximized separately in each pedigree with the maximum lod score obtained on the pooled data, following standard-likelihood theory (Rao 1973). Lod scores computed for the pedigree of Kenwrick et al. (1986) on the basis of their reported data are presented in table 1B; our values depart slightly from those computed by the authors themselves, no doubt because we had to assume marker-loci gene frequencies that may be different from those that they used. For markers DXYS1, DXS15, and DXS52, the results of the tests for heterogeneity are reported in table 2. In all cases, heterogeneity is significant; the largest χ^2-value ($\chi^2_1 = 14.34$; $P < .005$) was observed for the most informative marker, DXS52.

The investigation of heterogeneity was pursued further by considering a complete genetic map of the long arm of the X chromosome. Previously characterized genotypic data for our reference linkage families (Drayna and White 1985), together with additional data for the loci DXS3 and DXS11 as well as data on the new locus pYNH3, were used to derive the genetic map illustrated in figure 2. With this genetic map assumed, heterogeneity was examined by means of a calculation of location scores (Lathrop et al. 1984), with all markers being used jointly (fig. 3). The joint analysis documented a significant heterogeneity in the linkage detected between the two pedigrees of X-linked HSP ($\chi^2_1 = 21.08$; $P < .00001$).

DISCUSSION

Delineation of X-linked HSP into pure and complicated forms, as can be done with autosomal HSP (McKusick 1986, p. 1454), is difficult because the X-linked form is so rare. Most published reports of X-linked HSP present a disease phenotype that includes mental retardation, optic-nerve defects, and

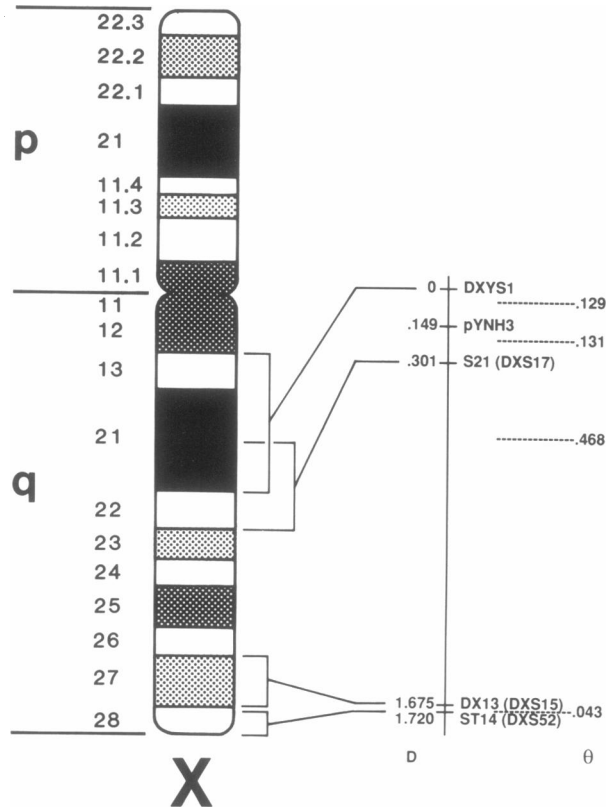

FIG. 2.—Genetic map of the markers linked to HSP in K313, showing recombination fractions (θ) and genetic distance (D; in Morgans) of each tested marker from DXYS1. Previously established physical locations of four markers are indicated on the ideogram.

upper-extremity involvement, as seen in complicated HSP (Blumel et al. 1957; Johnston and McKusick 1962; Barr and Gabriel 1966; Holmes and Shaywitz 1977; Harding 1981, 1983). Thurmon et al. (1971) described a pedigree of pure HSP that suggested X linkage, but they did not report neurological examination of carrier females. Zatz et al. (1976) reported a large kindred having 24 affected males with pure HSP; three carrier females in the family were clinically normal. Many of the affected males had reproduced, with no instances of male-to-male transmission in the 20 sons of affected fathers.

The segregation pattern and the phenotype described in K313 are consistent with the notion that this family, like that described by Zatz et al., represents an X-linked form of pure HSP. All 12 affected males have normal intelligence and lack the additional features of complicated HSP. They have experienced delayed motor milestones and spastic gait with gradual deterioration in adolescence, but upper extremities, intellect, speech, optic nerves, and longevity have been unimpaired. Three affected males have reproduced, and there has been no evidence of male-to-male transmission. Four affected males have uri-

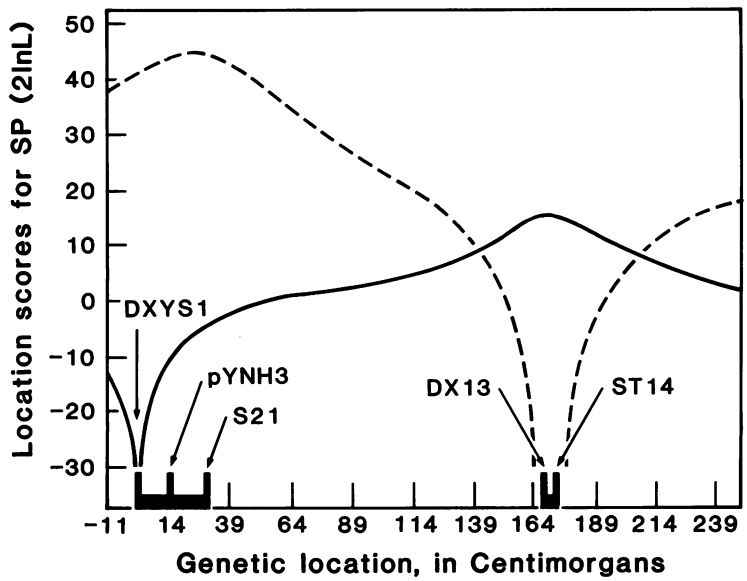

FIG. 3.—Support for the location of the gene for X-linked HSP in K313 (dashed lines) and the family reported by Kenwrick et al. (1986) (solid lines), relative to the markers mapped in fig. 2. 2lnL = twice the natural logarithm of the likelihood function.

nary symptoms, but that is common in pure HSP (Sutherland 1975; Harding 1981).

As already mentioned, Kenwrick et al. (1986) reported complete linkage of the DNA markers DXS15 and DXS52 to the disease in a family with X-linked HSP. All six affected males had spasticity and were mentally retarded. Congenital malformations and optic-nerve atrophy were also seen in some of the patients. Linkage analysis localized the mutation in this family to Xq28.

Our linkage studies on K313, on the other hand, indicate complete linkage of the disease gene to DNA markers pYNH3 and DXS17, located in the chromosome Xq21-22 region within a cluster of markers that are essentially unlinked to the chromosome Xqter region. Our results (see table 2, fig. 3) indicate, with a high degree of statistical significance, that a locus different from that reported for Kenwrick et al.'s family is involved in the X-linked form of HSP present in K313. The clinical case reports described by Kenwrick et al. conform to the phenotype of complicated HSP; our data confirm that an X-linked form of pure HSP also exists.

This result illustrates the power of linkage studies with DNA markers to examine the etiology of genetic disease in man. X-linked muscular dystrophy (Monaco et al. 1985) and cystic fibrosis (Beaudet et al. 1986) are examples of disorders that, in spite of a wide range of clinical presentation, are mapped genetically to unique loci by means of DNA technology. In contrast, the results reported here show locus heterogeneity for diseases having overlapping phenotypes. They also illustrate the potential complexities in the clinical application

of DNA markers, particularly in genetic diseases for which numerous extended pedigrees are not available.

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