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Allan-Herndon Syndrome. II. Linkage to DNA Markers in Xq21

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Summary

The original family with the Allan-Herndon type of X-linked mental retardation has been investigated for linkage by using DNA probes spanning the length of the X chromosome. Available for study, over 3 generations, were 13 affected males, three obligate carriers, and three normal sons of the obligate carriers. Initial disease-to-marker analysis suggested linkage to three markers (DXYS2 [7b], DXS250 [GMGX22], and DXS3 [p19-2]) located in Xq21. All three exhibited the same maximum lod score of 2.3 at a maximum theta of .05. Multipoint analysis using LINKMAP and a set of four DNA markers (DXYS1–DXYS2–DXS3–DXS94) gave a multipoint lod score of 3.58 for a location of the Allan-Herndon syndrome near locus DXYS1 (pDP34). Therefore, our data indicate that the gene for the Allan-Herndon syndrome is likely located in Xq21.

Introduction

Allan et al. (1944) described a large family with mental retardation in which only males were affected. Their paper is considered the first report of X-linked mental retardation (XLMR) not associated with the fragile X chromosome. Members of this family were recently reinvestigated, and the phenotype was determined to be unique (Stevenson et al., 1990). The phenotype has been designated the Allan-Herndon syndrome (AHS). The availability, over 3 generations, of 13 affected males, three obligate carriers, and three normal male offspring of two of the carrier females allowed us to pursue linkage analysis. Family members were typed for 20 DNA markers spanning the X chromosome. Standard two-point linkage analysis and multipoint analysis were conducted on the genotype data. The results indicate the disease gene is located in Xq21.

Material and Methods

Clinical Assessment of Patients

Individuals studied are indicated on the partial pedigree shown in figure 1. The clinical features of the patients are presented in detail elsewhere (Stevenson et al. 1990). Affected males are severely retarded and exhibit muscle hypoplasia, spastic paraplagia, and ataxia. The facies appear elongated with normal head circumference and bitemporal narrowing. Macroorchidism is not present, and statural growth is normal. Carrier females do not exhibit any of the clinical features.

Preparation of DNA and Blot Hybridization

Genomic DNA was isolated from peripheral blood by using a high-salt-precipitation method. In brief, white blood cells were lysed and digested overnight with proteinase K (220 μg/ml) according to a method described elsewhere (Schwartz et al. 1986), except that the incubation temperature was 42°C. The following day, a saturated sodium chloride solution was added in a ratio of 1 ml/3 ml of lysis solution, and the tubes were vigorously mixed for 15 s and then centrifuged at 4,500 rpm for 20 min at room temperature. The DNA was precipitated from the supernatant by adding an equal volume of isopropanol and was washed twice with 70% ethanol by inversion. Solubilization was accomplished at 37°C with gentle agitation. The isolated DNA was digested, separated by agarose-gel electrophoresis, transferred to a nylon membrane, prehybridized, and hybridized according to methods described elsewhere (Schwartz et al. 1986). The DNA probes were labeled

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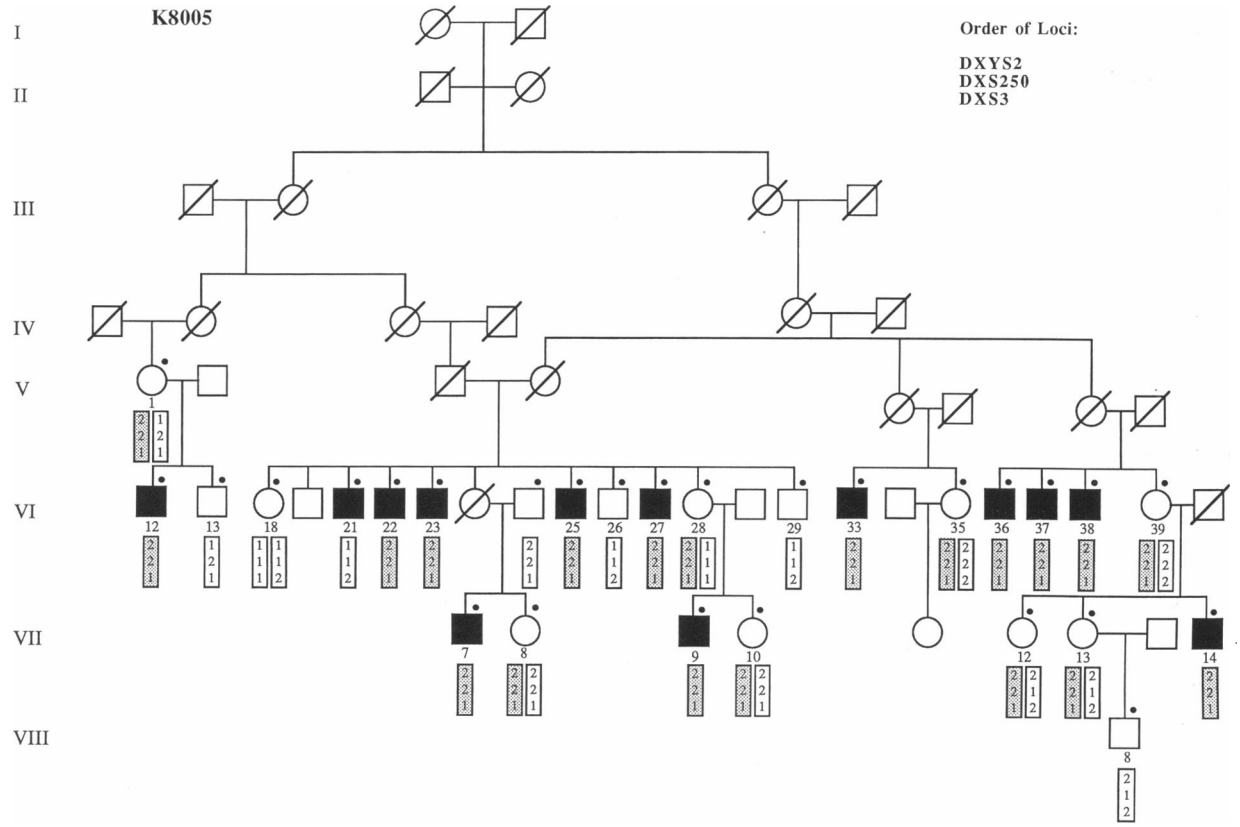

Figure 1 Partial pedigree of family with AHS. The numbering is consistent with the complete pedigree presented by Stevenson et al. (1990). ○• and □• = those members studied with DNA markers on the X chromosome. The genotype data for the three DNA markers exhibiting linkage are given below each individual. The haplotype apparently segregating with AHS, except for male VI-21, is indicated by stippled rectangles (▦).

by primer extension (Feinberg and Vogelstein 1983) to a specific activity of approximately 1×10^9 cpm/μg.

DNA Probes

Twenty DNA markers spaced along the length of the X chromosome were utilized for linkage analysis and are listed in table 1.

Linkage Analysis

Two-point disease-to-marker analysis was conducted using the computer program MLINK of the LINKAGE package (Lathrop and Lalouel 1984), and multipoint analysis was done using LINKMAP (Lathrop et al. 1985). The mutation rate and gene frequency were set at 0.3×10^{-5} and .0001, respectively.

Results

Two-point lod scores (Z) for the disease locus and for X chromosome markers, computed at different recombination fractions (θ) by using MLINK (Lathrop and Lalouel 1984), are listed in table 1. Of the 20 loci analyzed, three markers—p19-2 (DXS3), 7b (DXYS2), and GMGX22 (DXS250)—exhibited positive Z values >2, each reaching a maximum of 2.3 at $\theta = .05$. Individual genotypes for these markers are presented in figure 1. For each of these markers, person VI-21 was the single recombinant. A $\hat{Z}$ value >3 is considered as proof of linkage, while a $\hat{Z}$ value $2 > 3$ is suggestive of linkage (Botstein et al. 1980). Therefore, the $\hat{Z}$ values for these three probes suggest linkage of the disease locus to the region of Xq21.

Multipoint analysis, using the LINKMAP program, was undertaken to better place AHS in the region of Xq21. The five-point linkage analysis included AHS and the DNA markers DXYS1, DXYS2, DXS3, and DXS94. The marker DXS250, although physically mapped in the DXYS1-DXS17 region, proximal to DXS3 (Feil et al. 1989), was not included because of a lack of linkage information relative to the other markers. The position

Table 1

Two-Point Z Values between AHS and X Chromosome Probes

Probe	Locus	Location[a]	Z at θ of .001	.01	.05	.1	.2	.3	.4	$\hat{\theta}$	$\hat{Z}$
S232	DXS278	Xp22.3	−8.26	−4.21	−1.61	−.75	−.05	.13	.11	.330	.14
782	DXS85	Xp22.3	−1.04	−.07	.49	.64	.63	.49	.27	.142	.66
RC8	DXS9	Xp22.2	−2.87	−.93	.24	.55	.54	.22	−.08	.140	.61
pD2	DXS42	Xp22.2	−4.93	−2.05	−.23	.33	.52	.32	.07	.180	.52
pXUT23	DXS16	Xp22.2	.54	.53	.48	.43	.32	.21	.11	.001	.54
99-6	DXS41	Xp22.1	.91	.88	.78	.65	.43	.25	.11	.001	.91
C7	DXS28	Xp21.3	−8.64	−4.71	−2.10	−1.12	−.32	−.02	.06	.402	.06
pERT87-15	DXS164	Xp21.2	−6.31	−2.31	.16	.78	1.06	.74	.15	.182	1.06
754	DXS84	Xp21.1	−9.32	−6.39	−4.58	−3.95	−2.41	−1.20	−.45	.637	.23
L1.28	DXS7	Xp11.4	−3.57	−1.63	−.44	−.10	−.04	−.14	−.12	.707	.15
p8	DXS1	Xq11.2	.09	.08	.06	.04	.01	.00	.00	.001	.09
GMGX22	DXS250	Xq13-q22	1.09	2.00	2.37	2.24	1.64	.85	.03	.052	2.37
pDP34	DXYS1	Xq21.3	.73	.70	.60	.49	.28	.12	.03	.001	.73
7b	DXYS2	Xq21.3	1.04	1.96	2.31	2.18	1.56	.74	−.07	.050	2.31
p19-2	DXS3	Xq21.3	1.06	1.97	2.32	2.18	1.58	.85	.10	.050	2.32
pXG-12	DXS94	Xq22	−4.26	−2.34	−1.05	−.57	−.21	−.08	−.02	.550	.00
S9	DXS17	Xq22	−9.37	−5.67	−2.46	−1.22	−.26	.05	.07	.734	.26
C11	DXS144E	Xq26	−2.55	−1.55	−.85	−.57	−.34	−.21	−.10	.199	.44
pX45h	DXS19	Xq26	−4.82	−2.87	−1.44	−.84	−.32	−.09	.00	.448	.01
St14	DXS52	Xq28	−12.4	−8.50	−5.49	−3.49	−1.58	−.63	−.19	.532	.01

[a] Taken from Kidd et al. (1989), except for GMGX22 (Feil et al. 1989).

of the four marker loci were fixed, and the genetic distances between them were calculated, using Haldane's mapping function, based on previously published recombination frequencies (Arvelier et al. 1987; Keats et al. 1989). The results are graphed in figure 2. The multipoint analysis suggested that AHS is located proximal to the DXYS2 and DXS3 markers, near DXYS1, with a multipoint Z value of 3.58.

Discussion

The concept of XLMR gained acceptance over 40 years ago with the publication of two large families which exhibited "sex-linked" mental retardation (Martin and Bell 1943; Allan et al. 1944). Richards et al. (1981) restudied the Martin-Bell family and showed that the males had the fragile X syndrome. The family studied by Allan et al. (1944) has recently been reinvestigated (Stevenson et al. 1990). The affected males in 7 generations of this family are severely retarded and have dysarthria, muscle hypoplasia, ataxia, and normal testicular measurements. The condition exhibited by the affected males appeared to be a distinctive XLMR syndrome and was therefore referred to as AHS.

In order to establish linkage of AHS to DNA markers on the X chromosome, 27 members of the family were studied. This group included all of the living affected males, three normal brothers of the affected males, and three carrier females (fig. 1). Of the 20 DNA markers utilized, three markers (7b [DXYS2], GMGX22 [DXS250], and p19-2 [DXS3]) had $\hat{Z}$ values >2 ($\hat{Z}$ = 2.32 at $\hat{\theta}$ = .05) which is suggestive of linkage (table 1). Utilizing LINKMAP (Lathrop et al. 1985), the location of AHS was placed against a fixed map of four marker loci (DXYS1, DXYS2, DXS3, and DXS94). A multipoint lod score of 3.58 was obtained when AHS was situated near DXYS1 in Xq21 (fig. 2).

Linkage of other XLMR syndromes has been established for markers in Xq21 (Wieacker et al. 1987; Miles and Carpenter, in press). However, these syndromes do not appear to be the same as AHS. Patients with the Wieacker-Wolff syndrome (Wieacker et al. 1985) have milder mental retardation, congenital foot deformities, and normal deep-tendon reflexes. Males with Miles-type mental retardation (Miles and Yeager 1979; Miles and Carpenter, in press) have severe mental retardation, microcephaly, assymetric facies, hypogonadism, joint hypermobility, and digital arches.

Mental retardation in association with spastic paraplegia has been found in other X-linked disorders

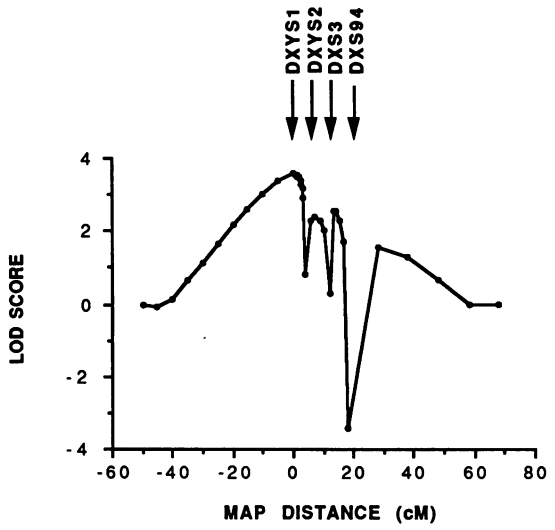

Figure 2 Logarithm of the odds of linkage of AHS: multipoint analysis vs. map of markers in Xq21. The assumed fixed order was centromere–DXYS1–DXYS2–DXS3–DXS94–telemere. The genetic distances, calculated as in the text, were based on the following recombination frequencies: DXYS1–.04–DXYS2–.08–DXS3–.06–DXS94. Multipoint Z values correspond to location scores divided by 4.6.

(Stevenson et al. 1990). Three of these syndromes—mental retardation with clasp thumb (Willems et al. 1989), complicated spastic paraplegia (Kenwrick et al. 1986) and MASA syndrome (Winter et al. 1989)—have been linked to the same Xq28 markers. Although it is possible that these three disorders may represent allelic conditions, our linkage results indicate that AHS cannot be included in this group of X-linked spastic paraplegia syndromes.

In conclusion, the family first investigated by Allan et al. (1944) and restudied by Stevenson et al. (1990) can be clinically distinguished from other XLMR disorders exhibiting linkage to Xq21. The linkage data reported here distinguish the family from other XLMR disorders with spasticity, except for those families not yet studied with DNA markers (Davis et al. 1981; J. A. Edwards, personal communication; R. A. Gorlin, personal communication). Therefore AHS appears to be a distinctive XLMR syndrome located in Xq21. The results reported here now allow for the development of experimental strategies to isolate and characterize the gene responsible for this syndrome.

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