Further Localization of X-linked Hydrocephalus in the Chromosomal Region Xq28

Patrick J. Willems,* Lieve Vits,* Peter Raeymaekers,† Joke Beuten,* Paul Coucke,*
Jeanette J. A. Holden,‡ Christine Van Broeckhoven,† Stephen T. Warren,§ Michal Sagi,‖
David Robinson,# Nick Dennis,** Kenneth J. Friedman,†† Dorothy Magnay,§§
Stanislas Lyonnet,‖‖ Bradley N. White,## Bärbel H. Wittwer,***
Arthur S. Aylsworth,‡‡ and Sigrid Reicke†††

Departments of *Medical Genetics and †Neurogenetics, University of Antwerp–UIA, Antwerp; ‡Cytogenetics and DNA Research Laboratory,
Ongwanada, Kingston, Canada; §Department of Biochemistry and Pediatrics, Howard Hughes Medical Institute, Emory University School of
Medicine, Atlanta; ‖Department of Human Genetics, Hadassah University Hospital, Jerusalem; #Wessex Regional Genetics Laboratory, Salisbury;
**Department of Child Health, Southampton General Hospital, Southampton, England; ††Division of Molecular Pathology and ‡‡Department
of Pediatrics and the Brain and Development Research Center, University of North Carolina, Chapel Hill; §§Department of Human Genetics,
University of Newcastle-upon-Tyne, England; ‖‖INSERM Unité 12, Hôpital des Enfants Malades, Paris; ##McMaster University, Hamilton,
Canada; ***Institute for Medical Genetics, Public Hospital, Magdeburg, Germany; and †††Institut für Humangenetik der Universität des Saarlandes,
Homburg, Germany

Summary

X-linked hydrocephalus (HSAS) is the most frequent genetic form of hydrocephalus. Clinical symptoms of HSAS include hydrocephalus, mental retardation, clasped thumbs, and spastic paraparesis. Recently we have assigned the HSAS gene to Xq28 by linkage analysis. In the present study we used a panel of 18 Xq27-q28 marker loci to further localize the HSAS gene in 13 HSAS families of different ethnic origins. Among the Xq27-q28 marker loci used, DXS52, DXS15, and F8C gave the highest combined lod scores, of 14.64, 6.53 and 6.33, respectively, at recombination fractions of .04, 0, and .05, respectively. Multipoint linkage analysis localizes the HSAS gene in the telomeric part of the Xq28 region, with a maximal lod score of 20.91 at 0.5 cM distal to DXS52. Several recombinations between the HSAS gene and the Xq28 markers DXS455, DXS304, DXS305, and DXS52 confirm that the HSAS locus is distal to DXS52. One crossover between HSAS and F8C suggests the HSAS gene to be proximal to F8C. Therefore, data from multipoint linkage analysis and the localization of key crossovers indicate that the HSAS gene is most likely located between DXS52 and F8C. This high-resolution genetic mapping places the HSAS locus within a region of <2 Mb in length, which is now amenable to positional cloning.

Introduction

X-linked hydrocephalus (HSAS) is an X-linked recessive condition (McKusick 30700; [McKusick 1988, pp. 1313–1314]) characterized by hydrocephalus and stenosis of the aqueduct of Sylvius, mental retardation, clasped thumbs, and spastic paraparesis (Bickers and Adams 1949; Edwards et al. 1961; Fried 1972;

Renier et al. 1982; Willems et al. 1987). HSAS is the most frequent genetic form of hydrocephalus, accounting for up to 25% of male cases not associated with meningocoele (Burton 1979).

The basic biochemical defect responsible for HSAS is still unknown, and the genes encoding HSAS or any other form of hydrocephalus have not yet been isolated. Until recently, prenatal diagnosis depended on the ultrasonographic detection of hydrocephalus, but reliable exclusion of HSAS cannot be achieved, as hydrocephalus may be absent antenatally (Rogers and Danks 1983). Furthermore, carrier assessment was not possible, since obligate carriers are asymptomatic.
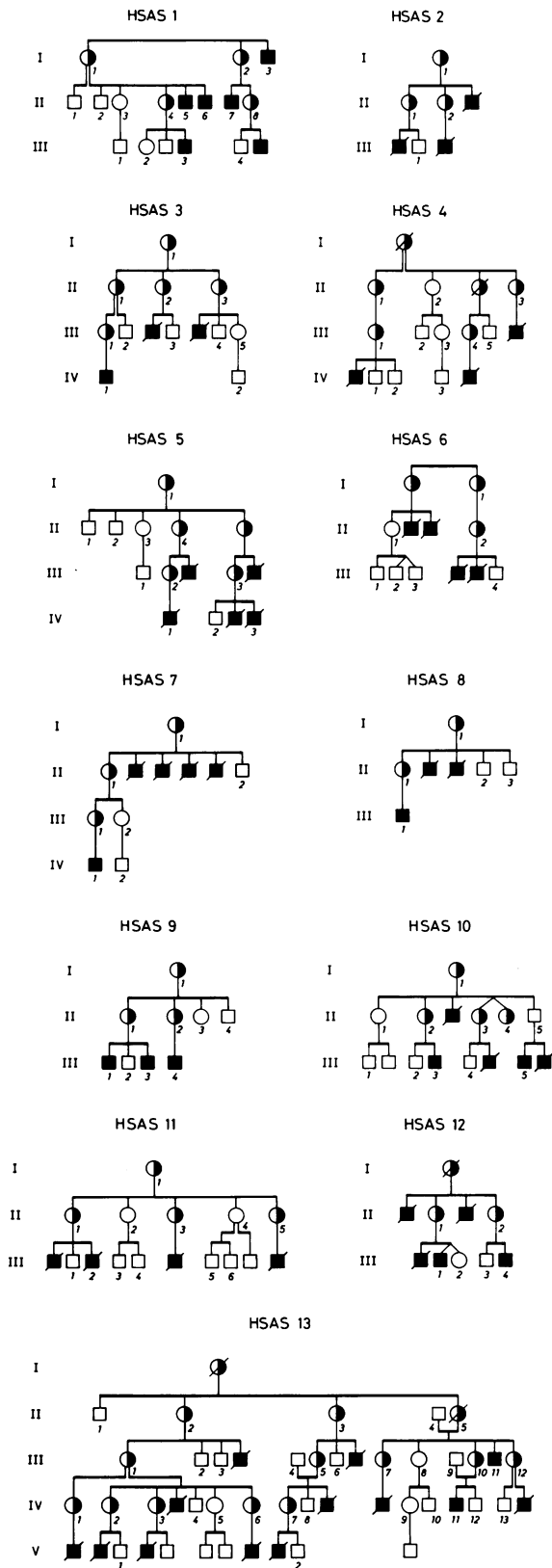
To provide a molecular genetic approach to carrier detection and prenatal diagnosis, we have previously conducted linkage analysis of HSAS to marker loci dispersed over the X chromosome. This resulted in the localization of the HSAS gene to chromosome Xq28 (Willems et al. 1989, 1990), which was confirmed recently in additional HSAS families (Holden et al. 1990; Lyonnet et al. 1991; Serville et al., in press).

In the present report we extended our linkage study to a total of 13 HSAS families originating from different parts of the world, to address the question of genetic heterogeneity of HSAS. Furthermore, we used a panel of 18 Xq27-q28 marker loci to perform multipoint linkage analysis and to identify key crossover events, as a step toward positional cloning of the HSAS gene.

Patients and Methods

Patients

Linkage analysis was carried out in 13 HSAS families of different ethnic origin (fig. 1 and table 1). Criteria for inclusion in this study were the presence of at least two male patients with hydrocephalus and mental retardation in different generations consistent with X-linked inheritance. Although we identified several additional families with male sibs affected with hydrocephalus and mental retardation, these families were not included in this study, as they may represent the autosomal recessive form of hydrocephalus (Teebi and Naguib 1988; Willems 1988). In family 5, two pregnancies were terminated in view of fetal hydrocephalus detected by serial prenatal ultrasound, and DNA was extracted from abortus material. DNA analysis was performed on 20 affected patients, 50 obligate carriers, and 34 unaffected sons of obligate carriers. Linkage of the X-linked hydrocephalus gene to Xq28 markers has been partly reported elsewhere for families 1–4 (Willems et al. 1989, 1990), families 8 and 10 (Holden et al. 1990), and family 13 (Lyonnet et al. 1991; Serville et al., in press).

RFLP Analysis

Southern blot analysis of DNA extracted from peripheral blood, Epstein-Barr–transformed lymphoblastoid cells, or, in the case of terminated pregnan-

Figure I   Pedigrees of the families with HSAS. Affected males (■) and obligate heterozygotes (◑) are indicated. The individuals available for DNA analysis are marked with Arabic numerals.

Table I

**Families Affected with X-linked Hydrocephalus
That Are Included in the Present Linkage Study**

Pedigree	Country of Origin	Reference(s)
HSAS 1	The Netherlands	Willems et al. 1987
HSAS 2	United Kingdom	Cassie and Boon 1977
HSAS 3	United States	
HSAS 4	The Netherlands	Renier et al. 1982
HSAS 5	Israel	
HSAS 6	United Kingdom	Edwards et al. 1961
HSAS 7	United States	Friedman et al. 1991
HSAS 8	United States	Holden et al. 1990
HSAS 9	United States	
HSAS 10 ...	United States	Holden et al. 1990
HSAS 11 ...	Germany	
HSAS 12 ...	Germany	
HSAS 13 ...	France	Lyonnet et al. 1991; Serville et al., in press

cies, abortion material was performed according to standard procedures. Twenty-one different probes from 18 loci localized to chromosome Xq27-q28 were used in this study: cX55.7, cX33.2, 4D8, RN1, VK21C, 346.8, U6.2, U6.2-20E, St35.691, DK9.5, F814 (DXS52), MN12, cpX67, DX13, HK13, Fr5, G6PD, F814 (F8C), probe C (F8C), 482.6 (F8C), and 767 (fig. 2 and table 1). RFLP analysis was performed in the laboratories of K.J.F. (HSAS family 7), J.J.A.H. (HSAS families 8–10), S.L. (HSAS family 13), and P.J.W. (HSAS families 1–6 and 11–12).
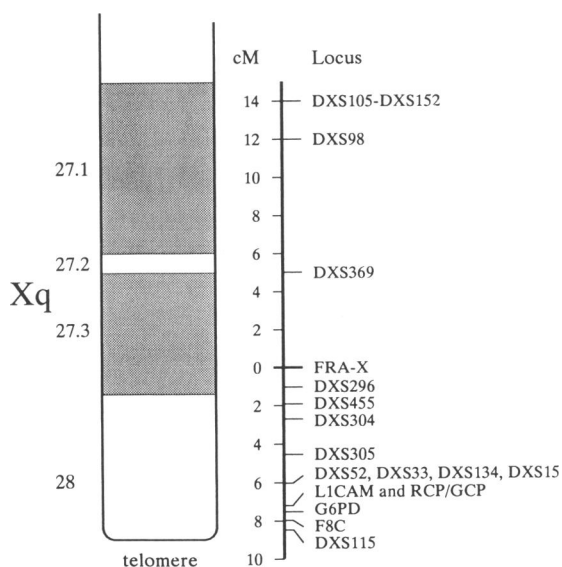

Figure 2   Map of chromosome Xq27-q28 region. The position of the marker loci used in this study is discussed in Results.

Linkage Analysis

Two-point and multipoint linkage analyses were performed using the computer programs MLINK and LINKMAP from the computer package LINKAGE version 5.1 (Lathrop and Lalouel 1984). A disease frequency of .0001 was assumed for HSAS. Males younger than 5 years old whose clinical signs were not sufficient to support the diagnosis HSAS were not included, as mental retardation can be the only clinical abnormality in HSAS (Willems et al. 1987). If IQ tests or computed-tomography (CT) scan of the brain were not performed, penetrance was considered to be 90% in healthy at risk males, since the presence of hydrocephalus (Willems et al. 1987) and mental retardation (Opitz et al. 1965) can be minimal. If these tests were performed, penetrance was considered to be complete.

Results

Polymorphic Markers

Twenty-one polymorphic probes from 18 marker loci from the chromosomal region Xq27-q28, including three new polymorphic markers, were used in this linkage study (table 2). Their locations and relative positions are shown in figure 2. By use of pHK13, a HindIII/EcoRI fragment derived from the neural adhesion molecule L1CAM (Djabali et al. 1990), an MspI RFLP was identified in the L1CAM locus (Willems et al. 1991). MspI detects a two-allele polymorphism with fragment length of either 2.5 kb (allele 1) or 2.0 kb (allele 2) and with constant bands of 1.9 and 1.4 kb (fig. 3). Gene frequencies, as studied in 154 unrelated Caucasian alleles, were .95 for allele 1 and .05 for allele 2. A BclI RFLP in the glucose-6-phosphate dehydrogenase (G6PD) gene was also studied. This RFLP was previously reported in a small set of nonrandomly selected Middle Eastern subjects (Kurdi-Haidar et al. 1990). As G6PD RFLPs are known to be confined to certain populations (Fey et al. 1990), we studied a larger group of 128 randomly selected Caucasians, which showed frequencies of .80 (allele 1) and .20 (allele 2). The G6PD RFLP can be detected by PCR with previously published PCR primers (Kurdi-Haidar et al. 1990). After BclI digestion of amplified fragments and subsequent PAGE, two allelic fragments—one of 203 bp (allele 1) and one of 180 bp (allele 2)—were detected (fig. 3) (Willems and Vits, in press). Fr5 detects fragments of both the red-color-pigment (RCP) and green-color-pigment (GCP) genes, and a three-allelic RsaI RFLP has been reported (Feil

Table 2

Combined Lod Scores for Two-Point Linkage between the X-linked Hydrocephalus Gene and Xq27-q28 DNA Markers

| MARKER (Locus)	LOCALIZATION	LOD SCORE AT θ OF							θ_{max}	Z_{max}
		.00	.01	.05	.10	.20	.30	.40		
cX55.7 (DXS105)	Xq27.1-q27.2	− ∞	− 5.46	− 2.84	− 1.73	− .75	− .31	− .10	...	...
cX33.2 (DXS152)	Xq27.1-q27.2	− ∞	− 4.54	− 2.41	− 1.50	− .67	− .27	− .08	...	...
4D8 (DXS98)	Xq27.1-q27.2	− .43	− .40	− .30	− .21	− .10	− .04	− .01	...	...
RN1 (DXS369)	Xq27.2-q27.3	− ∞	− 1.16	.86	1.41	1.38	.89	.33	.14	1.50
VK21C (DXS296)	Xq27.3-q28	− ∞	.29	1.80	2.17	1.99	1.38	.62	.12	2.20
346.8 (DXS455)	Xq28	− ∞	− 1.24	.82	1.45	1.55	1.14	.53	.16	1.61
U6.2/U6.2-20E (DXS304) ...	Xq28	− ∞	− .63	.71	1.16	1.30	1.05	.59	.18	1.31
St35.691 (DXS305)	Xq28	− ∞	2.42	3.80	4.00	3.45	2.42	1.20	.09	4.00
DK9.5 (DXS523)	Xq28	− ∞	− .07	.54	.70	.67	.49	.25	.13	.72
F814 (DXS52)	Xq28	− ∞	13.91	14.60	13.86	11.23	7.83	3.94	.04	14.64
MN12 (DXS33)	Xq28	− 1.14	− .81	− .35	− .14	.03	.08	.06	.32	.08
cpX67 (DXS134)	Xq28	4.83	4.73	4.32	3.78	2.66	1.53	.54	0	4.83
DX13 (DXS15)	Xq28	6.53	6.40	5.86	5.13	3.59	2.06	.77	0	6.53
HK13 (L1CAM)	Xq28	1.38	1.36	1.26	1.13	.86	.56	.26	0	1.38
Fr5 (GCP/RCP)	Xq28	1.09	1.07	.98	.87	.63	.39	.16	0	1.09
PCR primers (G6PD)	Xq28	1.69	1.68	1.62	1.50	1.17	.79	.39	0	1.69
F814/482.6/probeC (F8C) ...	Xq28	− ∞	6.19	6.33	5.89	4.58	3.04	1.44	.05	6.33
767 (DXS115)	Xq28	.47	.47	.44	.40	.30	.20	.10	0	.47
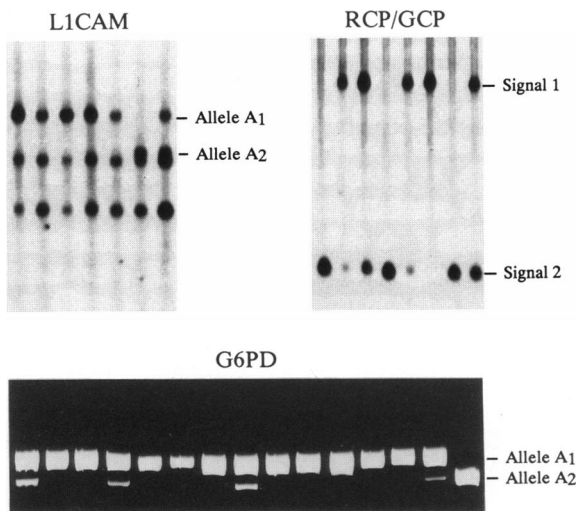
Figure 3   *Top left panel, MspI* RFLP on a Southern blot hybridized with pHK13 (L1CAM), showing two alleles—one of 2.5 kb and one of 2.0 kb—and constant fragments of 1.9 and 1.4 kb. *Top right panel,* Fr5 (RCP/GCP), showing fragments of 5 and 2 kb. Allele A shows fragment 1 (5 kb) only, allele C shows fragment 2 (2 kb) only, and allele B shows fragment 1 (strong signal) and fragment 2 (weak signal). *Bottom panel, BclI* digestion of PCR-amplified fragments of the G6PD gene, showing PCR fragments of 203 and 180 bp on PAGE.

et al. 1990). This RFLP is difficult to type in females, as different genotypes give very similar signals on Southern blots. Using Fr5, we identified a *BglII* RFLP consisting of two fragments—one of 5 kb and one of 2 kb—defining three alleles (A = 5 kb; B = 5 + 2 kb, and C = 2 kb). This *BglII* RFLP probably corresponds to the *RsaI* RFLP reported elsewhere (Feil et al. 1990), and genotypes are not really easier to type (fig. 3).

Linkage Analysis

Thirteen families segregating HSAS are shown in figure 1. In previous studies of families 1–4 (Willems et al. 1989, 1990), families 8 and 10 (Holden et al. 1990), and family 13 (Lyonnet et al. 1991; Serville et al., in press), the HSAS gene was mapped to Xq28 by using the three polymorphic Xq28 marker loci DXS52, DXS15, and F8C. In the present study we examined six additional HSAS families from different parts of the world and expanded the number of Xq27-q28 marker loci used in the linkage analysis to 18. Table 2 presents two-point lod scores for linkage between these markers and the HSAS gene. Tight linkage was found between HSAS and Xq28 markers. The peak two-point lod scores for linkage with HSAS were

obtained with DXS52 (Z_{max} = 14.64 at θ = .04), DXS15 (Z_{max} = 6.53 at θ = 0) and F8C (Z_{max} = 6.33 at θ = .05) (table 2).

To refine the position of the HSAS gene in the Xq27-q28 region we performed two consecutive multipoint linkage analyses. Analysis A included marker loci DXS369, DXS455, DXS304, and DXS52 (fig. 4, *left panel*), whereas analysis B included DXS52, DXS15, and F8C (fig. 4, *right panel*). The relative positions of the different Xq27 markers, as shown in figure 2, was deduced from genetic linkage studies in families with the fragile-X syndrome (Keats et al. 1989). The distances and relative positions of the different Xq28 markers were based on the physical map of the Xq28 region as reported by Poustka et al. (1991). Although DXS52 and DXS15 are separated by less than 100 kb, genetic analysis of five recombinational events between DXS52 and DXS15 suggests a position of DXS52 proximal to DXS15 (Veenema et al. 1987; Brown et al. 1988; Bell et al. 1989). Analysis of the recombinational events between DXS15 and DXS52 in HSAS families 3 and 8 adds more evidence for the distal position of DXS15 relative to DXS52 (fig. 5). In family 8 the recombinational event in which DXS52 cosegregated with DXS33 and in which DXS134 cosegregated with DXS15 is compatible with the order DXS52(VNTR)–DXS33-DXS134–DXS15. Therefore, the relative order and recombination fractions between adjacent loci as used in our multipoint linkage analysis are DXS369–(7.0 cM)–DXS455–

Figure 4 Multipoint linkage analysis of the HSAS gene with Xq27-q28 markers. The relative position of the marker loci was as shown in fig. 2. Two different multipoint analyses, A (*left panel*) and B (*right panel*), were performed. Analysis A included marker loci DXS369, DXS455, DXS304, and DXS52, whereas analysis B included DXS52, DXS15, and F8C.

(0.5 cM)–DXS304–(3.5 cM)–DXS52, in the left panel of figure 4, and DXS52–(0.5 cM)–DXS15–(1.5 cM)–F8C, in the right panel of figure 4. In the analysis represented in the left panel of figure 4, a Z_{max} of 15.62 was observed 4 cM distal to DXS52. Furthermore, the HSAS gene can be excluded with odds of 1.2 × 10^5:1, 1.4 × 10^7:1, 3.3 × 10^9:1, and 1,318:1 for the regions proximal to DXS369, between DXS369 and DXS455, between DXS455 and DXS304, and between DXS304 and DXS52, respectively. The second analysis (fig. 4, *right panel*) included three markers from the distal Xq28 region. The most likely position of the HSAS gene is between DXS52 and F8C at DXS15, with a Z_{max} of 20.96. The regions proximal to DXS52 and distal to F8C are excluded with odds of 1,479:1 and 79:1, respectively. Although the relative distance between the loci used in the multipoint linkage analysis is not yet completely certain and relies greatly on the physical mapping data of Poustka et al. (1991), this does not weaken the conclusion that the HSAS gene most likely is located between DXS52 and F8C. This position of the HSAS gene is further confirmed by analysis of the recombinational events (fig. 5). Several crossovers between HSAS and Xq28 marker loci were observed in the different families, when family 12, which does not show evidence for linkage to Xq28, is not taken into account. Three recombinations between the HSAS gene and DXS52 (fig. 5, cols. 3A, 8, and 13A) suggest a position of the HSAS gene distal to DXS52, whereas one crossover between HSAS and F8C (fig. 5, col. 3B) suggests a position of HSAS proximal to F8C. None of these meioses showed a recombination with DXS15. Therefore, the most probable location of the HSAS gene is between DXS52 and F8C. Furthermore, the recombination in family 8 occurred also with DXS33. If the order Xcen–DXS52(VNTR)–DXS33–DXS15–F8C is accepted, then the HSAS gene must be located in the DXS33–F8C region, which is 1–2 cM (Poustka et al. 1991).

Twelve of the 13 families showed consistent positive lod scores in two-point and multipoint linkage analysis with the Xq28 markers. In family 12, lod scores with Xq28 markers were always negative, because of the segregation of the same Xq28 haplotype from an obligate carrier (II-2) to her affected son (III-4) and his healthy brother (III-3). This haplotype was also shared by their affected cousin (III-1), indicating that the unaffected individual (III-3) was recombinant. This individual showed no clinical signs of HSAS but refused further IQ testing or CT scanning of the brain. To test
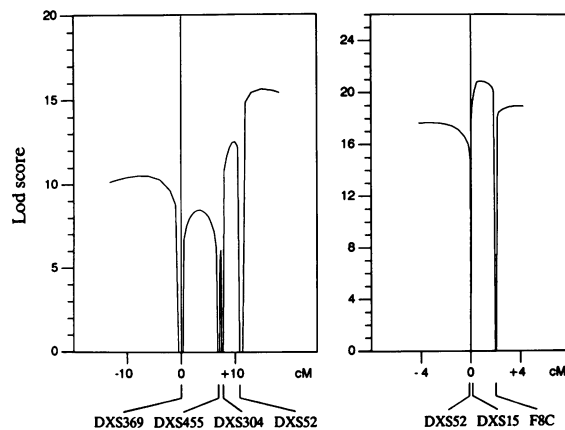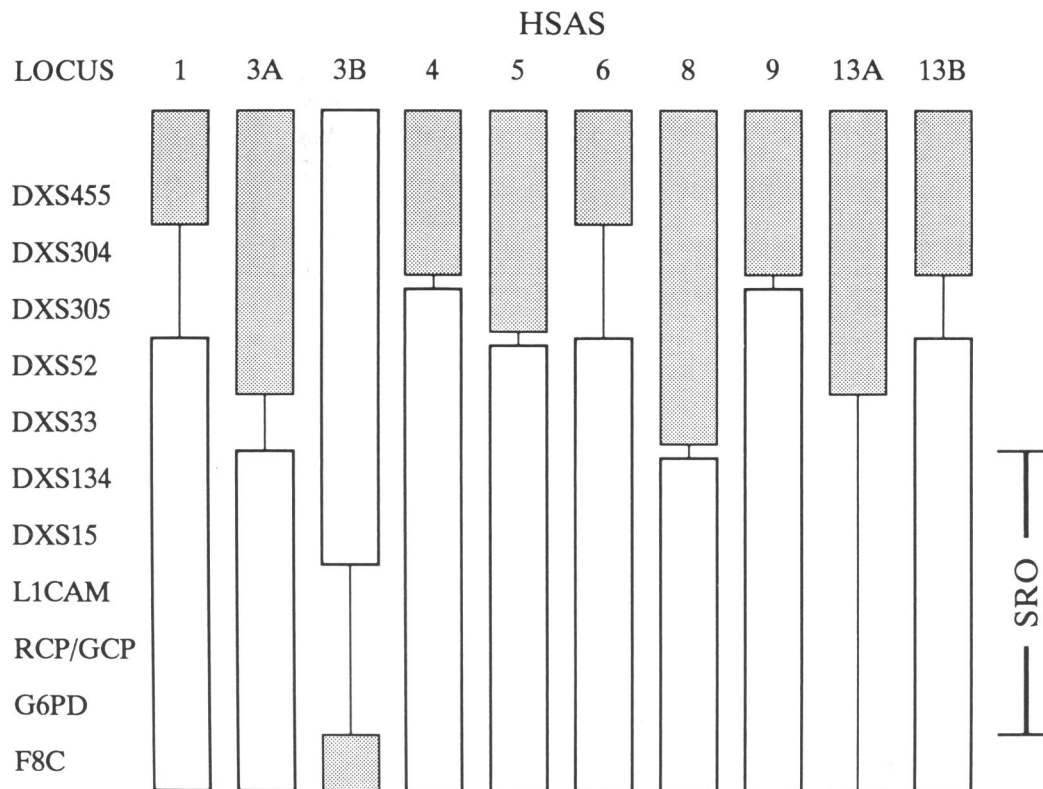
Figure 5   Recombinational events between HSAS and Xq28 marker loci in different HSAS families. Shaded bars indicate recombinant regions, single lines indicate noninformative segments, and unshaded bars indicate nonrecombinant regions.

the hypothesis of genetic heterogeneity versus homogeneity, the multipoint linkage analysis from the right panel of figure 4 was recalculated for each family separately and was used in the computer program HOMOG (Ott 1985). When, as in the right panel of figure 4, a penetrance for at-risk males who did not have IQ testing or brain CT scanning was set at 90%, no suggestion of genetic heterogeneity was observed. However, if penetrance was set at 100%, significant heterogeneity ($\chi^2 = 5.93$; $P = .0074$) and a Z_{max} of 21.05 with DXS15, with 92% of the HSAS families linked, were found.

Discussion

This study improves the assignment, in Xq28, of the gene responsible for X-linked hydrocephalus (Willems et al. 1989, 1990). The question of genetic heterogeneity could not unequivocally be resolved. Only family 12 shows negative lod scores, because of a recombination, with all informative Xq28 markers, in

an unaffected individual. There are several possible explanations for these results. First, a double crossover between the HSAS gene and the Xq28 markers might have occurred. However, this is highly unlikely, because of the small genetic distances between the analyzed markers. Second, the clinical diagnosis in this individual might be incorrect. This presumably normal individual might still have inherited the disease gene as hydrocephalus (Willems et al. 1987), and mental retardation (Opitz et al. 1965) might be minimal; and neither CT scan of the brain nor IQ testing were performed. Third, HSAS might be genetically heterogeneous and thus, in family 12, unlinked to Xq28. However, it seems clear that, in the large majority of studied families, HSAS is linked to Xq28, allowing for reliable carrier detection and diagnosis of HSAS by DNA analysis in multiple affected and Xq28-linked families.

Multipoint linkage analysis and analysis of recombinational events map the HSAS gene to the telomeric portion of Xq28, between DXS52 and F8C, with lod

scores >20. The genetic distance between DXS52 and F8C has been a topic of discussion, as these polymorphic markers have been used extensively in carrier detection and prenatal diagnosis of various genetic disorders located in Xq27-q28. The most probable distance between DXS52 and F8C is 1–2 cM, on the basis of genetic (Mulligan et al. 1987) and physical studies (Kenwrick and Gitschier 1989; Poustka et al. 1991). The localization of the HSAS gene within this relatively small region by genetic studies such as reported here is an important step toward the cloning of the HSAS gene. The Xq28 region between DXS52 and F8C contains several expressed genes, such as the G6PD gene, the RCP gene, the GCP gene, and the neural adhesion molecule (L1CAM) gene. The L1CAM gene is of particular interest, as it codes for a neural adhesion molecule involved in adhesion, migration, and outgrowth of neuronal cells (Moos et al. 1988), making it a candidate gene for HSAS. However, Southern blot analysis and pulsed-field analysis of genomic DNA of six unrelated HSAS patients has not yet revealed major gene rearrangements in the L1CAM gene. We also searched for RFLPs in the L1CAM gene and report here an MspI RFLP. Although the PIC value of this RFLP is low, it might be useful in refined localization of Xq28 disease genes.

The boundaries of the HSAS region currently rely solely on linkage data, as large-scale chromosomal rearrangements made visible by cytogenetic techniques or pulsed-field mapping have not yet been discovered. This is in line with the almost total absence of reported visible deletions of Xq28, suggesting that these may be lethal mutations. Nevertheless, cloning of the HSAS gene by positional cloning is feasible now, as the candidate region is only 1–2 Mb, and a large set of cosmid and YAC contigs are available from A. Poustka and D. Schlessinger.

The chromosomal band Xq28 contains many disease genes, including those for adrenoleukodystrophy, myotubular myopathy, X-linked cardioskeletal myopathy (Barth syndrome), Emery-Dreifuss muscular dystrophy, incontinentia pigmenti, hemophilia A, Hunter syndrome, dyskeratosis congenita, red/green colorblindness, nephrogenic diabetes insipidus, X-linked spastic paraparesis, G6PD deficiency, and MASA (mental retardation, aphasia, shuffling gait, and adducted thumbs) syndrome. We have previously suggested that the MASA syndrome (Gareis and Mason 1984; Yeatman 1984; Kenwrick et al. 1986; Winter et al. 1989; Schrander-Stumpel et al. 1990; Fryns et al. 1991) might be due to an allelic variation of the

HSAS disease gene (Willems et al. 1989, 1990). This is based upon (a) the presence of macrocephaly (Winter et al. 1989) and hydrocephaly (Schrander-Stumpel et al. 1990; Fryns et al. 1991) in MASA families and (b) the absence of hydrocephalus in some mentally retarded members of families affected with X-linked hydrocephalus (Willems et al. 1987). Although the localization of MASA syndrome to Xq28 has been established firmly now, with combined lod scores for linkage with DXS52 that are >15 at $\theta = 0$, a detailed regional localization of the MASA gene has not yet been reported, because of the paucity of recombinations between this disease and Xq28 marker loci (Kenwrick et al. 1986; Winter et al. 1989; Schrander-Stumpel et al. 1990; Fryns et al. 1991; Legius et al. 1991; Rietschel et al. 1991). Only one recombinant between MASA and an Xq28 marker (F8C) has been reported, localizing the MASA gene proximal to F8C (Schrander-Stumpel et al. 1990). Although both diseases map in Xq28, the possibility that MASA syndrome and X-linked hydrocephalus are allelic variants will remain unresolved until the identification of the responsible gene(s) and mutation(s) in both diseases.

Acknowledgments

References

Bell MV, Patterson MN, Dorkins HR, Davies KE (1989) Physical mapping of DXS134 close to the DXS52 locus. Hum Genet 82:27–30

Bickers DS, Adams RD (1949) Hereditary stenosis of the aqueduct of Sylvius as a cause of congenital hydrocephalus. Brain 72:246–262

Brown WT, Gross A, Chan C, Jenkins EC, Mandel JL, Oberle I, Arveiler B, et al (1988) Multilocus analysis of the fragile X syndrome. Hum Genet 78:201–205

Burton BK (1979) Recurrence risk for congenital hydrocephalus. Clin Genet 16:47–53

Cassie R, Boon AR (1977) Sex-linked hydrocephalus. J Med Genet 14:72–73

Djabali M, Mattei M-G, Nguyen C, Roux D, Demengeot J, Denizot F, Moos M (1990) The gene encoding L1, a neural adhesion molecule of the immunoglobulin family, is located on the X chromosome in mouse and man. Genomics 7:587–593

Edwards JH, Norman RM, Roberts JM (1961) Sex-linked hydrocephalus: report of a family with 15 affected members. Arch Dis Child 36:481–485

Feil R, Heilig R, Mandel JL (1990) A 195-kb cosmid walk encompassing the human Xq28 color vision pigment genes. Genomics 6:367–373

Fey MF, Wainscoat JM, Mukwala EC, Falusi AG, Vulliamy TJ, Luzzatto L (1990) A PvuII restriction fragment length gene is an African-specific marker. Hum Genet 84:471–472

Fried K (1972) X-linked mental retardation and/or hydrocephalus. Clin Genet 3:258–263

Friedman KJ, Hostetler D, Highsmith WE Jr, Kepten E, Silverman LM, Aylsworth AS (1991) Linkage studies in a four generation family with X-linked hydrocephalus associated with flexed index fingers. Proceedings of the 23d annual March of Dimes Clinical Genetics conference, Vancouver, July 7–10

Fryns JP, Spaepen A, Cassiman JJ, Van den Berghe H (1991) X-linked complicated spastic paraplegia, MASA syndrome and X-linked hydrocephaly due to congenital stenosis of the aqueduct of Sylvius: a variable expression of the same mutation at Xq28. J Med Genet 28:429–431

Gareis FJ, Mason JD (1984) X-linked mental retardation associated with bilateral clasp thumb anomaly. Am J Med Genet 17:333–338

Holden JJA, Ridgway P, White BN, Young JC, Chalifoux M, DiTullio K, Wiens R, et al (1990) Further evidence localizing the X-linked hydrocephalus locus to Xq28. Am J Hum Genet 47 [Suppl]: A183

Keats B, Ott J, Conneally M (1989) Report of the Committee on Linkage and Gene Order. Cytogenet Cell Genet 51: 459–502

Kenwrick S, Gitschier J (1989) A contiguous, 3-Mb physical map of Xq28 extending from the colorblindness locus to DXS15. Am J Hum Genet 45:873–882

Kenwrick S, Ionascescu V, Ionascescu G, Searby C, King A, Dubowitz M, Davies KE (1986) Linkage studies of X-linked recessive spastic paraplegia using DNA studies. Hum Genet 73:264–266

Kurdi-Haidar B, Mason PJ, Berrebi A, Ankra-Badu G, Al-Ali A, Oppenheim A, Luzzatto L (1990) Origin and spread of the glucose-6-phosphate dehydrogenase variant (G6PD-Mediterranean) in the Middle East. Am J Hum Genet 47:1013–1019

Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. Am J Hum Genet 36:460-465

Legius E, Higgins JV, Kaepernick L, Cassiman JJ, Fryns

JP, Glover TW (1991) Fine mapping of X-linked clasped thumb and mental retardation (MASA syndrome) in Xq28. Am J Hum Genet 49 [Suppl]: A1959

Lyonnet S, Pelet A, Briard ML, Serville F, Le Marec B, Pfeiffer RA, Hors MC, et al (1991) Further linkage data at the X-linked hydrocephalus locus with seven polymorphic DNA markers of Xq28. Am J Hum Genet 49 [Suppl]: A1959

McKusick VA (1988) Mendelian inheritance in man: catalogs of autosomal dominant, autosomal recessive, and X-linked phenotypes. The Johns Hopkins University Press, Baltimore

Moos M, Tacke R, Scherer H, Teplow D, Früh K, Schachner M (1988) Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature 334:701–703

Mulligan LM, Grover HJ, Blanchette VS, Giles AR, Lillicrap DL, Phillips A, Holden JJA, et al (1987) Recombination between the factor VIII gene and the DXS52 locus gives the most probable genetic order as centromere-Fra(X)-DXS15-DXS52-F8C-telomere. Am J Med Genet 26:751–760

Opitz JM, Segal AT, Klove H, Mathews CH, Lehrke RC (1965) X-linked mental retardation: study of a large kindred with 20 affected members. J Pediatr 67:713–714

Ott J (1985) Analysis of human genetic linkage. The Johns Hopkins University Press, Baltimore

Poustka A, Dietrich A, Langenstein G, Toniolo D, Warren ST, Lehrach H (1991) Physical map of human Xq27-qter: localizing the region of the fragile X mutation. Proc Natl Acad Sci USA 88:8302–8306

Renier WO, Ter Haar BGA, Slooff JL, Hustinx TWJ, Gabreëls FJM (1982) X-linked congenital hydrocephalus. Clin Neurol Neurosurg 84:113–123

Rietschel M, Friedl W, Uhlhaas S, Neugebauer M, Heimann D, Zerres K (1991) MASA syndrome: clinical variability and linkage analysis. Am J Med Genet 41:10–14

Rogers JG, Danks DM (1983) Prenatal diagnosis of sex-linked hydrocephalus. Prenat Diagn 3:269–270

Schrander-Stumpel C, Legius E, Fryns JP, Cassiman JJ (1990) MASA syndrome: new clinical features and linkage analysis using DNA probes. J Med Genet 27:688–692

Serville F, Lyonnet S, Pelet A, Reynaud M, Louail C, Munnich A, Le Merrer M. X-linked hydrocephalus: clinical heterogeneity at a single gene locus. Eur J Pediatr (in press)

Teebi AS, Naguib KK (1988) Autosomal recessive nonsyndromal hydrocephalus. Am J Med Genet 31:467–470

Veenema H, Carpenter NJ, Bakker E, Hofker MH, Ward AM, Pearson PL (1987) The fragile X syndrome in a large family. III. Investigations on linkage of flanking DNA markers with the fragile site Xq27. J Med Genet 24:413–421

Willems PJ (1988) Heterogeneity in familial hydrocephalus. Am J Med Genet 31:471–472

Willems PJ, Brouwer OF, Dijkstra I, Wilmink J (1987) X-linked hydrocephalus. Am J Med Genet 27:921–928

Willems PJ, Dijkstra I, Van der Auwera BJ, Vits L, Coucke PJ, Raeymaekers P, Van Broeckhoven C, et al (1989) Assignment of X-linked hydrocephalus to Xq28. Am J Hum Genet 45 [Suppl]: A167

——— (1990) Assignment of X-linked hydrocephalus to Xq28 by linkage analysis. Genomics 8:367–370

Willems PJ, Vits L. PCR detection of a BclI RFLP in the G6PD gene of Caucasians. Hum Genet (in press)

Willems PJ, Vits L, De Boulle K (1991) MspI RFLP in the L1CAM gene in Xq28. Nucleic Acids Res 19:5448

Winter RM, Davies KE, Bell MV, Huson SM, Patterson MN (1989) MASA syndrome: further clinical delineation and chromosomal localisation. Hum Genet 82:367–370

Yeatman GW (1984) Mental retardation-clasped thumb syndrome. Am J Med Genet 17:339–344