

# Refining the genetic location of the gene for X linked hydrocephalus within Xq28

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## Abstract

**The most common inherited form of hydrocephalus, X linked hydrocephalus (HSAS), is characterised by mental retardation, adducted thumbs, and spastic paraplegia. Genetic analysis has mapped the locus for HSAS to subchromosomal band Xq28 within a region of approximately 2 megabases of DNA. In order to refine the location of the disease gene we have conducted genetic linkage analysis with Xq28 marker loci in four additional HSAS families. A lod score of 4.26 with polymorphic marker *DXS52* (St14) confirms the linkage of HSAS to Xq28. Identification of a recombination event between the HSAS gene and Xq28 loci *F8C* and *DXS605* (2-19) reduces the size of the interval likely to contain the disease locus to about 1.5 megabases, the distance between *DXS605* and *DXS52*. The locus for neural cell adhesion molecule, *L1CAM*, maps within this interval and therefore represents a candidate gene for HSAS.**

(*J Med Genet* 1993;30:214-17)

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Received 27 August 1992.  
Revised version accepted  
29 September 1992.

X linked recessive hydrocephalus is the most common genetic form of congenital hydrocephalus, occurring in approximately 1/30 000 male births (McKusick 30700<sup>1</sup>). The primary diagnostic features of mental retardation and enlarged cerebral ventricles are often accompanied by adducted thumbs and spastic paraplegia.<sup>2</sup> The frequent observation of aqueductal stenosis led to the acronym HSAS (X linked hydrocephalus with stenosis of the aqueduct of Sylvius) although this malformation is now considered to be secondary to the basic defect.<sup>3</sup> A variety of other cerebral malformations, including absence of the corpus callosum and defects in the septum pellucidum, have occasionally been reported in this condition. Genetic analysis has placed the locus for HSAS in subchromosomal band Xq28 close to polymorphic locus St14 (*DXS52*) and the gene for coagulation factor VIII (*F8C*).<sup>4-8</sup>

HSAS exhibits marked clinical variation within and between families and its features overlap with those of another Xq28 linked disorder, MASA syndrome (mental retardation, aphasia, spastic paraplegia, and adducted thumbs). Since genetic analysis has also placed the gene for MASA syndrome close to *DXS52* and *F8C* it has been suggested that the two disorders are caused by different mutations at the same locus.<sup>9-11</sup> Indeed hydro-

cephalus has been observed in at least one MASA syndrome family.<sup>10</sup>

Attempts to refine the location of the HSAS locus within Xq28 have relied on the analysis of rare examples of meiotic recombination between the disease locus and Xq28 markers. A recent summary of these and additional data, incorporating information from 13 HSAS families,<sup>12</sup> concluded that the relevant gene lies between *DXS52* and *F8C*, an interval of about 2 megabases (Mb) by comparison with a physical map of the region.<sup>13</sup>

In order to identify further recombinants we have conducted genetic linkage analysis with Xq28 markers in four additional HSAS pedigrees.

## Materials and methods

### FAMILIES

Families with at least two affected males in more than one sibship or generation were selected for analysis. No male to male transmission was noted and female carriers were asymptomatic.

### Family H1

Subject II.7 was born with a normal head circumference but developed massive hydrocephalus within the first few weeks of life. He died of pneumonia aged 9 months. II.5 died aged 47 and despite a normal head circumference was found to have grossly enlarged cerebral ventricles at necropsy. He was mentally retarded (IQ 45) and suffered from epilepsy and spastic diplegia, requiring crutches from 6 years of age. II.8 is now 49 years old, mentally retarded, has spastic diplegia, and walks with a shuffling gait. His head circumference is within the normal range (55.5 cm) but a brain CT scan showed grossly enlarged ventricles. III.2 had a normal head circumference at birth but developed progressive hydrocephalus requiring shunting. He is now 25 with mild spastic diplegia, mental retardation, and has suffered from epilepsy since the age of 22. DNA was not available for II.7 and only paraffin embedded tissue was available for II.5.

### Family H2

Subjects II.9, III.6, and IV.1 were stillborn with hydrocephalus but no further details are available. Fetal scanning of IV.2 at 28 weeks' gestation showed marked dilatation of the lateral and third, but not fourth, cerebral ventricles with the cortical mantle reduced to 3 mm in

depth. IV.6 was diagnosed as having hydrocephalus during labour and lived for 27 hours, a ventricular tap having been conducted before delivery. Necropsy showed gross dilatation of the lateral, third, and possibly fourth ventricles. Patient V.1 was diagnosed prenatally and terminated at 20 weeks. Necropsy confirmed enlargement of the cerebral ventricles. DNA samples were not available for II.9, III.6, and IV.1 and paraffin embedded tissue sections were the only source of DNA for IV.6.

#### Family H3

Patient IV.6 is 6 years old and was born by caesarean section at 35 weeks after prenatal diagnosis of hydrocephalus. He had a grossly enlarged head and a subsequent CT scan showed dilatation of the lateral and third ventricles with a thin cerebral mantle. A ventriculo-peritoneal (VP) shunt was fitted. He is mentally retarded, and has adducted thumbs and spastic diplegia. Patient IV.8 died at 2 weeks of age and necropsy showed hydrocephalus.

#### Family H4

Patient II.1 is now 23 years old, mentally retarded, has adducted thumbs, and walks with a shuffling gait. No scans have been performed. II.4 was born with hydrocephalus, is now 13 years of age, and has received 23 operations since birth for VP shunt maintenance. His head circumference is normal. He is mentally retarded with an unsteady gait, left sided weakness, and adducted thumbs. Patient III.1 was diagnosed prenatally with hydrocephalus and delivered by caesarean section. His head was enlarged and a CT scan showed dilatation of the lateral and third ventricles only. Choroid plexus coagulation and VP shunt insertion were performed. He had adducted thumbs and left sided weakness and died aged 10 months. Necropsy showed collapse of both cerebral hemispheres.

#### DNA POLYMORPHISMS

Blood samples were obtained with informed consent from available family members and DNA was extracted using routine methods. Where paraffin embedded tissue was the source of DNA, extraction was performed according to the following procedure: 250 mg of tissue (in 10  $\mu$ m sections) was suspended in 250  $\mu$ l extraction buffer (75 mmol/l NaCl, 25 mmol/l EDTA). This was subjected to three cycles of heating to 65°C for five minutes plus vortexing for one minute. After collection by centrifugation the sample was incubated for three days at 50°C in the presence of proteinase K (300  $\mu$ g/ml) and SDS (0.5%). Aliquots of this crude extract were used directly for polymerase chain reactions. Families were typed for DNA polymorphisms *DXS52* (St14, VNTR<sup>14</sup>), *F8C* (*BclI* restriction digest<sup>15</sup>), *DXS605* (2–19, *EcoRI*<sup>16</sup>), and *DXS707* (2–55, *MspI*<sup>16</sup>) using PCR and digestion with the appropriate restriction enzyme. The sequences of oligonucleotide primers for *DXS605* and

*DXS707* were a personal communication from Daniella Toniolo.

#### LINKAGE ANALYSIS

Data obtained by polymorphic typing were analysed using the LIPED<sup>17</sup> and LINKMAP<sup>18</sup> computer programmes for two point and multipoint analyses respectively. Confidence intervals were obtained by taking values of the recombination fraction corresponding to a lod score one unit less than the maximum.

#### Results

Linkage analysis was conducted on the HSAS families shown in fig 1 using a sample collection of 36 subjects that included 10 affected males and 11 obligate carriers. Two point lod scores for X linked hydrocephalus versus Xq28 polymorphic marker loci are given in the table. No recombinants were observed between *DXS52* and the disease locus and a lod score of 4.26 supports the Xq28 location of the HSAS gene. A single recombinant between *HSAS* and marker loci *F8C* and *DXS605* was observed in family H4 and the haplotypes of this family are shown in fig 2. The relative order of marker loci in Xq28 is derived from Poustka *et al.*<sup>13</sup> Since the disease segregates with *DXS52* in family H4 and not with *DXS605* and *F8C* these data imply that the HSAS gene is located proximal to *DXS605*. For multipoint analysis we used the marker order *DXS52*, *DXS605*, *F8C* with recombination fractions of 0.05 and 0.001 respectively taken from published linkage data<sup>19</sup> on the assumption that the genetic location of *DXS605* is the same as *G6PD*. *DXS707* was not included in this analysis as its location is uncertain. The LINKMAP analysis gave a maximum location score of 24.2 with *HSAS* coincident with *DXS52*. This position was favoured by odds of 10<sup>6</sup>:1 compared to a location between *DXS605* and *F8C*, and by odds of 80:1 compared to a location distal to *F8C*.

#### Discussion

We have conducted genetic linkage analysis on four new X linked hydrocephalus families. The affected males in our study vary markedly in their clinical presentation both within and between families, an observation that has been made for other HSAS pedigrees.<sup>2</sup> The presence of adducted thumbs in addition to spastic paraplegia and mental retardation in two families highlights the overlap of HSAS with MASA syndrome.

A lod score of 4.26 obtained using *DXS52* indicates close linkage of the disease locus in these families with Xq28 markers. Lack of linkage to Xq28 has been shown for only one out of 13 families analysed by Willems *et al.*<sup>2</sup> and absence of recombination with *DXS52* implies that the families in our study exhibit the Xq28 linked form of the disorder. There was no evidence of heterogeneity for families H1 to H4 with *DXS52* yielding lod scores of 1.25, 1.51, 0.6, and 0.9 respectively.

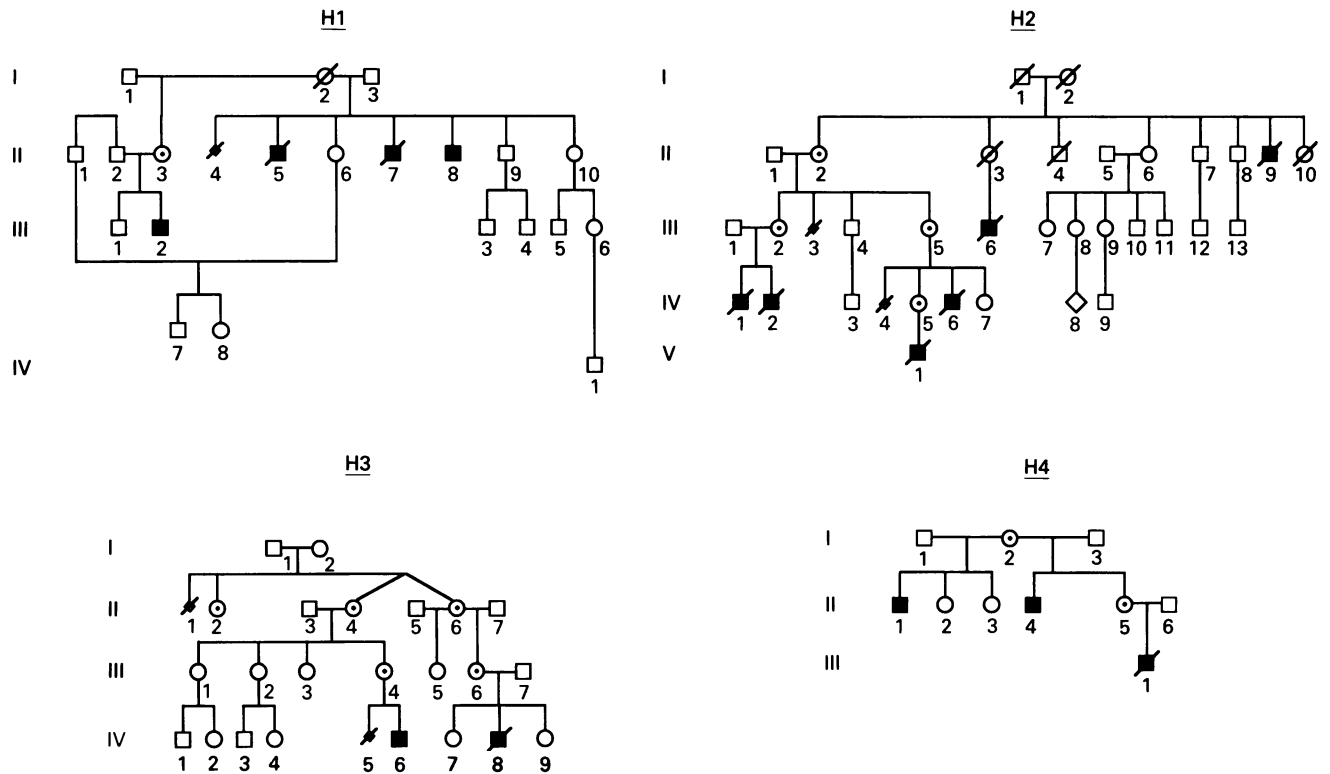


Figure 1 Pedigrees of HSAS families. Affected (dark square) and carrier (circle with central dot) subjects are indicated.

Location of the HSAS gene within Xq28. Two point lod scores between X linked hydrocephalus (HSAS) and the DNA markers DXS52 (St14), DXS707 (2-55), DXS605 (2-19), and F8C.

Locus	Lod score Z at recombination fraction $\theta$							Zmax	$\theta_{max}$	Confidence interval for $\theta$
	0	0.001	0.05	0.1	0.2	0.3	0.4			
DXS52	4.26	4.25	3.85	3.43	2.54	1.62	0.74	4.26	0.0	0.0-0.12
DXS707	1.62	1.61	1.44	1.26	0.90	0.55	0.23	1.62	0.0	
DXS605	-∞	-0.23	1.25	1.32	1.12	0.78	0.39	1.32	0.09	
F8C	-∞	-1.69	-0.10	0.09	0.18	0.15	0.08	0.18	0.21	

A single recombination event indicates that the HSAS gene lies proximal to polymorphic loci DXS605 and F8C. Polymorphic locus DXS605 is situated 20 kb downstream of the gene for glucose-6-phosphate dehydrogenase (G6PD<sup>16</sup>), approximately 1.5 Mb distal to DXS52 and 500 kb proximal to F8C. A study by Willems *et al*<sup>12</sup> indicated that the HSAS gene lies in the interval between F8C and DXS52, a distance of about 2 Mb. Our results are consistent with this location but narrow down the size of the relevant region to the distance between DXS605 and DXS52, approximately 1.5 Mb. Thus, a direct search for candidate genes should focus on this reduced interval.

The most likely candidate identified to date is the gene for neural cell adhesion molecule, L1CAM. This highly conserved, cell surface glycoprotein is involved in neural cell migration and development of the neuromuscular junction.<sup>20</sup> Since the L1CAM locus is physically located between DXS605 and DXS52<sup>21</sup> our data indicate that the L1CAM gene should be examined directly in cases of X linked hydrocephalus.

We would like to thank Tim Cox for his support and advice during this study; Michael Patton, Rob Elles, Malcolm Taylor, John Crolla, Karen Temple, Rose Buchanan, Alan Barker, and P C Reynolds for assistance in collecting clinical samples; Stephen Siggers and Daniella Toniolo for providing oligonucleotide sequences. SK and MJ are supported by the Medical Research Council Human

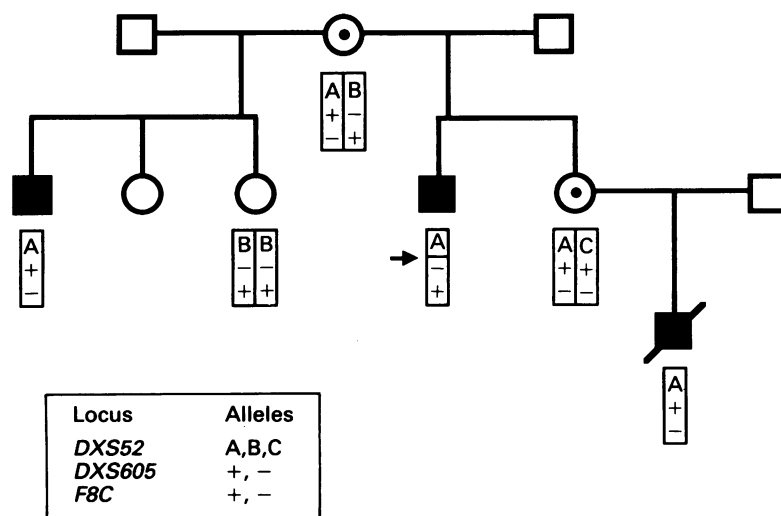


Figure 2 Xq28 haplotypes for family H4. DXS52 (St14) alleles A, B, and C represent PCR products of different sizes. For other loci + indicates presence of the appropriate restriction site and - indicates its absence. Recombination between DXS52 and more distal loci is indicated by an arrow.

Genome Programme and EF is funded by the Wellcome Trust.

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