# *Short reports*

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## A new X linked neurodegenerative syndrome with mental retardation, blindness, convulsions, spasticity, mild hypomyelination, and early death maps to the pericentromeric region

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**Abstract**

**We report on a family with an X linked neurodegenerative disorder consisting of mental retardation, blindness, convulsions, spasticity, and early death. Neuropathological examination showed mild hypomyelination. By linkage analysis, the underlying genetic defect could be assigned to the pericentromeric region of the X chromosome with a maximum lod score of 3.30 at θ=0.0 for the DXS1204 locus with DXS337 and PGK1P1 as flanking markers.**

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Keywords: XLMR; hypomyelination; early death; pericentromeric region

X linked mental retardation (XLMR) comprises a heterogeneous group of disorders with an estimated cumulative birth prevalence of 1/600 males.[1] This high frequency, as well as clinical, genetic, and recent molecular findings, indicate that there are numerous X linked genes which control the development of the central nervous system as well as its cognitive and adaptive functioning. In their recent update of XLMR genes, Lubs *et al*[2] have listed 105 disorders with mental retardation and other associated features. Many of these have neurological symptoms.[2–4] Here, we report on a family with an apparently new X linked neurodegenerative disorder with early lethality which maps to the pericentromeric region of the X chromosome.

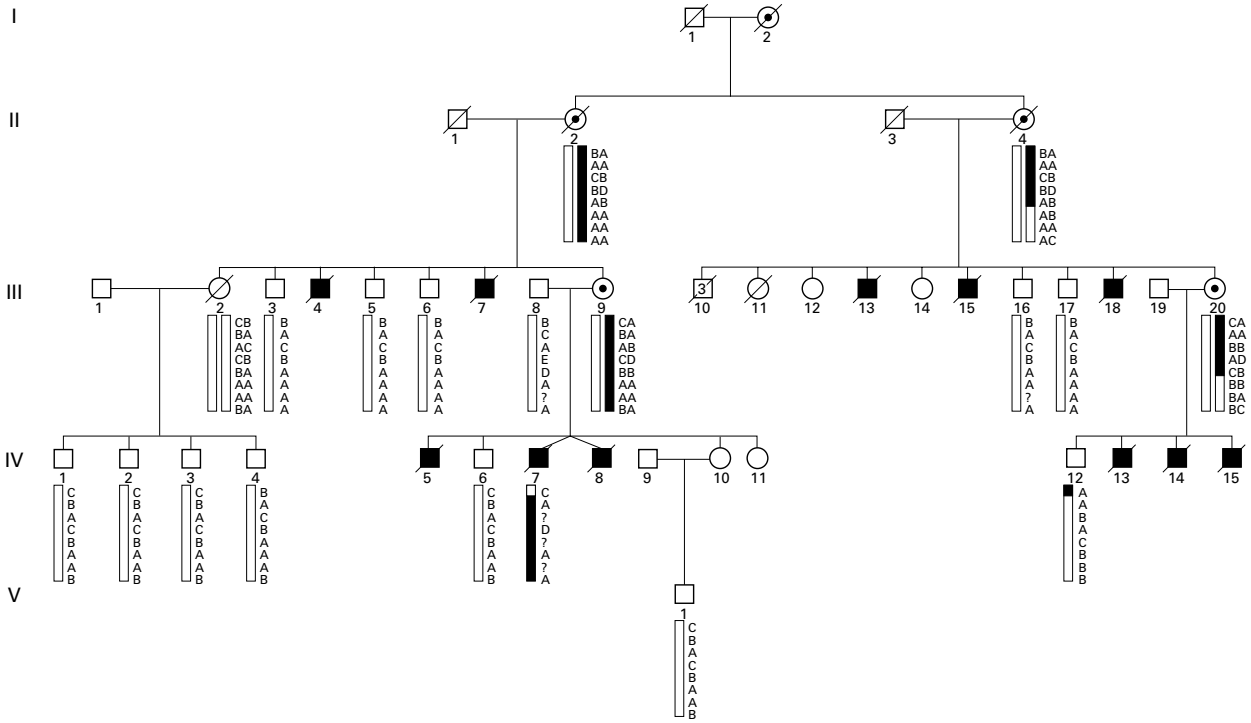


*Figure 1 Pedigree of the family. Haplotypes are shown, which have been constructed from informative markers in the pericentromeric region. Haplotypes of people in the second generation who had died were deduced (Cyrillic version 2.0). The cosegregating haplotype has been marked by a black bar.*

## Materials, methods, and results

The family (fig 1) was ascertained when IV.10 was referred for genetic counselling. At that time all patients had been dead for at least 20 years. Therefore, the clinical information had to be retrieved from limited medical records, which were only available for IV.5, IV.7, and IV.8. Necropsy had been performed on IV.7 and IV.8. The neuropathology was re-evaluated for this study.

### GENETIC ANALYSIS

From relevant members of the family venous blood was sampled and DNA was isolated according to the procedure of Miller *et al*.[5] DNA from patient IV.7 could only be isolated from a postmortem specimen. Markers were analysed by the amplification of 50 ng of genomic DNA with the appropriate primers (GDB, Isogen Bioscience BV, The Netherlands). Amplification involved 35 PCR cycles of one minute at 94°C, two minutes at 55°C, and three minutes at 72°C, which was carried out in a 15 µl reaction mixture containing 0.06 U Super*taq* in 1 × Super*taq* buffer (HT Biotechnology Ltd, England) and in the presence of [$\alpha^{32}$P]dCTP. Subsequently, labelled fragments were separated on 6.6% denaturing polyacrylamide gels. After electrophoresis, gels were exposed overnight to Kodak X-Omat S film to visualise the allelic bands. Linkage data were evaluated with the program LINKAGE[6] using the Mlink option. Calculations were based on complete penetrance and a disease allele frequency of 0.0001. Map locations, genetic distances, and allele frequencies of the marker loci were obtained from the Genome Database and from the reports of Nelson *et al*[7] and Dib *et al*.[8]

### CLINICAL REPORT

In IV.5, IV.7, and IV.8, pregnancy and delivery had been uneventful and all were born with bilateral pes calcaneovalgus. The first neurological signs were noticed at about the age of 3 months. From that age, the disorder took a progressive course with gradual loss of vision, development of spastic tetraplegia and scoliosis, convulsions, secondary microcephaly, unexplained febrile episodes, severe mental retardation, and failure to thrive. There was neither stridor nor nystagmus. Hearing was apparently normal. The zygosity status of the twins IV.7 and IV.8 is unknown.

Proband IV.5 was born at 38 weeks'gestation with a weight of 3000 g and OFC of 36 cm. Apart from bilateral pes calcaneovalgus which was treated conservatively, no other anomalies were noticed. Gradually the above mentioned signs and symptoms, bilateral flexion contractures of the proximal interphalangeal joints, and bilateral strabimus became apparant. The OFC at 12 months was 44 cm and at 21 months 45 cm. He never sat, crawled, nor had any speech. At the age of about 1 year his mother reported loss of interest in his environment and loss of some skills, like reaching for toys. He underwent several additional investigations. Ophthalmological examination showed pale fundi, the EEG showed an epileptic focus in the left frontotemporal region, and skeletal age was retarded. Unbanded chromosomes were normal. He died at the age of 29 months during a febrile episode.

IV.7 died at the age of 16½ months of aspiration pneumonia. There were no records of further investigations. Born at 36 weeks' gestation with a weight of about 2500 g his twin brother IV.8 followed the same course as the proband. He died at the age of 26 months, also from aspiration pneumonia. Ophthalmological examination showed pale fundi; the EMG was normal, and so were the unbanded chromosomes. Metabolic analysis showed no abnormalities. Lysosomal enzymes (in 1971) were normal.

Obligate and possible carriers were all functioning normally.

### PATHOLOGY REPORT

In patients IV.7 and IV.8 the brain appeared small for age (900 and 980 g, normal mean weight about 1000 and 1050 g, respectively). Only minor abnormalities were noted on macroscopic examination: relatively thin gyri and a somewhat grey colour of the white matter in IV.7, relatively delicate optic nerves and corpus callosum and slightly enlarged lateral

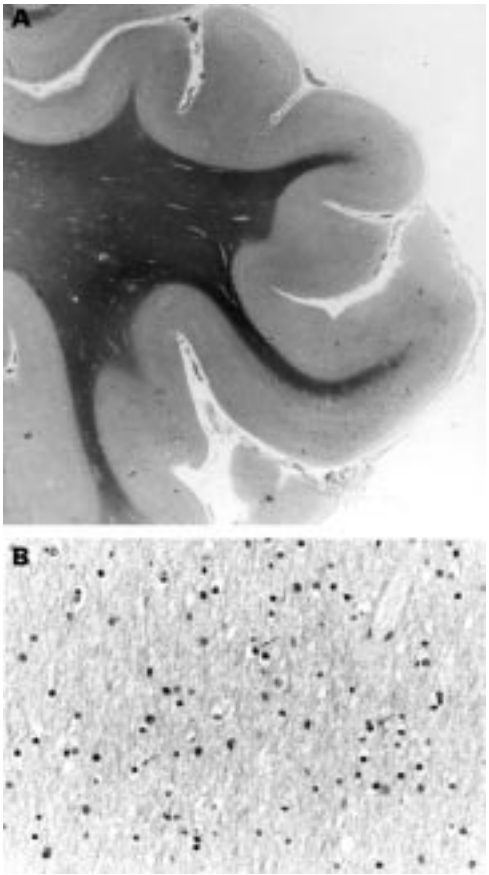


*Figure 2 (A) Histological section of the frontal cerebral cortex and white matter of patient IV.7; the white matter shows a gradual decrease in myelin staining from the subcortical to the more central area. (B) Higher magnification of central white matter shows evenly distributed oligodendroglial cells with small, round, dark nuclei (see arrows) and absence of inflammation (combined luxol fast blue and haematoxylin-eosin (LFB-HE) staining).*

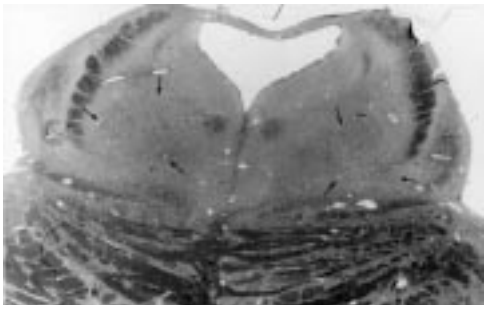

*Figure 3 (A) Histological section of the dorsal part of the pons in patient IV.7; note the symmetrical, spongy change in the area of the central segmental tract (see arrows). (B) Higher magnification of this tract shows pronounced microvacuolar change without gliosis or inflammatory infiltrate (combined LFB-HE staining).*

ventricles in IV.8. The spinal cords were not available for further examination. Microscopically, in both patients the cerebral and cerebellar white matter showed areas of mild myelin pallor with a gradual transition to the normal white matter (fig 2). The subcortical white matter was relatively spared. The myelin pallor was neither accompanied by infiltration of inflammatory cells (including macrophages) nor by a remarkable loss of oligodendroglial cells, while gliosis was mild or absent. In both patients, the central tegmental tract showed pronounced spongy changes (fig 3). A similar change was focally present in the centre of the occipital lobe and around the central nuclei. Histochemical staining for the detection of storage of metabolic products was negative. Apart from aspiration pneumonia in both patients, necropsy did not show conspicuous abnormalities in other organs. The brain pathology in the two brothers was categorised as mild hypomyelination.

*Table 1 Results of two point linkage analysis*

| | *Lod scores (θ)* | | | | | |
|---|---|---|---|---|---|---|
| *Marker* | *0.0* | *0.05* | *0.1* | *0.2* | *0.3* | *0.4* |
| DXS1060 | −∞ | −2.12 | −1.29 | −0.56 | −0.22 | −0.05 |
| GHGXg | −∞ | −0.82 | −0.38 | −0.09 | −0.02 | −0.01 |
| DXS443 | −∞ | −0.86 | −0.62 | −0.41 | −0.27 | −0.14 |
| DMD | NI | NI | NI | NI | NI | NI |
| DXS7 | −∞ | 0.67 | 0.80 | 0.75 | 0.55 | 0.28 |
| DXS538 | −∞ | −1.70 | −0.92 | −0.27 | −0.03 | 0.04 |
| MAO A | 0.37 | 0.33 | 0.30 | 0.24 | 0.17 | 0.09 |
| DXS1003 | −∞ | 0.89 | 1.00 | 0.89 | 0.63 | 0.28 |
| DXS337 | −∞ | 0.60 | 0.94 | 0.98 | 0.73 | 0.32 |
| DXS426 | 0.83 | 0.76 | 0.69 | 0.53 | 0.36 | 0.19 |
| DXS6941 | NI | NI | NI | NI | NI | NI |
| DXS573 | 2.86 | 2.61 | 2.35 | 1.80 | 1.19 | 0.52 |
| DXS1204 | 3.30 | 3.00 | 2.69 | 2.05 | 1.35 | 0.61 |
| ALAS2 | 2.43 | 2.21 | 1.98 | 1.51 | 0.99 | 0.43 |
| AR | NI | NI | NI | NI | NI | NI |
| PGK1P1 | −∞ | 0.29 | 0.46 | 0.49 | 0.39 | 0.21 |
| DXS339 | 0.29 | 0.26 | 0.23 | 0.18 | 0.13 | 0.07 |
| DXS453 | −∞ | 0.80 | 0.90 | 0.80 | 0.58 | 0.28 |
| DXS559 | −∞ | 1.23 | 1.34 | 1.24 | 0.95 | 0.54 |
| DXYS1X | −∞ | −0.55 | −0.34 | −0.20 | −0.13 | −0.07 |
| DXS3 | 0.38 | 0.34 | 0.31 | 0.24 | 0.17 | 0.09 |
| DXS458 | −∞ | −0.18 | −0.01 | 0.06 | 0.05 | 0.03 |
| DXS454 | 0.38 | 0.34 | 0.31 | 0.24 | 0.17 | 0.09 |
| DXS178 | NI | NI | NI | NI | NI | NI |
| COL4A5 | −∞ | 0.67 | 0.80 | 0.75 | 0.55 | 0.28 |
| DXS456 | 0.35 | 0.31 | 0.28 | 0.22 | 0.16 | 0.09 |
| DXS424 | −∞ | 0.16 | 0.35 | 0.41 | 0.32 | 0.17 |
| HPRT | −∞ | −1.65 | −0.88 | −0.27 | −0.05 | 0.01 |
| DXS294 | −∞ | −0.18 | −0.01 | 0.06 | 0.05 | 0.03 |
| DXS984 | −∞ | −0.18 | −0.01 | 0.06 | 0.05 | 0.03 |
| DXS292 | −∞ | −0.26 | −0.07 | 0.04 | 0.04 | 0.02 |
| FRAXAc2 | −∞ | 0.45 | 0.58 | 0.51 | 0.31 | 0.09 |
| DXS1113 | −∞ | −2.33 | −1.26 | −0.37 | −0.01 | 0.09 |
| DXS1108 | −∞ | −2.71 | −1.63 | −0.69 | −0.26 | −0.06 |

NI = not informative.

*Table 2 X linked mental retardation disorders associated with neurological features and early death*

| *Name* | *MIM/ref* | *Locus* |
|---|---|---|
| Adrenomyodystrophy | 300270 | — |
| ALD | *300100 | Xq28† |
| Arthrogryposis | *301830 | — |
| Arts | 301835 | Xq21.33−q24 |
| Baar-Gabriel | 312890 | — |
| Bertini | /26 | Xp22.33−pter |
| Cantu | 308830 | — |
| Gustavson | *309555 | Xq24−q26 |
| Holmes-Gang | *309530 | — |
| HSAS | *308840 | Xq28† |
| Hunter | *309900 | Xq27.3−q28† |
| Juberg-Marsidi/ATR-X | *309590 | Xq13.3† |
| Lenz | *309800 | — |
| Lowe | *309000 | Xq25−q26† |
| Lesch-Nyhan | *308000 | Xq26.1† |
| Menkes | *309400 | Xq13.3† |
| Paine-Seemanova | 311400 | — |
| PMD | *312080 | Xq21−q22† |
| Pyruvate dehydrogenase deficiency | *312170 | Xp22† |
| Schmidley | 301790 | — |
| VACTERL with hydrocephalus | 314390 | — |
| Wittwer | /27 | Xp22.3−pter‡ |

ALD = adrenoleucodystrophy.
PMD= Pelizaeus-Merzbacher disease.
ATR-X= α thalassaemia retardation X linked.
HSAS= hydrocephalus owing to stenosis of the aqueduct of Sylvius.
† = gene cloned.
‡ = tentative assignment.

LINKAGE DATA

Pedigree and clinical findings suggest that we are dealing with a novel X linked neurodegenerative disorder with mental retardation, blindness, convulsions, spasticity, mild hypomyelination, and early death.

To determine the location of the gene(s) involved, linkage analysis was performed with 34 highly polymorphic markers distributed along the entire X chromosome (table 1). Significant lod scores were obtained only with markers from the pericentromeric region. A maximum lod score of 3.30 at θ=0.0 was obtained with marker DXS1204. To locate the genetic defect more accurately, haplotypes were constructed with markers from the relevant region (fig 1). In this way, DXS337 and PGK1P1 were defined as closest flanking markers, spanning a distance of about 13 cM. Thus, our results show that the genetic defect underlying the neurodegenerative disorder in this family is located in the pericentromeric region of the X chromosome, Xp11.3-q12.

## Discussion

Initially, a severe form of Pelizaeus-Merzbacher disease (PMD) had been suspected in this family. However, the gene for PMD (PLP) is in Xq21-q22.[9] None of the X linked neurological disorders with early death has been localised to the pericentromeric region Xp11.3-q12 (table 2). Therefore, it appears that this is the first X linked neurodegenerative disorder which maps to the pericentromeric region. The disorder described here does not show overt clinical resemblance to any of the other known but as yet unassigned X linked neurodegenerative disorders. Neither in reviews of XLMR

classified by clinical manifestations[3 4 10] nor in the latest update of XLMR[2] could a disorder like the one we describe be found.

Hypomyelination is a feature of PMD and the families reported by Schmidley *et al*[11] and Arts *et al*.[12] Although clinically different, PMD and Arts syndrome have been mapped to overlapping X chromosomal regions, Xq21-q22 and Xq21.33-q24[13] respectively, while in the family of Schmidley *et al*[11] no linkage study was performed. Watanabe *et al*[14] described a family with "Pelizaeus-Merzbacher disease" with, on neuropathological examination, a surprising amount of preserved myelin sheaths and mature oligodendrocytes and in which much later a normal PLP gene was shown.[15] It would be interesting to perform linkage analysis in this large family to see whether its genetic defect colocalises with that of the family described here. A spongy state of the central tegmental tract was found by Satoh *et al*[16] in 11 out of 22 necropsied cases with a variety of developmental disabilities and a history of infantile spasms, indicating that this change is a secondary and non-specific phenomenon.

The pericentromeric region harbours many of the syndromal and non-specific XLMR loci[2] and therefore a considerable proportion of the (unknown) number of XLMR genes will be in this region. Apart from genes of known syndromes like Wiskott-Aldrich syndrome and Aarskog syndrome, several genes have been mapped to the DXS337-PGK1P1 interval, like ARAF1, SYN1 (synapsin1), SYP (synaptophysin), ELK1, and a cluster of zinc finger genes ZNF21, 41, 81, and 157, which are all possible candidate genes for neurological/neurodegenerative disorders. *A-raf* deficient mice displayed neurological and gastrointestinal defects and early postnatal death.[17] *Synapsin-I* deficient mice showed impairment of axonal development and of synaptogenesis in hippocampal neurones,[18] and impairment of synaptic vesicle clustering and of synaptic transmission and increased seizure propensity.[19] Synaptophysin is an integral membrane protein of small synaptic vesicles in brain,[20] while ELK1[21] and ZNF21, 41, 81, and 157[22] are as transcription factors putative candidate genes for developmental disorders. Davies *et al*[23] postulated the presence of a contiguous gene syndrome including an XLMR gene in Xq11-q12. Recently this gene has been identified as oligophrenin-1.[24] Up to now inherited neurodegenerative disorders all appeared to be the result of single gene defects. Therefore, we feel that the disorder in this family is more likely the be the result of pleiotropic effects of a single gene.

Sequence tagged sites with expression in brain are becoming increasingly available and will allow identification of more candidate genes in the defined interval.[25]

In conclusion we describe a family with a new X linked neurodegenerative disorder with early death which maps to the pericentromeric region.

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