

Original articles

A linkage study of a large pedigree with X linked centronuclear myopathy

J Starr, M Lamont, L Iselius, J Harvey, J Heckmatt

Abstract

Centronuclear myopathy (CNM) is a muscle wasting disorder that occurs in three distinct forms. Previous studies have shown linkage between the X linked form of the disease and the Xq28 probes ST14, DX13, and F8C. Our study on a previously unreported, three generation, X linked CNM family confirms linkage between these markers and the CNM locus ($Z=3.21$, $\theta=00$). However, results from the laboratory of J-L Mandel (Samson and Hanover, personal communication) on a number of X linked CNM families exclude genetic linkage from the region Xq26-qter, suggesting genetic heterogeneity in this condition.

Centronuclear myopathy derives its name from the predominance of centrally placed nuclei seen on histological examination of muscle in affected persons. The condition was originally called 'myotubular' myopathy because of its resemblance to myotubes in developing fetal muscle.

The condition was first described by Spiro *et al.*¹

Wessex Regional Cytogenetics Unit, Salisbury General Hospital, Salisbury, Wiltshire SP2 7SX.

J Starr, J Harvey

Department of Child Health, Southampton General Hospital, Southampton.

M Lamont

Department of Community Medicine, CRC Research Group in Genetic Epidemiology, Southampton General Hospital, Southampton.

L Iselius

Department of Paediatrics and Neonatal Medicine, Hammersmith Hospital, London.

J Heckmatt

Correspondence to J Starr.

They reported a boy aged 12 years with congenital progressive muscle weakness. Since then about 80 cases have been reported, with varied clinical course and modes of inheritance. Heckmatt *et al.*,² reporting eight cases, suggested that there may be three distinct forms.

(1) An autosomal dominant form that is relatively mild, with symptoms usually becoming evident in the third decade. It is very slowly progressive, affecting mainly proximal muscle groups, and is compatible with a normal life span (McKusick No 16015).

(2) Juvenile autosomal recessive. This type becomes evident in the first year of life with retarded physical milestones, and patients may become wheelchair bound in the second decade (McKusick No 25520).

(3) Severe X linked form. Polyhydramnios and reduced fetal movements are common in pregnancies with affected males. At birth there is often severe hypotonia and difficulty in establishing respiration. Stillbirth or perinatal death is common. Female carriers of the condition have been shown to have an excess of central nuclei on muscle biopsy. This, however, is variable; Keppen *et al.*³ reported a normal biopsy in the mother of two affected males (McKusick No 31040).

There have been no published studies that determine the chromosomal localisation of the two autosomal forms of the disease. However, a previous study of two X linked centronuclear myopathy families by Thomas *et al.*⁴ has provided strong evidence for linkage between the disease locus and three Xq28 probes, ST14, DX13,⁵ and F8C.⁶ Our study on a previously unreported three generation family provides additional data to support these findings.

Methods

DNA ANALYSIS

Leucocyte DNA extraction was carried out using a salt precipitation method.⁷ DNA samples were digested to completion with the relevant restriction enzyme, and the fragments separated by electro-

phoresis in a 0.8% agarose gel. Southern blots were performed using Hybond membranes (Amersham). The filters were hybridised with the three Xq28 probes, ST14, DX13, and F8C. The probes were labelled with ^{32}P dCTP using a random primed DNA labelling kit (Boehringer, Mannheim). The filters were washed to a final stringency of $0.1 \times \text{SSC}$ at 65°C and exposed to Kodak X-omat AR film at -80°C using intensifying screens.

LINKAGE ANALYSIS

Lod scores were calculated using the program LINKAGE (V3.5) with two point, sex linked data.⁸ A gene frequency of 0.001 was assumed for the mutant allele. Penetrance was taken as complete in males with the mutant allele.

Clinical details

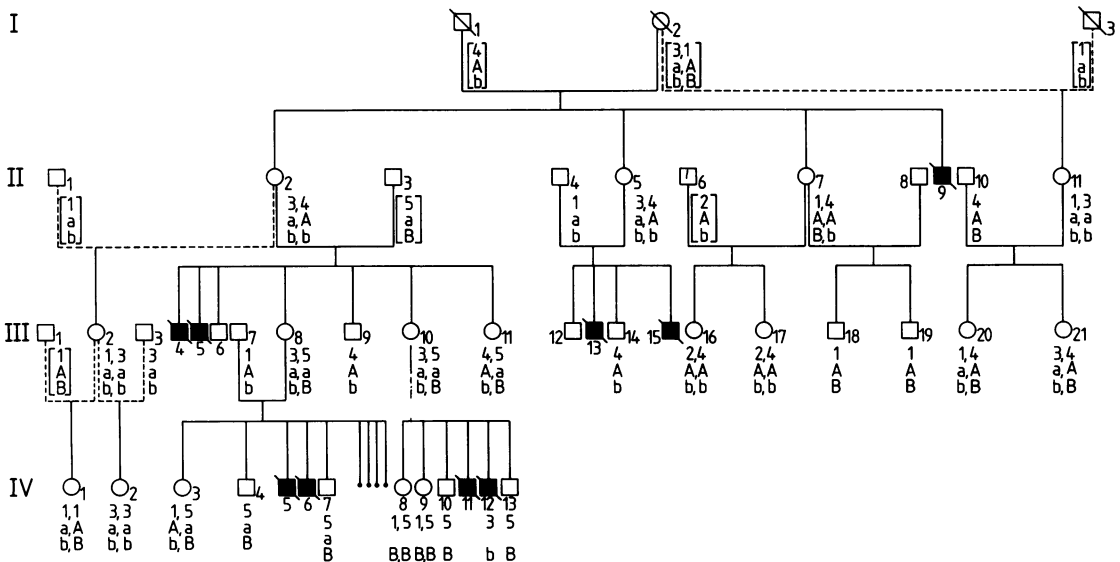
The pedigree of the family is shown in the figure. A member of the family (III.8) was referred to us for genetic counselling after the diagnosis of X linked centronuclear myopathy in her sister's son (IV.12). The consultand (III.8) had a daughter, two normal sons, and two sons who were affected. She had also had three spontaneous abortions at 12 weeks and an abortion at 18 weeks shortly after an amniocentesis done because of maternal age. In neither of the affected pregnancies had she been aware of reduced fetal movements. They were both born at home. One male was stillborn at term, but with no malformations. The other, born at 41 weeks' gestation, had a

birth weight of 2888 g and was admitted to hospital because of severe hypotonia, respiratory problems, and dysmorphic features. He had marked pectus carinatum and very slender ribs on x ray. His karyotype was 46,XY and his creatine kinase 18 IU/l. He was diagnosed as having severe birth asphyxia. He was put on a ventilator but remained floppy and unreactive and died at the age of 10 days. A further five maternal relatives, all male, died in the newborn period of respiratory problems.

The affected child on whom the diagnosis of CNM was established (IV.12) was born prematurely at 31 weeks. He was severely hypotonic, had difficulty in establishing respiration at birth, and was put on a respirator. He was weaned off the ventilator at 5 weeks of age but remained very floppy. In the subsequent months he had several more apnoeic episodes, and at 9 months of age was given a negative ventilator to use at night.

A needle biopsy of the quadriceps muscle performed at 10 months showed classical features of centronuclear myopathy with the presence of large, round, centrally placed nuclei in a significant proportion of fibres with routine histological stains and on histochemical preparations, aggregation of oxidative enzyme activity in the central part of the fibre, and absence of myofibrils with negative reaction for ATPase in the central region of the fibres. He died at 11 months of age of respiratory failure.

Blood samples were obtained from 30 family members, including four known obligate carrier females, 13 females at risk of being carriers, and one of the 10 affected males.



The family pedigree. Genotypes are indicated using numbers and letters as follows: 1 to 5, ST14; aA, DX13; bB, F8C.

Linkage analysis between X linked centronuclear myopathy and ST14, DX13, and F8C with lod scores for different recombination frequencies.

	Recombination frequency							θ_{\max}	Z_{\max}
	0.00	0.05	0.10	0.20	0.30	0.40			
ST14, DX13, F8C	3.21	2.95	2.67	2.06	1.41	0.72	0.00	3.21	

Results

DNA RESULTS

The 30 family members were studied with the three Xq28 probes previously described. The RFLP results were combined to obtain a chromosomal haplotype and the linkage phase was established. The figure shows that all the healthy male offspring of the obligate carrier females have inherited a different chromosomal haplotype from the affected male.

LINKAGE ANALYSIS

There were no recombinants between the disease locus and any of the markers DX13, ST14, and F8C. The maximum lod score was 3.21 at $\theta=0.00$ with the one lod unit support 0.00 to 0.18 (table).

CARRIER STATUS DETERMINATION

On the basis of the linkage analysis we were able to investigate the carrier status in a number of females. Of the 13 females at risk, we identified four who have a high probability of being carriers and nine who have a very low probability of being carriers.

Discussion

The results from this family, together with those of Thomas *et al* (this issue), show a linkage between X linked centronuclear myopathy and the Xq28 probes ST14, DX13, and F8C, suggesting that in these three families the gene causing the X linked centronuclear myopathy is very close to the end of the long arm of the X chromosome. However, results from the laboratory of J-L Mandel (Samson and Hanover,

personal communication) on a number of families with X linked centronuclear myopathy have excluded linkage from Xq26 to Xqter. These contradictory findings suggest that X linked centronuclear myopathy may be heterogeneous and caused by mutation at at least two separate loci on the X chromosome. Recently it has been suggested, on the basis of linkage, that there are at least two separate loci on Xp for X linked retinitis pigmentosa, the two forms being clinically indistinguishable. Studies of many more families with X linked CNM are necessary to establish the detailed phenotype, frequency, and geographical distribution of the two mutations.

We wish to thank Dr P Jacobs and Professor N Morton for useful advice and discussion; Professor V Dubowitz for providing access to clinical material; and Greta Curtis, Anne Hayter, and Christine Patch for visiting family members and obtaining samples for this study.

Note added in proof

Recent additional results, from a live born, healthy son of obligate carrier III-8, support our linkage data, increasing the lod score to $Z_{\max}=3.51$, $\theta_{\max}=0.00$.

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