European Journal of Human Genetics (2000) 8, 125–129

www.nature.com/ejhg

ARTICLE

Linkage of X-linked myopathy with excessive autophagy (XMEA) to Xq28

Laurent Villard[1], Vincent des Portes[2], Nicolas Levy[1], Jean-Pierre Louboutin[3], Dominique Recan[4], Michelle Coquet[5], Brigitte Chabrol[6], Dominique Figarella-Branger[6], Jamel Chelly[2], Jean-François Pellissier[6] and Michel Fontes[1]

[1]INSERM U491, Université de la Méditerrannée, Faculté de Médecine La Timone, Marseille; [2]INSERM U129, Faculté de Médecine Cochin, Paris; [3]CJF INSERM 96-01, Hôpital GR Laennec, Nantes; [4]Laboratoire de Biochimie Génétique, Paris; [5]Laboratoire d'Anatomie Pathologique, Hôpital Pellegrin, Bordeaux; [6]Laboratoire de Biopathologie Nerveuse et Musculaire, Faculté de Médecine La Timone, Marseille, France

X-linked myopathy with excessive autophagy (XMEA, MIM 310440) is a rare inherited mild myopathy. We have used 32 polymorphic markers spanning the entire X chromosome to exclude most of the chromosome except the Xq28 region in a large XMEA family. Using three additional families for linkage analysis, we have obtained a significant two-point lod score with marker DXS1183 (Z = 2.69 at θ = 0). Multipoint linkage analysis confirmed the assignment of the disease locus with a maximal lod score of 2.74 obtained at recombination fraction zero. Linkage of XMEA to the Xq28 region is thus firmly established. In addition, we have ruled out the Emery-Dreifuss muscular dystrophy to be allelic with XMEA by direct sequencing of the emerin gene in three of our families. *European Journal of Human Genetics* (2000) **8**, 125–129.

Keywords: XMEA; myopathy; X chromosome; linkage; EDM

Introduction

X-linked myopathy with excessive autopathy (XMEA, MIM 310440) is a rare inherited mild myopathy segregating as an X-linked trait. The first family was reported by Kalimo *et al* in 1988 who described a new disorder characterised by a slowly progressive myopathy segregating as an X-linked recessive trait.[1] This condition was characterised by juvenile onset and slow progression of the disorder, which seems predominantly to affect proximal muscles. Upon histological studies, the muscle does not display acute necrosis but shows an excess of autophagic processes and exocytosis of the phagocytosed material. This condition has been named X-linked myopathy with excessive autophagy (XMEA). A genetic analysis in this first reported family suggested linkage to Xq28, although the lod score (Z = 0.9) was not significant.[2] This work excluded XMEA to be allelic with the DMD/BMD locus, but clearly raised the question of allelism with the Emery-Dreifuss muscular dystrophy (EDM) because the gene for this later condition is located in the putative linkage area (Xq28), and because it is also responsible for a mild myopathy.

In 1995, another family was reported by Villanova *et al* in which the disorder was named X-linked vacuolar myopathy.[3] These authors described in this and additional papers[4–6] the deposition of membrane attack complex (MAC) on affected muscle fibres, as well as calcium accumulation in the sarcolemma.

All these data have raised two questions: is the *XMEA* gene really located in Xq28, and are XMEA and EDM allelic disorders, involving different mutations in the emerin gene?

In this paper, using four families affected with XMEA, we demonstrate linkage of the condition to Xq28 (Z = 2.69 at θ = 0 for DXS8103). Moreover, all the other regions of the X chromosome are excluded. Finally, we rule out the hypothesis stating that this condition could be allelic with the Emery-Dreifuss muscular dystrophy since we did not find any mutation in the *EDM* gene of three of our families.

Correspondence: Dr Michel Fontès, INSERM U491, Faculté de Médecine La Timone, 27 Bd Jean Moulin, 13385 Marseille, Cedex 5, France. Tel: +33 491784477; Fax: +33 491804319; E-mail: fontes@medecine.univ-mrs.fr
Received 9 July 1999; revised 6 October 1999; accepted 15 October 1999

Materials and methods

Families and clinical presentation

Four families have been used in this study. The first family has already been reported on and extensively studied.[3–6] The three additional families are new and unrelated, with a total of seven affected boys, and present similar clinical and histopathological features to the first family. All patients' medical history was characterised by a slow progression of limb girdle muscle weakness beginning in infancy, without calf hypertrophy, mental retardation or cardiac involvement. Increased levels of CPK was noted in all cases. Muscle biopsies showed the same pathological features of vacuolar myopathy (Figure 1), due to invagination of damaged muscle membranes and phagocytosed material. A muscle biopsy of an unaffected mother showed the same pathological presentation in only a few fibres.

Markers used

For the exclusion mapping, and haplotyping in Xq28, the following markers were used (from Xpter to Xqter, together with the genetic distances between the markers when known): DXS402 – DXS1228 – DXS996 – (2.9 cM) – DXS1223 – (7.4 cM) – DXS1224 – (15.7 cM) – DXS1226 – (7.2 cM) – DXS985 – (2.2 cM) – DXS1214 – (10 cM) – DXS1068 – (9.9 cM) – DXS993 – (10.9 cM) – DXS1003 – (9.9 cM) – DXS991 – (0.4 cM) – DXS1204 – (6.6 cM) – DXS1275 – (4.4 cM) – DXS1196 – (7 cM) – DXS990 – (7.9 cM) – DXS1231 – (2.4 cM) – DXS1230 – (5.8 cM) – DXS1059 – (18.4 cM) – DXS1001 – (10.9 cM) – DXS1047 – (13.4 cM) – DXS1205 – (1 cM) – DXS1227 – (8.9 cM) – DXS8106 – (1.1 cM) – DXS8073 – (2.8 cM) – DXS8028 – (2.3 cM) – DXS1200 – (4 cM) – DXS1215 – (3.9 cM) – DXS1193 – (4.8 cM) – DXS8011 – (0.1 cM) – DXS1684 – (2 cM) – DXS8103 – (5.6 cM) – DXS1108.

DNA studies

For all DNA studies, informed consent was obtained for each family. PCR reactions were performed and microsatellites markers were used as recommended by Généthon. Samples were loaded on a 6% polyacrylamide gel and run for 3 h 30 min at 45W. The gel was then transferred to Hybond N^+ filters, and subsequently hybridised with a biotynylated CA probe in gold buffer (Amersham). Filters were then revealed in a buffer containing streptavidin/peroxydase, according to the instructions of the manufacturer (ECL kit, Amersham). Polymorphic alleles were visualised using Kodak Biomax films.

Linkage analysis

Linkage analysis was performed using the LINKAGE package.[7] Two-point lod scores were calculated with the MLINK program of the package and maximal lod scores with the

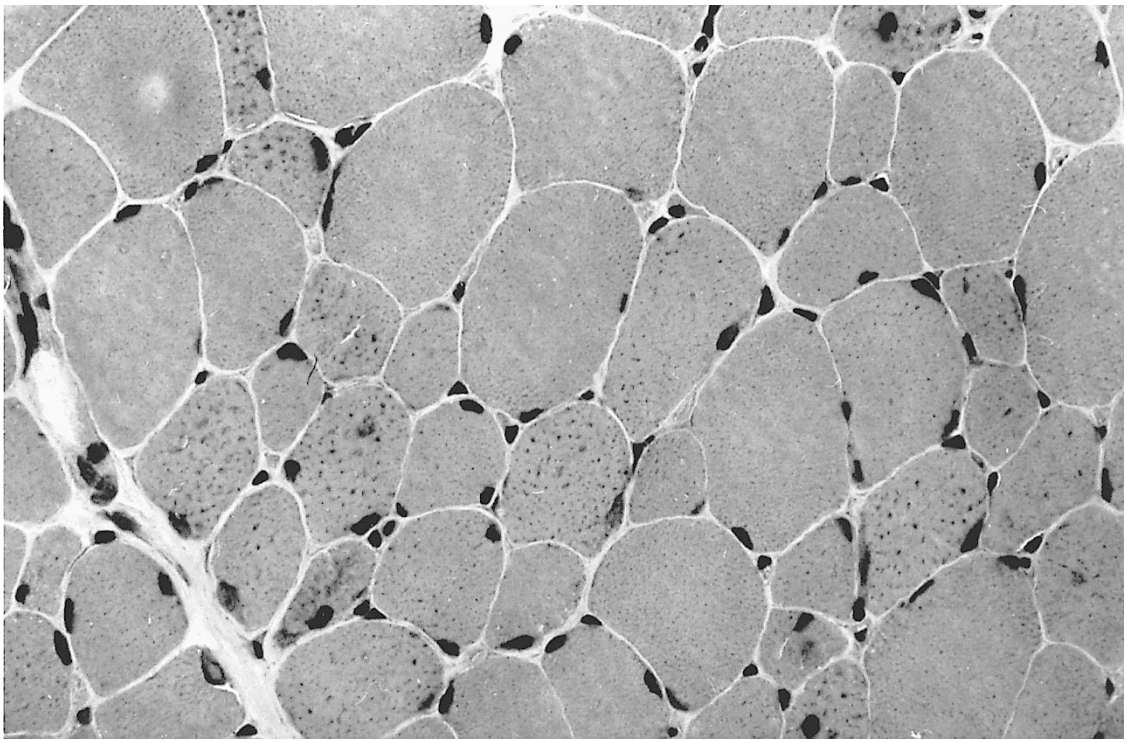


Figure 1 Muscle biopsy showing the affected fibres of various sizes with intracytoplasmic basophilic vacuoles (haematoxylin-eosin, $\times$ 300) for patient 3-1 (first affected child, family 3).

LODSCORE program. Multipoint linkage analysis was performed using the GENEHUNTER program.[8]

Sequencing of the emerin gene

For one affected member of families 1,3 and 4 the emerin transcript was amplified starting from lymphocyte RNA using primers already described.[9] Amplification products were directly sequenced and the sequence compared to the wild-type emerin transcript (GenBank accession number NM 000117).

Results

Exclusion mapping of the X chromosome

In a first step, we used the large family described by Villanova *et al* to construct an exclusion map of the X chromosome. For this purpose, we analysed the segregation of 32 polymorphic markers, distributed along the chromosome. From the constructed haplotypes and lod scores (data not shown), we deduced that all the chromosome was excluded, excepted the Xq28 region. Three markers (DXS8011, DXS1684, DXS8103) showed no recombination with the disease, with lod scores ranging from 1.30 to 1.48. A recombination between DXS1193 and DXS8011 was observed in one meiosis, potentially placing the XMEA locus between DXS1193 and the telomere. Unfortunately, the lod score, obtained with six informative meioses, was low ($Z = 1.48$ at $\theta = 0$ for DXS8103), and was not conclusive regarding a potential linkage to Xq28.

Linkage between XMEA and Xq28

In order to increase the lod score, we collected three additional families fulfilling all the inclusion criteria, especially the histopathological features. Interestingly, in a relatively short time, four additional families were collected in France (one is a nuclear family which has not been included in the linkage study presented below) which will raise the point of the prevelance of this disorder as discussed below.

We have performed genetic linkage analysis in the four families using a set of six markers located in Xq28. No additional recombination was detected by haplotypes construction (Figure 2). The maximal lod score is now of 2.69 ($Z = 2.69$ at $\theta = 0$ for DXS8103). (Table 1), thus establishing linkage of the XMEA locus to Xq28. Multipoint linkage analysis gave the same results with a slightly higher lod score ($Z = 2.74$ at $\theta = 0$, data not shown).

Search for mutations in the emerin gene

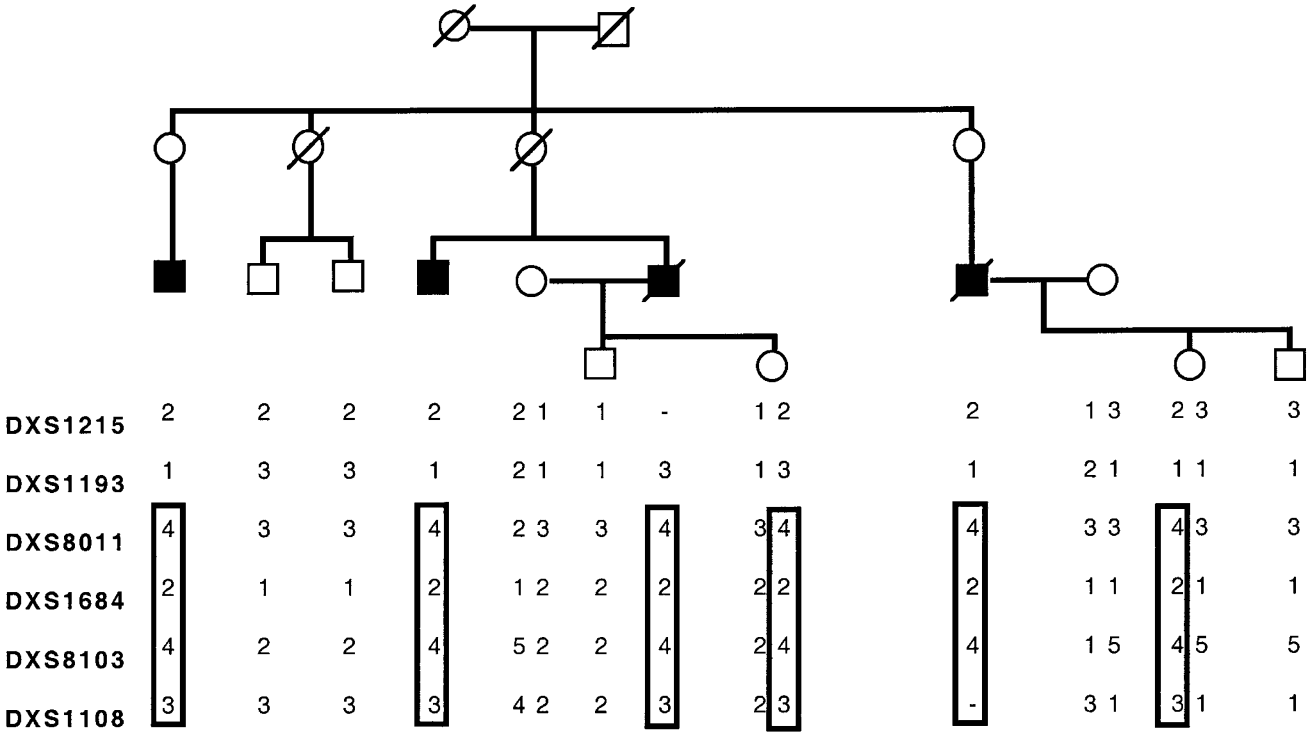
Several genes involved in inherited muscular disorders (Emery-Dreifuss muscular dystrophy, myotubular myopathy and Barth syndrome) have been identified in this region of the human X chromosome. Based on the phenotypes, only EDM could be compatible with the clinical presentation of the XMEA patients (an hypothesis already proposed by Saviranta *et al* in 1988). We have thus sequenced the emerin gene coding region in three patients from three different families (families 1, 3 and 4). No difference from the published sequence of the normal gene has been found. We can thus probably exclude XMEA as a disorder allelic with the Emery-Dreifuss muscular dystrophy.

Discussion

Our results clearly demonstrate that the XMEA locus can only be located in Xq28, as all other regions of the X chromosome have been excluded. More precisely, a maximal two-point lod score of 2.69 with no recombination was obtained with DXS8103. A proximal recombinant has been detected between DXS1193 and DXS8011, which places the XMEA locus between DXS1193 and the telomere of the long arm of the X chromosome, in an approximately 8 Mb interval[10] corresponding to 12.5 cM according to the Généthon human genetic linkage map.

Genes involved in three disorders affecting the muscle are located in this region: myotubular myopathy, Barth syndrome, and Emery-Dreifuss muscular dystrophy (EDM). The first two syndromes are severe myopathies occurring in early childhood, with additional clinical signs, and are thus not likely to be allelic with XMEA. On the other hand, Emery-Dreifuss muscular dystrophy is a mild myopathy, as Saviranta *et al* have already pointed out, although EDM patients present elbow and neck contractures together with cardiac involvement, clinical signs which are not found in XMEA.[2] However, it has been noted that localisation, and maybe function, of the emerin is not the same in skeletal and cardiac muscle.[11] It was thus possible that different mutations in the emerin gene could affect differentially the skeletal and the cardiac isoforms of the protein, leading to two different myopathies. To rule out this possibility, we sequenced the coding region of the *EDM* gene in three of our families. No mutation was found. This probably excludes allelism between *EDM* and *XMEA*, as rare mutation events (like mutations in regulatory regions, which represent less than 1% of the mutations listed in the Human Gene Mutation Database) are not likely to occur independently in three different families. We are now sequencing additional genes which have been localised in this gene-rich region of the X chromosome, especially those which are expressed in muscle.

Finally, a point should be made regarding the prevalence of the disorder. In 10 years, only two families have been reported, originating from two different countries. It was thus suspected that this disorder will be extremely rare. In six months, we have collected four additional French families, and two additional candidate families are under clinical exploration. This probably means that the disorder is not as rare as was previously thought, and is likely to be under or misdiagnosed. The point is that the clinical presentation is mild and not very specific if muscle biopsies are not



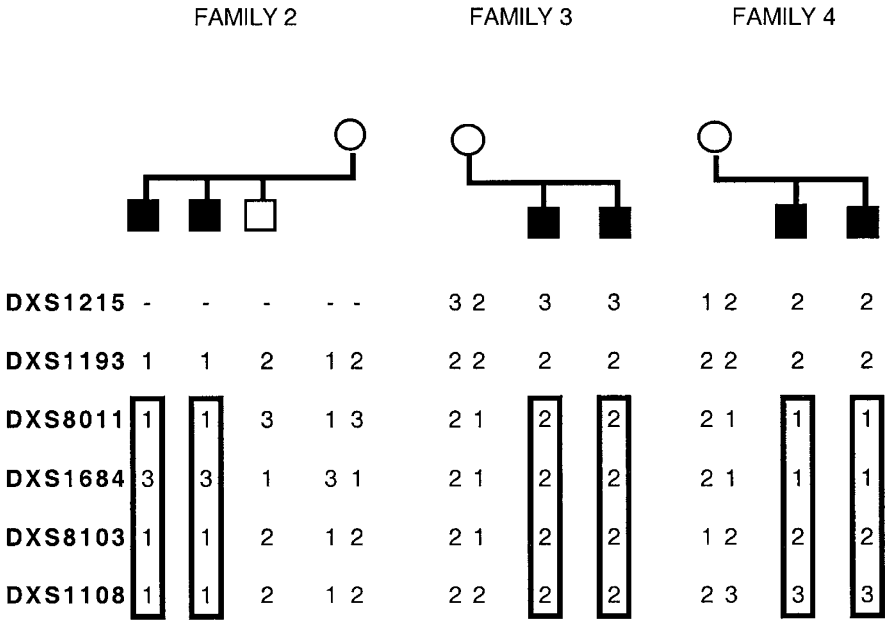


Figure 2 Pedigree of the four families. Only informative subjects are represented together with their haplotypes in the critical Xp28 region indicated below their pedigree symbol. The corresponding markers are indicated on the left from Xq27.3 to Xqter. The only condordant region within these pedigrees is boxed. The genotype of the two deceased affected males from the first family was deduced from the haplotype of their respective wife, son and daughter.

Table 1 Linkage results obtained using the four families

	Recombination				
Marker	0.0	0.01	0.05	0.1	0.2
DXS1215	0.71	0.69	0.61	0.51	0.32
DXS1193	–	0.01	0.58	0.70	0.62
DXS8011	2.51	2.45	2.23	1.96	1.39
DXS1684	2.59	2.54	2.31	2.03	1.44
DXS8103	2.69	2.63	2.40	2.11	1.51
DXS1108	1.03	1.00	0.90	0.77	0.51

performed. The specificity lies in the histopathological presentation which is highly characteristic. Without this criteria, the disorder can probably be confused with other disorders like Becker myopathy or storage myopathies. We hope that the identification of the gene involved in this disorder will allow the investigation of patients with unclassified myopathy, such as BMD patients without anomalies in the *DMD* gene.

Acknowledgements

We would like to thank Dominique Hillaire for assistance with the linkage analysis and the Cell/DNA core facilities of La Timone Hospital for lymphoblastoid cell lines and DNA extraction. L Villard and V des Portes contributed equally to this work.

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