

X-linked myopathy with excessive autophagy

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LAMP-2	lysosome-associated membrane protein-2
MAC	membrane attack complex
OMIM	Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/omim)
XMEA	X-linked myopathy with excessive autophagy
XVM	X-linked vacuolated myopathy

Definition of entity

The first family with *X-linked myopathy with excessive autophagy* (XMEA) was described in 1988 by Kalimo et al (5) (OMIM 310440). It is a slowly progressive inherited myopathy characterised by membrane bound sarcoplasmic vacuoles. Therefore, it has been also reported as X-linked vacuolated myopathy, XVM (10). So far, 16 families have been identified in Europe and North America (4).

XMEA patients are males with onset in early childhood. The affected boy is unable to keep up with his companions in running and climbing. Serum creatine kinase values are elevated already during the first years of life by 2.5 to 3 times and may, during adolescence, rise to 10 to 15 times normal. Proximal muscles of the lower limbs are mainly affected, but shoulder girdle muscles are also involved. No other organs appear to be diseased, and the patients have no cardiomyopathy or cognitive deficits. XMEA is slowly progressive and ambulation is usually preserved until later age, but some patients become wheelchair bound around 50 to 60 years of age. XMEA patients have a normal life expectancy. Female carriers are usually without symptoms or only very mildly affected.

EMG findings are characteristic with abundant myotonic and high frequency discharges, without clinical myotonia. This is observed even in clinically unaffected muscles. MRI discloses fatty degeneration with typical

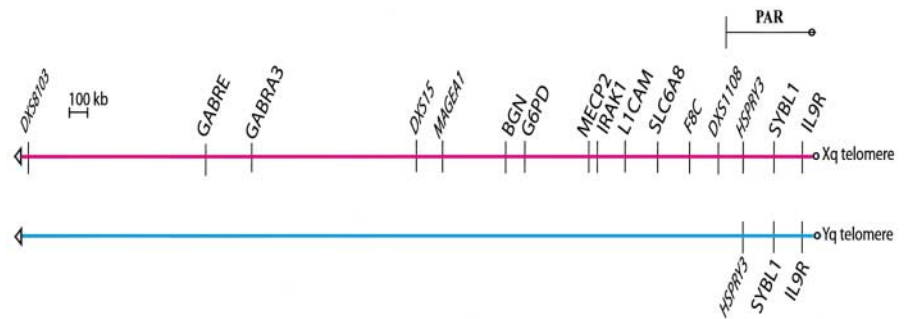


Figure 1. Map sketch of the XMEA gene locus in Xq28 (to scale). Pink, the X chromosome; Blue, the Y chromosome; PAR, the Xq pseudoautosomal region (the X and Y chromosomes have the same sequence and contain the same genes in this region). Some genes in the PAR (e.g. IL9R) are expressed from both the X and Y chromosomes and are therefore not candidates for this X-linked disease. Others (HSPRY3 and SYBL1), are expressed only from the X chromosome and remain therefore positional candidates (2).

distribution. (Jääskeläinen et al, unpublished data).

Molecular genetics and pathophysiology

Genetic linkage was recently established between XMEA and markers in a 10 cM span of the most telomeric band of the long arm of chromosome X (Xq28) (1, 11). A recent unpublished observation of a recombination event in a new family defines marker DXS8103 as the centromeric limit of the XMEA region. The telomeric limit is in the extreme tip of the chromosome within the Xq pseudoautosomal region (PAR) (Figure 1). Based on the completed human genome sequence (<http://www.celebra.com>), the segment between DXS8103 and PAR contains 5 million base-pairs of DNA coding for at least 100 genes.

Two main pathogenic mechanisms have been suggested. As the name implies, Kalimo et al (5) suggested that the vacuoles are autophagosomes clearing sarcoplasm of debris after a sublethal injury to the myofibre, and the debris is further extruded from the fibres between the multiple layers of basal lamina. This view is supported by the presence of lysosomal enzymes in both the vacuoles and around the fibres and by the immunopositivity of XMEA

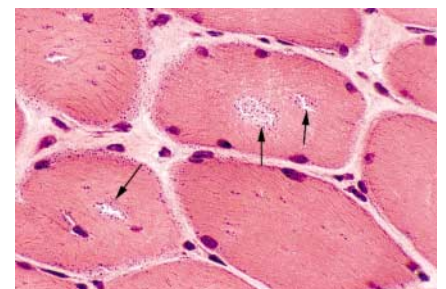


Figure 2. The vacuoles of XMEA (arrows) contain delicate basophilic granules, which are also present around the affected fibres between the multiplied layers of basal lamina, cf. Figure 6. H & E. $\times 80$

vacuoles for LAMP-2, a protein in lysosomal membranes considered to be important in autophagy (8, 9). Even if autophagy appears to have a key role in XMEA, it may be only secondary to a still unknown primary injury. Villanova et al (10) suggested a “reverse” pathogenesis, ie, the injury is induced by deposition of MAC on myofibres with secondary invagination and/or endocytosis, one of the arguments being that immunopositivity for dystrophin and laminin should not exist on lysosomal membranes. On the other hand, basal lamina is also present in the vacuoles of LAMP-2 deficiency (8), and MAC and calcium deposition may be only secondary to the debris around the affected myofibres (Meri, personal communication).



Figure 3. The size variation of myofibers is increased and fibre splitting occurs. Numerous dystrophin-positive vacuoles (arrows) are present in the sarcoplasm of most fibres. Anti-dystrophin 2. $\times 52$

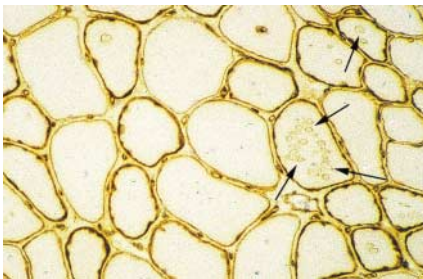


Figure 4. In an adjacent section, many (examples indicated by arrows) but not all vacuoles are weakly positive for muscle specific laminin 2 (merosin), which identifies basal lamina. Note that the affected fibres are surrounded by thickened basal lamina (cf. Figure 6). Anti-laminin 2. $\times 52$

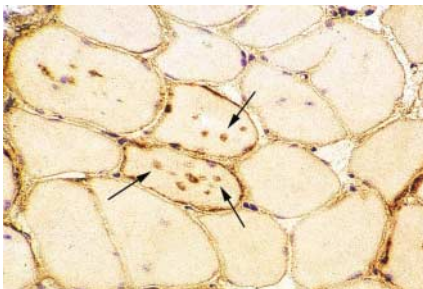


Figure 5. (D) $\alpha 1$ -antitrypsin-positivity is seen within the vacuoles (arrows) and surrounding the affected fibres. $\alpha 1$ -antitrypsin.

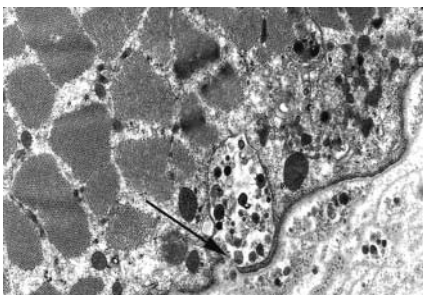


Figure 6. Electron micrograph of affected fibres. Granular debris is present both within a small subsarcolemmal vacuole opening to the surface of the fibre (arrow) and between the multiple layers of basal lamina around the fibre. $\times 2640$

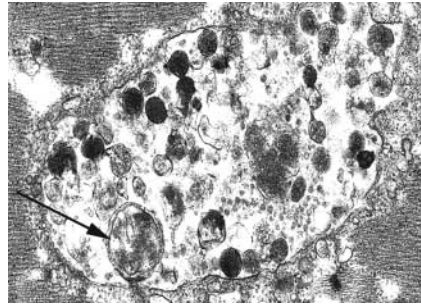


Figure 7. Another vacuole deep in the sarcoplasm contains a degenerating mitochondrion (arrow), small vesicles and granular debris. The vacuole is surrounded by a single membrane without a definite basal lamina. $\times 8000$

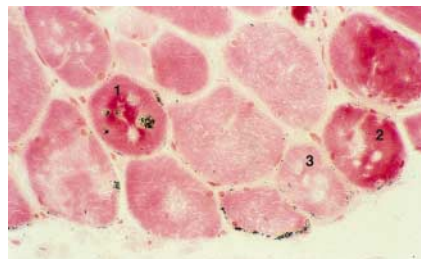


Figure 8. Black deposits of calcium are seen both around some affected fibres and within the vacuoles of one fibre (fibre 1), while in other fibres (fibres 2-3) the vacuoles are negative. $\times 80$

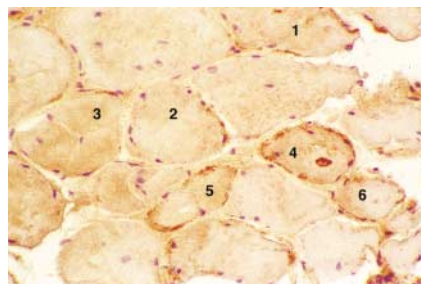


Figure 9. Immunoreactivity for complement complex 5b-9 (membrane attack complex, MAC) is present on the sarcolemma of fibres 1-6 and within one vacuole in fibre 4. $80\times$.

Structural changes

Fibre size variation of both fibre types is markedly increased. Definite necrosis, phagocytosis, inflammation, or fibrosis are not observed at younger ages, but later, fibrosis and fat accumulation occur. The key feature is vacuolation of sarcoplasm (Figure 2). In contrast to common rimmed vacuoles, these are bound by a dystrophin positive membrane (Figure 3). Many of the vacuoles are also immunopositive for laminin, and the affected fibres are surrounded by multiple layers of basal lamina (Figure 4); both features being

discernible in detail by electron microscopy (Figures 6, 7). The vacuoles contain cell debris (degenerating organelles, granular material, and membrane whorls), which is also present between the layers of the multiplied basal lamina, and both compartments stain positively for acid phosphatase and other lysosomal enzymes (Figure 5). The surface of the affected fibres is also decorated by deposited calcium (Figure 8) and complement C5b-9 membrane attack complex (MAC; Figure 9) (5, 6, 10).

Of the numerous forms of inherited muscular dystrophies only one, Danon's disease (3), exhibits the principal pathological feature seen in XMEA, namely vacuoles decorated by antibodies to dystrophin and laminin and containing lysosomal enzymes (chapter 8.1.2). Moreover, all Danon patients have cardiomyopathy and many have mental retardation (7, 8). Like XMEA, Danon's disease is X-linked, but the 2 conditions are not allelic, the latter resulting from defects in LAMP-2, the gene of which maps to Xq24 (8). The possibility that Emery-Dreifuss muscular dystrophy, the gene of which is also located in the Xq28 region, were allelic with XMEA despite its clearly different clinical picture has been excluded by direct sequencing of the emerin gene (11).

Future perspectives

Establishing the definite pathogenesis of this peculiar inherited myopathy must await identification of the gene defect. The Xq28 locus contains a vast number of genes, and an international collaborative effort is underway to study candidate genes for mutations. This work would however be greatly advanced through the identification of recombination events in new families.

References

1. Auranen M, Villanova M, Muntoni F, Fardeau M, Scherer SW, Kalimo H, Minassian BA (2000) X-linked vacuolar myopathies: two separate loci and refined genetic mapping. *Ann Neurol* 47: 666-669
2. Ciccodicola A, D'Esposito M, Esposito T, Gianfrancesco F, Migliaccio C, Miano MG, Matarazzo MR, Vacca M, Franze A, Cuccurese M, Cocchia M, Curci A,

Terracciano A, Torino A, Cocchia S, Mercadante G, Pannone E, Archidiacono N, Rocchi M, Schlessinger D, D'Urso M (2000) Differentially regulated and evolved genes in the fully sequenced Xq/Yq pseudoautosomal region. *Hum Mol Genet* 9: 395-401

3. Danon MJ, Oh SJ, DiMauro S, Manaligod JR, Eastwood A, Naidu S, Schliselfeld LH (1981) Lysosomal glycogen storage disease with normal acid maltase. *Neurology* 31: 51-57

4. ENMC (2000) European Neuromuscular Centre, Workshop 77, <http://www.enmc.org/workshops/reports.html>

5. Kalimo H, Savontaus ML, Lang H, Paljärvi L, Sonninen V, Dean PB, Katevuo K, Salminen A (1988) X-linked myopathy with excessive autophagy: a new hereditary muscle disease. *Ann Neurol* 23: 258-265

6. Louboutin JP, Villanova M, Lucas-Heron B, Fardeau M (1997) X-linked vacuolated myopathy: membrane attack complex deposition on muscle fiber membranes with calcium accumulation on sarcolemma. *Ann Neurol* 41: 117-120

7. Muntoni F, Catani G, Mateddu A, Rimoldi M, Congiu T, Faa G, Marrosu MG, Cianchetti C, Porcu M (1994) Familial cardiomyopathy, mental retardation and myopathy associated with desmin-type intermediate filaments. *Neuromuscul Disord* 4: 233-241

8. Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, Mora M, Riggs JE, Oh SJ, Koga Y, Sue CM, Yamamoto A, Murakami N, Shanske S, Byrne E, Bonilla E, Nonaka I, DiMauro S, Hirano M (2000) Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). *Nature* 406: 906-910

9. Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lullmann-Rauch R, Janssen PM, Blanz J, von Figura K, Saftig P (2000) Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* 406: 902-906

10. Villanova M, Louboutin JP, Chateau D, Eymard B, Sagniez M, Tome FM, Fardeau M (1995) X-linked vacuolated myopathy: complement membrane attack complex on surface membrane of injured muscle fibers. *Ann Neurol* 37: 637-645

11. Villard L, des Portes V, Levy N, Louboutin JP, Recan D, Coquet M, Chabrol B, Figarella-Branger D, Chelly J, Pellissier JF, Fontes M (2000) Linkage of X-linked myopathy with excessive autophagy (XMEA) to Xq28. *Eur J Hum Genet* 8: 125-129