CONGENITAL MYOPATHIES

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Congenital myopathies are a heterogeneous group of disorders characterized by muscle weakness and typical histopathological changes at muscle biopsy. In spite of recent advances on molecular genetics, their classification is still based on morphological criteria. Phenotypical and genetic heterogeneicity are common findings. The clinical symptoms usually appear in infancy, but adult-onset cases have been described. In this review, we focus on the current knowledges on congenital myopathies and we report our experience on adult-onset cases.

INTRODUCTION

Congenital myopathies (CMs) are muscular disorders characterized by early-onset and specific histopathological abnormalities (1) which over the years have led to a morphological classification. CMs are a relatively recent group of diseases, the first entity being described in 1956. They were included in the differential diagnosis of "floppy infant syndrome", particularly in cases with non fatal course. In recent years, advances in molecular genetics have modified the nosology, but muscle biopsy is still the cardinal tool for their definition. Clinical manifestations usually include neonatal hypotonia, delayed developmental milestones, difficulty in crying and feeding and variable degrees of muscle weakness. CMs occasionally manifest in adulthood with atypical or non specific clinical symptoms. Serum levels of CK are normal or slightly elevated; clinical course tends to stabilise, although fatal course is not excluded. There is clinical and even intrafamilial heterogeneity. About 40 entities have been described and are divided into: i) classical forms; ii) forms that are not clearly defined but usually recognised by researchers; iii) forms of uncertain nosography. The definition of CM includes specific morphological changes combined with muscle weakness. This review includes the prinicipal classical entities.

NEMALINE MYOPATHY

Nemaline myopathy (NM) is the most frequent CM (2). It is characterized by so-called rods in muscle fibres, which can be observed with modified Gomori stain; ultrastructurally they appear as fibrillar electrondense bodies related to the Z-line with alpha-actin as their major component. Clinical manifestations are heterogeneous, ranging from severe neonatal cases, to adult-onset paucisymptomatic forms. The following entities can be distinguished (3): i) severe neonatal; ii) congenital intermediate; iii) typical; iv) infantile-juvenile onset; v) adult-onset. In several cases there may also be cardiomyopathy. NM is usually characterized by diffuse weakness with particular involvement of proximal and respiratory muscles. In early-onset forms, difficulty in feeding, severe respiratory insufficiency and skeletal deformities are present. Course is usually slowly progressive with periods of stabilization. In early severe forms, respiratory failure is the main prognostic factor. Serum levels of CK are normal or slightly elevated. Differential diagnosis usually includes other CMs. The disorder has autosomal dominant or recessive transmission; sporadic cases due to "de novo" mutations of the skeletal alpha-actin (ACTA1) gene may occur. NM has remarkable genetic heterogeneity (4). To date, six genes responsible for most NMs have been identified; alpha-tropomyosin (TPM3), nebulin (NEB), alpha-actin (ACTA1), beta-tropomyosin (TPM2), troponin T1 (TNNT1) and muscle cofilin (CLF2). Most cases of NM are due to mutations of the ACTA1 and NEB genes (1). NM associated with NEB mutations seems to have a milder course. Homozygous mutations in the TPM3 gene were identified in four families with distal-myopathy-like NM. Heterozygous mutations in the TPM2 gene were identified in some patients with infantile-onset myopathy. Homozygous mutations in the NEB gene were recently associated with mild early-onset myopathy. Recessive mutations in the TNNT1 gene are responsible for severe forms of NM.

MYOTUBULAR MYOPATHY

Myotubular myopathy (MM) is a rare X-linked disorder with an estimated incidence of 1:50,000 (5). Diagnostic criteria include: male sex, neonatal onset, severe hypotonia, respiratory distress and associated signs such as swallowing disturbances, ophthalmoplegia and skeletal deformities. Prena-

tal onset includes polyhydramnios and reduced fetal movements. Prognosis may be fatal, depending on respiratory assistance in the first year of life. Milder forms with survival to adulthood have been reported. Female carriers may be asymptomatic or have mild myopathy, expression of which depends on the degree of inactivation of the X chromosome (6). Serum levels of CK are normal or slightly increased. The morphological hallmark is a high percentage (30-90%) of myofibres with centrally located nuclei. The nuclei are usually surrounded by a peripheral halo showing reduced enzyme oxidative activity (2). Immunocytochemistry shows increased expression of vimentin, desmin, utrophin and adhesion molecules. Pathogenesis is probably due to altered maturation of myofibres. This morphological change may resemble that of congenital myotonic dystrophy. The disease is caused by mutations in the myotubularin gene, a phosphatase expressed in most tissues, located at locus X28. About 200 mutations have so far been reported in about 300 families (7). Exons 4, 8 and 12 are "hot spots" for mutations.

CENTRONUCLEAR MYOPATHY

Centronuclear myopathy (CM) is characterized morphologically by centrally located nuclei in most fibres, associated with type I predominance and hypotrophy (2). It was long believed that CM and MM were different phenotypes of a same genetic entity. Now it is known that CM has autosomal dominant or recessive transmission, while MM is X-linked. Clinical manifestations are highly variable. Proximal and paraspinal muscles are particularly affected. Phenotypes with ophthalmoparesis, ptosis and distal myopathy are also known (8). Recessive forms usually have early-onset. Dominant CMs are usually associated with mutations in the dynamin 2 gene (DNM2), a protein involved in several cell functions (endocytosis, cell traffic, actin assembly etc.)(9). The amphyphysin gene was recently found responsible for recessive CM (10); the protein interacts with dynamin 2. Surprisingly, a "de novo" heterozygote mutation in the RYR1 gene was identified in a myopathy classified as CM.

CENTRAL CORE DISEASE AND MULTIMINICORE DISEASE

Central Core Disease (CCD) was the first CM identified (in 1956). It is histologically characterized by central or eccentric areas devoid of oxidative activity along the longitudinal axis of myofibres. Its estimated incidence is 3-5:100,000. Clinical presentation of the dominant forms usually occurs in infancy with motor delay and hypotonia. There is also wide intrafamilial clinical variability. Onset may occasionally be very severe with fetal akinesia (11). There is no correspondence between percentage of "cores" in muscle fibres and degree of muscle weakness. The distribution of weakness is typically proximal with particular involvement of the pelvic girdle and axial muscles. Ptosis and facial weakness may sometimes be predominant, but ophthalmoplegia and bulbar dysfunction are exceptional and even regarded as exclusion criteria. Orthopaedic complications such as hip dislocation, scoliosis, foot deformities and pes equinovarus are frequent. Apart from occasional mitral valve prolapse, cardiomyopathy is not typical of CCD associated with RYR1 mutations. Core-like changes were found in a group of patients with hypertrophic cardiomyopathy associated with mutations in the MYH7 (heavy beta-myosin chain) gene, but without skeletal myopathy (1). Association of cores and minicores in muscle biopsies has been reported in subjects with dilating cardiomyopathy and ACTA1 gene mutations (1). Respiratory distress is not typical of dominant CCD, but may be severe in the recessive form. CCD is considered allelic with susceptibility to malignant hyperthermia (MH), an anaesthesiological complication characterized by muscular rigidity, hyperpyrexia, rhabdomyolysis and metabolic derangement (12). Most patients with CCD and positive "in vitro" contracture test (IVCT) must be considered at risk for MH (12). Almost all CCD patients achieve unassisted walking; the disorder has a slowly progressive course with periods of stabilization. Serum levels of CK are normal or moderately increased. Muscle MR shows a characteristic pattern of muscle involvement; this finding may have diagnostic relevance.

As mentioned above, CCD is caused by RYR1 gene mutations (13); this gene is also implied in MH susceptibility. Since many subjects with MH susceptibility also show cores in muscle biopsies and CCD patients may be positive to IVCT, an association between CCD and MH was suspected several years ago. Most RYR1 gene mutations identified to date cause dominant CCD. Mutations in the N-terminal and central regions cause MHS phenotype, while mutations in the C-terminal region cause CCD phenotype (12). Recessive mutations may involve any region of the RYR1 protein and are usually associated with histological features of multiminicore. Due to the length of the RYR1 gene, "hot spot" regions are analyzed for mutations. Recent trends suggest to analyze larger portions of the gene for a more reliable diagnosis. Although the association between CCD and RYR1 mutations has been established, severe CCD syndromes exist in which molecular analysis failed to identify mutations in any part of the RYR1 gene.

As already mentioned, the diagnosis of CCD is based on observation of areas devoid of oxidative activity along the longitudinal axis of muscle fibres in muscle biopsies. These zones may be central or eccentric, single or multiple; they are present in type I fibres. The changes are believed develop postnatally; if muscle biopsy is performed at an early age, only predominance or uniformity

of type I fibres is sometimes observed. Ultrastructurally, the cores are characterized by absence of mitochondria, structural disorganization (unstructured cores) and accumulation of filamentous material. Structured cores maintain apparent sarcomeric structure. Differential diagnosis with respect to multiminicore myopathy (MM) may sometimes be difficult. MM is characterized by focal and multiple areas devoid of oxidative activity which do not involve the whole longitudinal axis of myofibres; they affect both types of fibres (2). A morphological continuum between the two pathological conditions was recently established. Although most cases of MM are associated with recessive mutations in the SEPN1 gene (selenoprotein), MM associated with recessive mutations in the RYR1 gene have also been reported (1). MM cases due to RYR1 mutations have particular and unusual clinical phenotypes, characterized by ophthalmoplegia, bulbar dysfunction and moderate respiratory distress, different from the phenotypes of classical CCD (14). Clinical phenotypes linked to recessive SEPN1 mutations are divided as follows:

i) bifid spine syndrome;

ii) classic with severe axial weakness, scoliosis and respiratory distress.

Ophthalmoplegia, shoulder girdle weakness and arthrogripposis may occasionally be found. Serum levels of CK are usually mildly elevated. The two conditions have different MRI patterns of muscle involvement: forms linked to the RYR1 gene show sparing of the gracilis and gastrocnemius muscles with respect to the sartorius and soleus, respectively. In forms linked to the SEPN1 gene, the gastrocnemius muscles are electively involved (15). Mutations in the RYR1 and SEPN1 genes have been found in about 50% of MM cases; other genes implicated in the disorder are still unknown.

ADULT-ONSET CONGENITAL MYOPATHIES

Experience of the Unit of Neurometabolic Diseases

Adult-onset congenital myopathies are fairly rare. The phenotype is extremely variable and non specific. We report our experience concerning diagnosis and management of adult-onset CMs.

Case 1

50 year-old woman, only child of unrelated parents.

25 years of age: severe neurosensorial deafness requiring hearing aid.

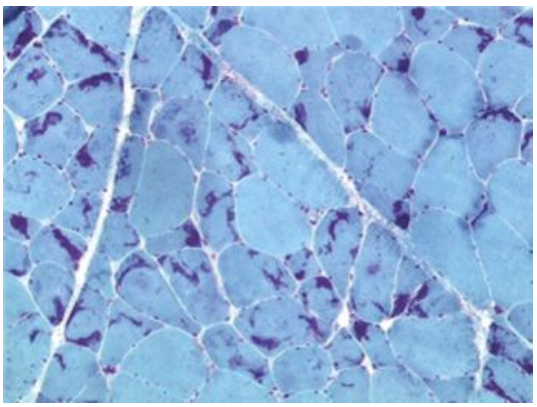
30 years of age: first walking difficulties and loss of balance; normal brain MR.

47 years: neurological examination showed weakness of pelvic girdle, mild tiptoe gait with retraction of Achille's tendon, mild ataxia, mild retraction of elbow and knee, absent deep tendon reflexes, distal hypotrophy of legs

Clinical and laboratory investigations also showed choroid-retinal inflammation, dilated cardiomyopathy and autoimmune thyroiditis. Normal serum CK. Muscle biopsy showed nemaline bodies in subsarcolemmal region of type I fibres (Fig.1). Oxidative stains showed core-like changes probably related to rod formation. Ultrastructural examination showed many filamentous structures arranged to form typical of rods. Molecular analysis of ACTA1 gene was negative.

This phenotype was particularly complex and unusual; in fact, the patient, apart from NM, showed neurosensory deafness, ataxia, ophthalmic disturbances and dilating cardiomyopathy. The clinical picture suggested mitochondrial myopathy. Since rods were observed mostly in type I fibres, we decided to perform molecular analysis of the alpha-tropomyosin gene. The test is currently in progress.

Fig.1: (Modified Gomori's stain)
Nemaline rods mostly in type I fibres



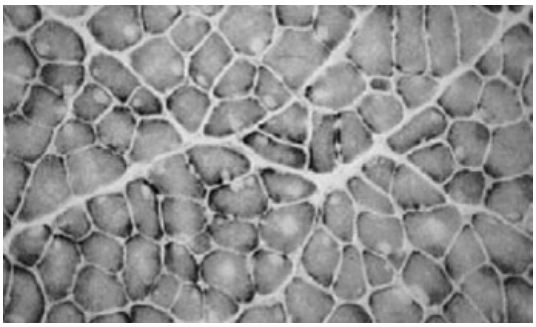
Case 2

70-year-old man.

60 years of age: cramps and pain in the legs with progressive difficulty in walking and climbing stairs. Family history negative for neuromuscular diseases. Serum CK 500-1886 IU/L. EMG evidence of diffuse myopathic signs. Neurological examination showed mild weakness of pelvic girdle, mild anserine gait and hypotrophy of right gastrocnemius muscle. Muscle biopsy showed cores in about 20% of type I fibres; predominance of type I fibres (fig. 2). Muscle CT showed fatty degeneration of vastus lateralis, tensor fasciae latae, semimembranosus, abductor magnus and biceps femoris muscles bilaterally, sparing antero-lateral muscles of thigh (Fig.3). Molecular analysis showed g.IVS105-70_71 insAT mutation in intron 105, probably a polymorphism and not responsible for the disorder. In this case, the morphology was typical of CCD, but the mutation is not regarded as pathogenetic. Furthermore, muscle CT scan did not show typical patterns of muscle involvement. Although CCD has not yet been asso-

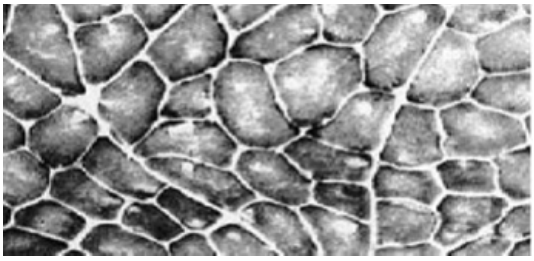
ciated with other genetic loci, genetic heterogeneity seems likely.

Fig.2: (NADH-TR stain)
Cores evident in about 20% of type I fibres.



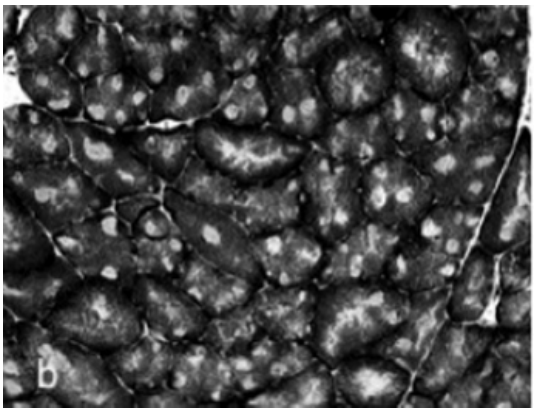
Case 3
72 year-old woman.
69 years of age: first weakness of lower limbs and walking difficulty. Serum CK 300-400 IU/L. Neurological examination showed mild waddling gait and weakness of pelvic girdle. Muscle biopsy showed cores in about 40% of type I fibres (fig 3). A sister who complained about asthenia had normal muscle biopsy and molecular analysis. Molecular analysis of proband showed a missense mutation 7372C>T (R2458C) in exon 46 of the RYR1 gene. This case was diagnosed as adult-onset CCD, although the clinical manifestations were non specific.

Fig.3: (NADH-TR stain)
"Core" structures in about 40% of type I fibres



Case 4
24-year-old woman with unrelated parents. The mother who had genetically-assessed type I myotonic dystrophy (600 CTG repeats in DM1 gene), died suddenly of heart failure (conduction disorder) at 50 years of age. At 10 years of age the proband experienced difficulty in walking and climbing stairs. At 22 years neurological examination showed weakness of pelvic girdle with positive Gowers manoeuvre, moderate calf hypertrophy, hyperlordosis, loose ligaments and reduced deep tendon reflexes. Serum CK was normal. EMG showed diffuse myopathic signs without myotonic phenomenon. Muscle biopsy showed slight variation in fibre size, 30% nuclear internalization and multiple small areas devoid of oxidative activity in type I and II fibres. Ultrastructural examination showed areas of structural disorganization with absence of mitochondria involving few sarcomeres. Molecular analysis detected a new missense mutation in exon 101 (14537C>T) of the RYR1 gene, leading to a A4846V substitution. This mutation was also detected in the mother, who unlike the proband, harboured a second mutation in the DM1 gene. This case suggests that rare disorders such as MM may segregate in families with relatively frequent neuromuscular disorders (16).

Fig.4: (NADH-TR stain)
Multiminicores in both types of myofibres.



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