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Clinical features of patients with dystrophinopathy sharing the 45-55 exon deletion of DMD gene

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Becker muscular dystrophy (BMD) was first described in 1953 by Emile Becker as a benign variant of Duchenne muscular Dystrophy (DMD). Compared with DMD, BMD is clinically more heterogeneous, with initial presentation in the teenage years and loss of ambulation beyond the age of 16 and a wide spectrum of clinical presentations, ranging from only myalgias and muscle cramps to exercise intolerance and myoglobinuria, asymptomatic elevation of serum creatin-kinase, or mild limb-girdle weakness and quadriceps myopathy. About 50% of patients become symptomatic by the age of 10 and the most part by the age of 20 years. However few patients can be free of symptoms till their fifties and cases of late-onset Becker Muscular Dystrophy have also been described.

In this report we describe the clinical features of patients with dystrophinopathy sharing a deletion of exons 45-55, occasionally or retrospectively diagnosed. These data are important for both the prognostic aspects of children presenting this dystrophin gene mutation, and for the genetic counseling in these families (reassuring them on the benign course of the disease), and last but not least to keep in mind a diagnosis of BMD in asymptomatic adults with mild hyperckemia.

Key words: Becker muscular dystrophy, dystrophin, asymptomatic BMD

Becker mucular dystrophy (OMIM 300376) is part of a spectrum of muscular disorders caused by pathogenetic variants in DMD gene encoding for dystrophin protein; this ranges in severity from asymptomatic increased levels of CK, cramps and myoglobinuria to progressive muscle diseases classified as Becker muscular dystrophy when skeletal muscle is primarily affected and as DMD gene-associated dilated cardiomyopathy (DCM) when the heart is primarily affected.

DMD gene (OMIM 300377), the largest found in nature measuring 2,5 Mb, was identified in 1987 through a positional cloning approach (1). It is composed by 79 exons and has 7 tissue specific promoters. Three promoters, localized upstream the first exon, control the transcription of the full length protein (dp427). They are named according to their main site of expression: (m - muscle), which induces transcription in skeletal and heart muscle but also in glial cells, (c - brain) specific for the brain and the retina as well, (p- Purkinje) which controls the expression in Purkinje cells and in muscle. The dystrophin gene also has at least 4 internal promoters, localized within introns, named according to the molecular weight of the produced protein: dp260 (retinal isoform, intron 29), dp140 (brain specific isoform, intron 44), dp116 (Schwann cells isoform, intron 44) and dp71 (general isoform, intron 55) (2). The shorter dystrophin proteins lack the actin-binding terminus but retain the cysteine-rich and carboxy-terminus domains that contain the binding sites for dystroglycan, distrobrevin and syntrophin. The genetic complexity is increased by the alternative splicing events; the spliced variants are formed both through the exclusion of some exons from the primary transcript (exon skipping) and

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by subversion of the reciprocal order of exons (exon scrambling) (2, 3). Taken together these mechanisms generate at least 18 transcripts (4).

Full-length dystrophin is a large rod-shaped protein with a molecular weight of 427kDa composed by 3685 amino acids organised in four structural domains: actin-binding domain, rod domain, cystein-rich domain and the COOH terminus region (4-6). These domains have different tri-dimensional structures and functional roles (7-11), as the protein is involved both in the interaction with integral membrane proteins (sarcoglycans, dystroglycans, syntrophin, and dystrobrevin complexes) assembled in the dystrophin-glycoprotein complex and in cellular communication and transmembrane signalling (12-14).

The most common mutational event is represented by intragenic deletions accounting for 65-70% of all mutations; duplications account for 10% of all mutations. Both might occur almost anywhere in the gene; however two deletion hot-spots are known – one located towards the central part of the gene, encoding for exons 45-55, and the other towards the 5', including exons 2-19.

There is no relation between size, region, domain of deletion and phenotype, which largely depends on whether or not the mutation disrupts the reading frame (15). Mutations which maintain the reading frame (in-frame mutations) generally result in abnormal but partially functional protein (BMD phenotype), while out of frame mutations cause a premature stop codon downstream, with the production of unstable mRNA that leads to a non-sense mediated decay and virtually undetectable levels of protein (DMD phenotype). However small amount of mRNA might escape this mechanism resulting in misfolded non functional proteins which exert heavy dominant negative effects (16).

The central and the distal rod-domains are likely to be functionally dispensable, as deletions in these domains have been associated with isolated hyperCkemia, myalgia and cramps, but not with weakness. This is the case of deletions in exons 32-44, 48-51 and 48-53, who had normal or near normal dystrophin concentrations (17-20). As a general rule deletions of large portions of *rod* domain result in BMD phenotype as long as they maintain the C and N-termini.

The reading frame hypothesis holds for over 90%; exceptions exist and involve both patients with BMD who carry out of frame deletions and DMD patients with in frame mutations, generally involving exons 3-7. The two mechanisms which may explain BMD phenotype in patients with out of frame deletions are the *exon skipping* event, occurring via an alternative splicing, and the presence of an additional translation start site located within exon 8 (21, 22). On the other hand, in-frame deletions disrupting the 5' actin binding domain may result in DMD phenotype (23).

In about 20-35% of dystrophynopathic patients sharing nonsense point mutations, small frameshifting deletions/insertions and splice site mutations have been identified (24); given the size of the gene, the identification of these mutations remain difficult.

BMD displays a high phenotypic variability ranging in severity from asymptomatic hyperCkemia, cramps and myoglobinuria to mild-moderate muscular involvement, characterized by a progressive symmetric muscle weakness and atrophy (proximal greater than distal) sparing calf muscles often hypertrophic; to be stressed that weakness of quadriceps femoris may be the only sign for a long time.

The clinical distinction between DMD and BMD is conventionally based on the age of wheelchair dependency: before age 13 years in DMD and after age 16 years in BMD; however BMD patients may remain ambulant until the late 40s and over.

Despite the milder skeletal muscle involvement, intractable heart failure from dilated cardiomyopathy is a common cause of morbidity and the most common cause of death; it may be the main clinical feature in patients affected by subclinical and mild BMD (25). Mean age at cardiomyopathy diagnosis is 14.6 years, similar to that in DMD (14.4 years) with the mean age of death the mid-40s (26, 27). In one study involving 28 individuals with subclinical and benign BMD between ages 6 and 48 years (28), 19 (68%) had myocardial involvement, although only two were symptomatic. Saito et al. (29) also demonstrated that of 21 individuals ranging from age 3 to 63 years (mean age 40 years), 33% had cardiac failure despite relatively mild skeletal muscle findings.

A significant role in the clinical variability of BMD patients likely relies on the structure of the internally truncated dystrophin produced by in-frame deletions in the central Rod domain. Indeed deletions leading to hybrid repeats should lead to more favourable phenotypes than deletions leading fractional repeats, although other factors may influence the clinical outcome, such as the presence/absence of binding factors, or other factors (SNPs and microRNAs) which could modulate the expression or the function of the protein (30). In particular, the deletion of exons 45-55 is considered to have a favourable prognosis, as the related phenotypes so far described, although limited to a small number of patients, range from asymptomatic patients to patients with only myalgia or exercise intolerance, mild BMD; however some cases of dilated cardiomyopathy have been also reported (31-35).

In this paper we wide the number of patients sharing this deletion, describing the clinical features of 9 new patients diagnosed at Cardiomyology and Medical Genetics of Second Naples University.

Patients and methods

Among the 249 BMD patients, regularly followed at our Clinical Service, Centre of Reference for muscular dystrophies for Campania and other Regions of Southern Italy, we retrospectively evaluated clinical data from nine patients in which a deletion of exons 45-55 in DMD gene has been found.

Clinical data included: family history, age at diagnosis and at last control, type of diagnosis (pre-clinical or clinical) and presence of muscle, cardiac and respiratory symptoms. The age of onset of myocardial and respiratory involvement was also noted.

Muscular involvement has been assessed through dynamic tests (Gower's time, time to get up from the floor and to climb 4 standard steps, in seconds) and since 2010 through North Star and 6MWT. Muscle strength was evaluated by manual MRC scale. Serum CK levels were also evaluated.

Myocardial involvement was assessed by evaluating ECG and Ecocardiography records, with a particular focus on ejection fraction (EF) and left ventricular volumes (VTD) adjusted for m2 (VTD/m2). We indicated as the onset of dilated cardiomiopathy an EF value < 50% and a VTD/m2 > 70.

Respiratory involvement was assessed through spirometric tests (FVC, absolute values and percentages). The onset of restrictive syndrome was considered for FVC values < 70%.

Results

The results are reported in Table I.

The average age of patients was 31,06 years (sd 24,10) at diagnosis, and 33,07 (sd 22,03) at the last control. For all of them but one the diagnosis was pre-symptomatic; however just one (D.G.) complained fatigue at the time of diagnosis but not at last control (19 years). Five patients were diagnosed after routine lab tests that showed high serum CK levels, while for four of them (T.A., T.R., M.C. and M.M.) the diagnosis was obtained retrospectively (T.R. maternal uncle of V.F.; M.C, T.A. and M.M respectively maternal grandfather, mother's cousin and mother's paternal uncle of S.A.). All patients have normal muscular strength (evaluated according MRC scale); the typical quadriceps hypotrophy and calf hypertrophy were observed in all. Serum CK levels ranged from 3,8 to 35,2x.

An initial ventricle dilation was observed at the last control in two patients, who presented a VTD/m2 > 70 :

Table I. Clinical features of patients with dystrophinopathy sharing the 45-55 exon deletion of DMD gene.

I.D.	Age at diagnosis	Age at last control	Symptoms at diagnosis	Family history	Diagnosis	Muscular involvement	Myocardial involvement	Respiratory involvement	CK
D.G.	11y 4m	19y 1m	Fatigue	Negative	Occasional	Calf hypertrophy	VTD/m2 70,6 EF 61%	No	5.5x
D.F.	14y 4m	17y 6m	No	Negative	Occasional	Quadriceps hypotrophy and calf hypertrophy	VTD/m2 65,4 EF 60,2%	No	24.4x
P.F.	49y 3m	49y3m	No	Negative	Occasional	Quadriceps hypotrophy and calf hypertrophy	VTD/m2 71 EF 63,3%	No	7.9x
S.A.	5y	8y	No	Negative	Occasional	Calf and quadriceps hypertrophy	EF 68%	No	23.4x
T.A.	39y 6m	39y6m	No	Negative	A posteriori	Quadriceps hypotrophy	VTD/m2 61,7 EF 66%	Obstructive	5.2x
M.C	62y3m	62y3m	No	Positive	A posteriori	Calf hypertrophy	VTD/m2 62 EF 64%		3.5x
M.M	66a	66a	No	Positive	A posteriori	Calf hypertrophy	VTD/m2 64,5 EF 63%		3x
V.F.	4y 9m	7y 2m	No	Positive	Occasional	Calf hypertrophy	EF 66,9%	No	35.2x
T.R.	34y 3m	36y 7m	No	Positive	A posteriori	Quadriceps hypotrophy and calf hypertrophy	VTD/m2 93,6 EF 53,5%	No	3.8x

Legenda

A posteriori: in these patients, maternal grandfathers of young children with BMD (< 10 years) the diagnosis was made after the occasional observation of hyperCkemia.

P.F. had a VTD/m2 of 71 at the age of 49 years, but his EF was 63,3%, and T.R. had a VTD/m2 of 93,6% at the age of 36 years, with EF of 53,5%.

Respiratory involvement (FVC was below 70%) was absent; only one patient had an obstructive syndrome related to cigarette smoke.

Discussion

Becker muscular dystrophy is a clinically heterogeneous disorder which may vary from asymptomatic forms to more relevant muscular and cardiac involvement (18, 31, 36). Genotype-phenotype correlation has been characterized in a previous study: deletions in the N-terminal region and in the rod-domain proximal to exon 45 have been associated with earlier onset of symptoms than mutations in the distal region (37). In particular patients sharing deletion of exons 45-55 seem to have a less severe muscular involvement, with only a few cases of dilated cardiomyopathy so far described (31-35).

Myocardial involvement, in particular, has been investigated in different studies so far; our group demostrated that a real, dilated cardiomyopathy is the most frequent type of myocardial involvement after the age of 20 and that the severity of cardiac involvement can be unrelated to that of skeletal muscle damage, confirming that cardiac dysfunction is a primary feature of Becker muscular dystrophy (38-41). Even in the absence of overt cardiomyopathy, there is an increased susceptibility to ventricular arrhythmias, whose severity appears closely related to the degree of left ventricular systolic dysfunction (42, 43). In addition we demostrated that BMD patients with deletions of exons 48-49 have a more severe cardiac involvement than others with different deletions (44). In another study Kaspar et al. focused on the correlation between the genotype and the age of onset of dilated cardiomyopathy in 3 different groups of BMD patients. They concluded that patients with deletions involving N-terminal domain (Group 1- deletion exons 2-9) and those causing a disruption of phase in rod domain (Group 2 – deletion exons 45-49) had an earlier onset of cardiomyopathy (early and mid 20's respectively) than that observed in case of in-phase deletions of rod-domain (Group 2) or disrupting hinge 3 domain (Group 3 – deletion exons 45-55) (mid 30's and 40's) (45). The not constant presence of cardiomyopathy seen in patients sharing deletion of exon 45-55 may be related to the different breakpoint locations eventually involving cardio-specific *cis* or *trans* regulatory elements (33, 34).

The clinical data reported herein are in keeping with previous observations, as none of our patients exhibited symptomatic muscle. However all patients showed as a constant feature quadriceps hypotrophy and calf hypertrophy. Furthermore only two patients showed at the last control (age 49 and 36, respectively) an increase in VTD/m2 values, suggesting an initial dilated cardiomyopathy to be monitored over time.

The definition of the clinical phenotype in patients carrying a deletion of exons 45-55 is important because its impact on several aspects of the disease: a) prognosis, often extremely benign, allowing less frequent follow-up and less aggressive therapies than in patients with more deleterious mutations; b) genetic counselling in these families, reassured about future pregnancies and prenatal diagnoses. The case of patients indirectly diagnosed in their sixties thanks to a positive family history (mother's father and uncle of S.A.) highlights the importance to extend the clinical assessment and eventually the molecular diagnosis on the paternal side of mothers of new very young diagnosed BMD patients.

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