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Chapter one

Signs and symptoms of Duchenne muscular dystrophy and Becker muscular dystrophy among carriers in the Netherlands: a cohort study

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Abstract

Background Carriers of Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) may show muscle weakness or dilated cardiomyopathy. Studies focusing on skeletal-muscle involvement were done before DNA analysis was possible. We undertook a cross-sectional study in a population of definite carriers to estimate the proportion and to assess the clinical profile of carriers with symptoms. We also assessed a possible correlation between genotype and phenotype.

Methods Carriers of DMD and BMD, aged 18-60 years, were traced through the files of the central register kept at the department of Human Genetics in Leiden, Netherlands. For each carrier who agreed to participate a medical history was taken, and muscle-strength assessment by hand-held dynamometry and manual muscle testing and cardiological assessment were done.

Findings Hundred and twenty-nine carriers of muscular dystrophy (85 DMD, 44 BMD) participated in the study. In 90 women from 52 (70%) families, 37 different mutations were found. 28 (22%) women had symptoms. Twenty-two (17%) had muscle weakness, varying from mild to moderately severe. Muscle weakness was found in carriers of DMD and BMD, but dilated cardiomyopathy was found only in seven (8%) carriers of DMD, of whom one had concomitant muscle weakness. There was an unexpectedly high proportion of left-ventricle dilation (18%). No genotype-phenotype correlation was found.

Interpretation Clinical manifestation of muscle weakness, dilated cardiomyopathy, or both can be found in about a fifth of carriers of DMD and BMD. If left ventricle dilation is taken into account, the proportion of carriers with symptoms is even higher, amounting to 40%.

Introduction

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive disorders caused by mutations in the dystrophin gene. It has long been known that carriers of DMD may also have symptoms of the disease. A summary of all case reports until 1970 was reported by Penn and colleagues.[2] The clinical picture of carriers with symptoms can vary from muscle pain and cramp on exertion at one end of the spectrum, to severe muscle weakness leading to wheelchair dependency on the other end.[3,4] If weakness is present, it is commonly mild, predominantly asymmetric and proximally distributed.[3,5,30,31] The pelvic girdle is more frequently and earlier affected than the shoulder girdle. Age of onset is also variable, ranging from the first to the fourth decade. Onset before the age of 15 usually leads to severe involvement.[3-5,30-32] Carriers of BMD rarely have symptoms—since the first report by Moser in 1971[6], only few cases have been described.[5,7-11,22,33]
Several mechanisms may cause symptoms in carriers of DMD and BMD. First, X-autosomal translocations with the breakpoint in the Xp-21 region can account for clinical signs.[34] These translocations lead to skewed X-inactivation of the normal dystrophin gene at an early stage of development. Second, X-inactivation plays a part in monozygotic twins discordant for myopathic symptoms of DMD. Uneven X-inactivation of the normal X-chromosome appears in the affected twin.[35-38] The same mechanism is suggested in symptomatic carriers: if X-inactivation is a random process, one might expect statistical outliers with varying degrees of symptoms of DMD. Evidence supporting this view has been derived from X-methylation studies, showing a picture of the activity state of X-chromosomes in carriers of the mutated dystrophin gene.[39-41]
Since 1967, abnormalities in electrocardiograms (ECG) like those seen in DMD patients[42] have been recognised in carriers of DMD.[4,22,30,43] It has become clear that severe cardiac involvement— dilated cardiomyopathy— can also occur in carriers, and may or may not be accompanied by muscle weakness.[22,25-29,44,45]
Two surveys have been done that showed that 2.5 - 7.8 % of carriers of DMD and BMD had clinical abnormalities.[3,5] Based on these results the prevalence of carriers with clinical abnormalities in the female population was estimated to be one per 45.000 and one per 100.000, respectively. However, one study[3] was executed before the discovery of the dystrophin gene and its protein product. In the second survey linkage analysis was used in a subgroup of the carriers, and some of them may therefore not have been definite carriers.[5] In those two studies cardiac involvement was not taken into account. In an Italian study, there was a high proportion of abnormal cardiac findings. Muscle weakness was not, however, systematically assessed.[29] These methodological shortcomings urged us to start a collaborative

study in a population of obligate and DNA proven carriers of DMD and BMD in the Netherlands. The objectives were to estimate the proportion of carriers who have symptoms, to assess the degree of severity of muscular and cardiac involvement, and to establish possible associations between phenotype and genotype.

Patients and Methods

Patients

Carriers of DMD and BMD were traced through the files at the Department of Human Genetics, University of Leiden, where registration of all families affected by DMD and BMD has been kept since 1982. Carriers were considered to be definite when: they were found to be obligate carriers after pedigree analysis (definite X-linked inheritance); when a mutation in the dystrophin gene was found; or when linkage analysis revealed a chance of more than 99% for carriership.[46-48] Furthermore, when linkage analysis was applied, it was taken to be informative only in carriers who belonged to large families.

Sporadic symptomatic carriers were not enrolled in our study, since selection bias would have been introduced, because one might expect that in absence of a family history of DMD or BMD, symptoms and signs have to be pronounced before they are recognised.

All carriers were contacted with the cooperation of the clinical geneticist who had given genetic counselling. Only carriers aged 18-60 years were invited to participate, because muscle strength is more or less constant in this age-group. Severe comorbidity had to be absent. All carriers who agreed to participate in the study were examined by EMH.

Methods

Investigations included full medical history, neurological examination, muscle-strength assessment by manual muscle testing with the use of the Medical Research Council scale [49], hand-held dynamometry[50] and a cardiological work-up consisting of electrocardiography and echocardiography to assess dilated cardiomyopathy (DCM). DCM was defined by a dilated left ventricle, corrected for body-surface area and impaired systolic function on echocardiographic examination. Systolic function was classified as impaired if fractional shortening was less than 28%, global hypokinesia was seen at two dimensional echocardiographic examinations, or both.[51,52]

The following categories of carriers were distinguished: (1) carriers with no history of frequent muscle pain, cramps and no muscle weakness or DCM; (2) carriers with regular muscle cramps and pains, affecting daily life activities, but without apparent muscle weakness; (3) carriers with muscle weakness in at least one muscle group as established by hand-held dynamometry, but not by manual muscle testing (mild weakness); (4) carriers with muscle weakness in at least one muscle group established both with manual muscle testing and hand-held dynamometry; moderate (MRC= 4) or severe (MRC<4) weakness; (5) carriers with DCM. We designated groups (3), (4), (5) as carriers with symptoms.
DNA-analysis was done in all carriers. Screening for deletions in the dystrophin gene was done with two PCR kits.[53,54] Genomic DNA from all carriers was also analysed by Southern blot and cDNA hybridisation utilising cDNA probes across the dystrophin gene.[55,56]
Serum creatine kinase activity (upper reference limit: 193 U/l) was measured at 37°C by the method proposed by the International Federation of Clinical Chemistry.[57]
The study was approved by the Medical Ethics Committee of the Academic Medical Centre, Amsterdam. Each participant gave informed consent to taking part in the study.

Statistical analysis

We analysed possible associations between variables with χ^2 tests and we used *t* tests to compare means of serum creatine kinase activity.

Table 1 *Skeletal muscle signs and symptoms in 127 carriers of Duchenne and Becker muscular dystrophy*

Clinical group	DMD		BMD		Total	
	n	%	n	%	n	%
No symptoms / signs	64	76	35	81	99	78
Myalgia / cramps	4	4.8	2	4.7	6	4.7
Muscle weakness	16	19	6	14	22	17
95% CI[a]	*10.6-27.4*		*3.6-24.4*		*10.7-23.7*	
Mild[b]	*8*	*9.5*	*2*	*4.7*	*10*	*7.9*
Moderate[c]	*8*	*9.5*	*4*	*9.3*	*12*	*9.4*
	84		43		127	100

[a] 95% confidence interval; [b] Measured only with hand-held dynamometry;
[c] Measured with manual muscle testing and hand-held dynamometry

Results

A list with 275 names of definite carriers (198 DMD, 77 BMD) aged 18–60 years was extracted from the files of the Department of Human Genetics, Leiden. Seven carriers had died or were too ill to be included, leaving 268 names (193 DMD, 75 BMD). For 16 carriers (14 DMD, two BMD) no address could be found, and 28 carriers (26 DMD, two BMD) were not invited for logistical reasons. A total of 224 letters (153 DMD, 71 BMD) were sent out. Forty-four carriers (31 DMD, 13 BMD) did not respond to the invitation. Fifty-one carriers (37 DMD, 14 BMD) refused to participate in the study. Finally, 129 carriers (85 DMD, 44 BMD), from 74 families, agreed to participate—129 (48%) of 268 of the carrier file in Leiden and 129 (58 %) of 224 of the posted letters.

All 129 carriers were seen by EMH between June, 1994, and July 1995. The mean age of the participants was 36.9 years (range 18–58 years; median 36; SD 9.5 years). In table 1 the proportion of muscle weakness for DMD and BMD carriers, respectively, is given. In two carriers muscle strength could not be properly assessed because of lack of cooperation. Therefore, they were excluded from the analysis of muscle weakness. Seven (8.2%) DMD carriers had dilated cardiomyopathy, one also had muscle weakness. Twenty-eight (22 %) carriers had symptoms according to our criteria (muscle weakness or dilated cardiomyopathy). However, in addition 23 carriers (18%) had echocardiographic evidence of left-ventricle dilation (with normal systolic function; table 2).

Table 2 *Dilated cardiomyopathy (DCM) and left ventricle (LV) dilation in DMD/BMD carriers*

	DMD		BMD		Total	
	n	%	n	%	n	%
DCM	7	8	0	0	7	5
LV dilation	16	19	7	16	23	18
	23	27	7	16	30	23

Twenty-two carriers with muscle weakness originated from 20 families. Four of these carriers were related—mother and daughter and two cousins. In eight (36.4%) carriers muscle pain or cramps were the first symptoms and six (27%) carriers had experienced muscle weakness as the presenting symptom. Eight (36.4 %) carriers did not report muscle weakness, but were identified during our study. Mean age at onset of symptoms was 33.6 years (16–48; SD 8.9). Mean time between onset of symptoms and time of current investigation was 4.9 year (1–21; SD 5.3). In

18 (81.8 %) carriers muscle weakness was predominantly asymmetric. Four carriers had symmetric muscle weakness. Nine (41%) carriers had weakness limited to shoulder girdle or upper arms, in five (23%) pelvic girdle or upper legs were affected, and in eight (36 %) upper and lower limbs were involved. The most commonly affected muscles are listed in table 3.

In 90 (69.8%) women from 52 families, 37 different mutations were found: 34 deletions and three duplications. They were located in the proximal part, in the rod domain and distal part of the dystrophin gene. In 24 (18.6%) individuals carriership was established by linkage analysis. Fifteen carriers (11.6%) in whom no mutation was found were obligate on the basis of pedigree analysis (definite X-linked inheritance). We did not find associations between site of mutation, age (18-60), muscle weakness or dilated cardiomyopathy.

Serum creatine kinase activity was raised (2–10 times the upper limit of normal) in 45 (53 %) carriers of DMD and 13 (30 %) carriers of BMD. Mean serum creatine kinase activity was 306 U/l (48–1860). There was no significant difference in mean activity between carriers with and carriers without muscle weakness.

Table 3 *Affected muscle groups in 22 carriers with muscle weakness*

Muscle groups	Left	Right	Total
Elbow flexors	11	9	20
Shoulder abductors	8	7	15
Knee extensors	7	6	13
Gluteal muscles	4	5	9
Elbow extensors	5	2	7
Intrinsic hand muscles	6	2	8
Psoas muscles	5	1	6
Hip abductors	4	4	8
Pectoral muscles	2	2	4
Other	11	3	14

Discussion

In this study we tried to include as many carriers within each family as possible to keep ascertainment bias to a minimum. However, we had no information about the carriers who did not respond to our invitation and only occasional information about the women who refused to participate. A selection bias may therefore have occurred towards carriers who had complaints and who wanted confirmation of their complaints by means of our study. On the other hand, eight (36%) carriers with muscle weakness did not have complaints beforehand but were recognised as having symptoms during our study.

The proportion of carriers manifesting with muscle weakness is higher than those reported in other studies. This discrepancy may be because we did not include possible carriers or because we applied hand-held dynamometry (which is more sensitive in detecting muscle weakness than manual muscle testing), which yielded ten (8%) carriers with symptoms.
Onset of symptoms did not occur before the age of 16 and mean age at onset was 33 years. Fourteen (70%) women who complained of myalgia, cramps, or muscle weakness did have muscle weakness. It is important to note there were no carriers with severe muscle weaknesses. However, individuals older than 60 years were not examined. In agreement with other studies muscle weakness was primarily proximal and asymmetric. However, in contrast to previous reports in nine (41%) women with muscle weakness only the shoulder girdle or upper arm was affected, which might also be explained by random X-inactivation.
Some groups have mentioned an increased proportion of carriers with symptoms among first-degree or more remote relatives.[3,4,58-62] Moser and Emery[3] found ten first degree cases in four families. In our study, of 34 pedigrees with more than one participating definite carrier per family (89 carriers in total), only two pedigrees had two carriers with symptoms. Two carriers of DMD were first-degree relatives and two BMD carriers were cousins. In the remaining 32 families, ten carriers with muscle weakness were the only individual with symptoms in the family. According to our data, familial occurrence of carriers with symptoms is a rare phenomenon.

In keeping with male patients, BMD carriers are less frequently and less severely affected than carriers of DMD. A small difference in symptoms was found between DMD and BMD carriers, 16 (19%) and six (14%) carriers respectively for muscle weakness and seven (8%) versus zero for dilated cardiomyopathy. Although dilated cardiomyopathy was found only in DMD carriers, left-ventricle dilation, which can be taken as the initial stage of DCM, was found in a proportion of carriers of DMD and BMD. The prevalence of dilated cardiomyopathy in our carrier population (5.4% or 5400 per 100 000) is higher than could be expected in the general female population (19.4 per 100 000).[63]
Of seven carriers with dilated cardiomyopathy, only one had myopathic symptoms, which might implicate that skewed X-inactivation is tissue specific.[28,45,64] In addition, other genetic factors may play a part in the development of dilated cardiomyopathy.[65,66]
We found that carriers who have symptoms make up 22% (28 women) of a cohort of definite carriers of BMD and DMD. Severe disabling muscle weakness among carriers is probably rare. We found no association between genotype and phenotype. Dilated cardiomyopathy occurred in carriers of DMD, although in only 8%

(seven patients) and generally without accompanying muscle weakness. However, in addition, in 18% (23 women) of carriers (both DMD and BMD) left ventricle dilation was found. Follow-up studies are needed to find out whether this condition is an early stage of dilated cardiomyopathy. On the basis of our results we strongly advocate cardiological investigation on a regular basis in carriers of DMD or BMD. Our study also shows that clinical geneticists should inform individuals who ask for genetic counselling about the increased risk for the potentially treatable cardiac complications.