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Chapter six

General Discussion

Since the earliest reports about Duchenne muscular dystrophy (DMD), some carriers are known to have signs or symptoms of DMD.[1,2] Reported clinical manifestations include calf hypertrophy, exertion dependent myalgia and cramps, and a variable degree of muscle weakness, ranging from slight asymmetric proximal muscle weakness with a predilection for the legs, to severe muscle weakness resembling the clinical picture of DMD boys.[3,4,31] Onset of muscle weakness in carriers is also variable and may start in childhood or develop in the fourth or fifth decade of life. Particularly in isolated cases of symptomatic carriers (girls without a family history of DMD), severe muscle weakness has been reported.[4,60] Symptomatic BMD carriers are rare. Only few instances of BMD carriers with signs or symptoms have been described since 1971.[6-9]
The identification of DMD in girls with X-autosomal translocations with the breakpoint in the Xp-21 region, has contributed to the discovery of the dystrophin gene.[34,147] These translocations cause non-random inactivation of the normal dystrophin gene at an early developmental stage. Skewed X-inactivation has also been suggested as cause for muscle weakness in DMD and BMD carriers.[39,40]
Other features recognised in dystrophinopathy carriers are 'DMD-like' electrocardiographic (ECG) abnormalities.[43,67,148] These ECG findings, including high R-waves in the right precordial leads and narrow deep Q-waves in lateral and inferior leads, have been reported since the sixties and some of them have been used as criterion to discriminate sporadic symptomatic carriers from girls with limb girdle dystrophy.[1] Only recently, it appeared that carriers could have dilated cardiomyopathy, without muscle weakness.[25,27,44,45] A large study of dystrophinopathy carriers even yielded a high proportion of females with cardiac involvement.[29] With the discovery of the dystrophin gene and its protein product dystrophin[77,78], it has become much easier to identify dystrophinopathy patients and carriers. Given the fact that previous studies were done in the premolecular-genetic era and did not include assessment of cardiac involvement [3,5], we initiated a survey among definite carriers of DMD and BMD in search of skeletal muscle and cardiac signs and symptoms.

Prevalence of symptomatic carriers

In two previous studies, the prevalence of carriers with muscle weakness was found to be 2.5% and 7.8%, respectively.[3,5] In a group of 129 definite carriers, we found that 22 carriers (17 %) had muscle weakness of mild to moderate severity (**chapter 1**). When dilated cardiomyopathy (DCM) is taken into account the proportion of symptomatic carriers rises to 22% (28 carriers) (**chapter 1**). The proportion of carriers with muscle weakness is two to seven times higher compared to the previ-

ously conducted surveys. One could argue that this is caused by selection bias. However, we have tried to include as many carriers from each family as possible. Furthermore, we did not include sporadic symptomatic carriers in our study, which would have artificially inflated the proportion of symptomatic carriers. Lastly, 36% of carriers with muscle weakness were identified as such during our study and did not have complaints beforehand. We used hand-held dynamometry to detect muscle weakness, which yielded ten carriers (8 %) with muscle weakness. This explains the main difference between our study and that of Moser and Emery.[3]

Profile and severity of skeletal muscle symptoms

It is noteworthy that in our study none of the carriers had severe muscle weakness (**chapter 1**). Muscle weakness was considered severe in the presence of weakness less than grade 4 (Muscle Research Council (MRC) scale) in at least one muscle group manually tested. Moderate was assigned to weakness MRC grade 4 in at least one muscle group, with or without abnormal hand-held dynamometry. Weakness was mild when manual muscle testing was normal (MRC grade 5), but hand-held dynamometry was abnormal. We did not designate carriers with frequent myalgia and cramps as symptomatic. However, one should bear in mind that this distinction in clinical groups on the basis of signs and symptoms does not necessarily relates to quality of life. It is conceivable that in individual cases some degree of muscle weakness, which is classified 'mild' in our study, is perceived 'severe' by a carrier. Carriers should therefore be informed, but the information has to be put in perspective. In our study, no carrier had become wheelchair-dependent. The chance to have some degree of muscle weakness is higher when there are complaints of muscle weakness, cramps or myalgia. It seems, therefore, important to take these complaints seriously and to look for muscle weakness very carefully in these cases.

In contrast to males with a dystrophinopathy, in whom muscle weakness starts in the proximal leg muscles, 41% of carriers had muscle weakness limited to the shoulder girdle or upper arms (**chapter 1**). Two other reports have also found selective involvement of arms in carriers.[5,30] In most other reports more (severe) involvement of limb girdle and upper legs was found.[3,4,31] In most studies however, only a selection of carriers was investigated.[4,31] Our results can easily be explained by random X-inactivation.

Prevalence of cardiac abnormalities

Several anecdotal reports have indicated that dystrophinopathy carriers can develop dilated cardiomyopathy[25,27,44,45], sometimes necessitating heart transplantation.[149] One Italian study, which included 197 definite carriers, showed cardiac abnormalities in 44.5%, designated as 'clinically evident myocardial damage', including 'preclinical stage', 'hypertrophic stage', arrhythmia and dilated cardiomyopathy.[29] Their criteria for cardiac involvement are in part comparable with ours. 'Preclinical stage' cardiac involvement, which included pathological R-waves on ECG, was present in 46% of carriers in their study. We found 47 carriers (36%) with typical (DMD-like) ECG changes, including three carriers (2.4%) with bundle branch blocks (**chapter 2**). Politano et al. found bundle branch blocks and /or severe tachyarrhythmias on 24-hour ECG, in five carriers (3%), designated as 'arrhythmia'. In addition they identified 50 carriers (31%) with 'hypertrophic stage' cardiac involvement, which was scored on the basis of an increased width of the intraventricular septum (IVS) and an increased ratio between IVS and the left ventricle posterior wall on echocardiography. We also used these criteria but found no instances of hypertrophic cardiomyopathy (**chapter 2**). Eighteen DMD/BMD carriers (11%) in the Italian study had dilated cardiomyopathy. Our study detected seven carriers (5.4%, all DMD) with dilated cardiomyopathy and 18% (23 cases) with left ventricle dilation (both DMD and BMD, **chapter 1 and 2**). Left ventricle dilation was not scored or found as such in the Italian study.[29] Since we, as mentioned above, could not confirm the hypertrophic abnormalities observed by them, different techniques in performing echocardiographies might explain these differences.

Significance of cardiac abnormalities

At present, the significance of left ventricle dilation is uncertain. It suggests involvement of the left ventricle and could be the initial stage of dilated cardiomyopathy. Interestingly, in a follow-up study of 27 BMD patients, we noticed that four (15%) BMD patients had developed left ventricle dilation as well (**chapter 4**). In an earlier population based prospective study, with a mean follow-up of 7.7 years, it was found that an increase in left ventricular internal dimension on echocardiography is a risk factor for congestive heart failure in men and women without a myocardial infarction.[150] Thus, ventricular dilation could be the initial compensatory response of the failing heart, which finally leads to dilated cardiomyopathy and

heart failure. Conversely, left ventricle dilation may remain unchanged for many years.
An important finding of the Italian study is the progression of cardiac abnormalities in 14 carriers during a mean follow-up period of 5 years.[29] In addition, four carriers died: three as a consequence of heart failure, and one carrier with dysrhythmias died suddenly. When we compare the prevalence of dilated cardiomyopathy in the carrier population (5400 per 100.000) with the prevalence of idiopathic dilated cardiomyopathy in the normal female population (19.4 per 100.000), it is obvious that cardiac abnormalities are a major concern in dystrophinopathy carriers. Carriership is a risk factor for developing dilated cardiomyopathy. Follow-up studies in carriers are needed to find out if left ventricle dilation progresses to dilated cardiomyopathy and if life expectancy in carriers is decreased compared with the normal female population.

Is it necessary to screen carriers for cardiac abnormalities?

In our opinion, our results and those of others justify to contact carriers, who have been counselled by clinical geneticists in the past, via their general practitioner or patient support group and inform them about this potentially treatable complication. All recently counselled carriers should be informed by their clinical geneticist, and be adviced to have regular cardiological examinations. We did not investigate carriers younger than 18 years, but two DMD carriers (younger than 16 years) with dilated cardiomyopathy have been reported.[29,44] Until more data are available it seems not justified to screen carriers younger than sixteen for dilated cardiomyopathy.

Should all female members of dystrophinopathy patients have their carrier status determined?

At present, the only reason for a women with a positive family history for DMD or BMD to have her carrier status determined is, when there is a wish for progeny. If carriership is determined only because of a 5.4 % chance on dilated cardiomyopathy, a positive DNA test could not predict possible cardiac abnormalities. Instead it would be preferable, to test women at risk for cardiac disease with a diagnostic test with sufficient sensitivity and specificity to detect all women with dilated cardiomyopathy or left ventricle dilation or to rule out dilated cardiomyopathy with

certainty. The best test would be the one that provides a very high negative predictive value with an adequate positive predictive value.

Table 1 *Predicted effect of several diagnostic tests on the prevalence of left ventricle dilation and/or dilated cardiomyopathy*

	Dilated cardiomyopathy prevalence 5.4%			Left ventricle dilation and DCM prevalence 23.3%		
	all ECG	typ ECG	SCK ↑	all ECG	typ ECG	SCK↑
sensitivity	7	43	71	60	43	63
specificity	54	64	55	57	66	57
PPV	8	6	9	30	28	41
NPV	97	95	97	82	79	67
1-NPV	3	5	3	18	21	33

DCM: dilated cardiomyopathy; all ECG: all electrocardiographic abnormalities; typ ECG: typical electrocardiographic abnormalities; SCK↑: increased serum creatine kinase activity; PPV: positive predictive value; NPV: negative predictive value

Tests for severe cardiac abnormalities

1. ECG

Unfortunately, despite the proportion of ECG abnormalities found in our study, the ECG is not sensitive and specific enough to screen for severe cardiac abnormalities. We found typical electrocardiographic abnormalities (which are seen in DMD patients) in 47 carriers (36%, **chapter 2**). Two out of seven carriers with dilated cardiomyopathy had a normal ECG and two had non-specific ECG changes (**chapter 2**). The sensitivity and specificity of typical ECG abnormalities for dilated cardiomyopathy is 43% and 64%, respectively (table 1). Given a prevalence of 5.4%, the positive predictive value and negative predictive value can be calculated as 6% and 95%, respectively. An abnormal ECG would thus change the probability on dilated cardiomyopathy from 5.4 to 6% with a positive test and to 5% with a negative test (normal ECG).

2. Raised serum creatine kinase activity

In our study 45% of carriers had raised serum creatine kinase (SCK) activity. Five out of seven (71%) carriers with dilated cardiomyopathy had raised SCK. When a raised SCK activity is used to test for dilated cardiomyopathy, the post-test probabilities are 9% (positive test) and 3% (negative test; table 1). However, high CK activity is more likely to arise from damaged muscle tissue than damaged myocard

tissue. This is reflected by the fact that none of our carriers had CK/CK-MB ratio above 6%.

3. Heart-specific proteins
Measurement of heart-specific proteins, i.e. cardiac Troponin T (cTnT) and cardiac Troponin I (cTnI), seemed to be an interesting option to detect minor myocardial damage in carriers (**chapter 5**). In particular cTnI was believed to be a cardiac specific marker for minor non-ischemic myocardial damage. However, none of our carriers, including the ones with cardiac abnormalities, had detectable cTnI. Heart specific proteins are, therefore, of no value for detection of non-ischemic myocardial damage in dystrophinopathy carriers.

4. Natriuretic peptides
More promising in the near future are the atrial (ANP) and brain natriuretic peptides (BNP), which are secreted by the atria and ventricles in response to increased transmural pressure. Recent reports have shown that these peptides, BNP more than ANP, are raised in plasma in patients with symptomatic and asymptomatic left-ventricle systolic dysfunction and can be used for screening in a general population.[151,152] It was found that targeted screening of individuals at high risk of developing left-ventricle systolic dysfunction increased sensitivity of ANP and BNP. Another study showed that BNP-guided treatment of heart failure reduced total cardiovascular events, and delayed time to first event compared with intensive clinically guided treatment.[153] In only one study these peptides were used to evaluate treatment with ACE-inhibitors and beta-blockers in 11 DMD patients.[154] It was concluded that BNP and ANP were helpful in diagnosis of DCM and in monitoring the effect of treatment. Whether natriuretic peptides are helpful to detect early left ventricle dilation in dystrophinopathy carriers and patients (instead of dysfunction), should be subject of future research.

Dilated cardiomyopathy in BMD patients

The course of heart involvement was assessed in patients with Becker muscular dystrophy (BMD; **chapter 4**). In a follow up study over 12.5 years among 27 patients it was found that progression of cardiac abnormalities occurred in 17 patients (63%), and nine patients (33%) had dilated cardiomyopathy with or without congestive heart failure, in four ultimately leading to death (**chapter 4**).[20] These results are in concordance with those of others.[21,23,24,75,102,103] Based on these findings heart abnormalities can be considered an important determinant of prog-

nosis in BMD patients.[111,128,155]
Some authors have suggested that typical mutations are associated with dilated cardiomyopathy, such as deletions including exons 45-46[105], 47[106], 48-49[24,103] and 2-7[114]. However, all these mutations are part of both hot spot regions for mutations in BMD/DMD. We found deletions of exons 2-5, exons 45-55, exons 45-47 and a duplication of exons 16-34 in BMD patients with left ventricle dilation or DCM. Other authors also failed to find an association between mutation site and DCM.[23,102] A mutation in the muscle promoter region caused DCM without muscle weakness (Ned Tijdschr Geneeskd, in press).[13,14,102,106,115,116] However, the same mutation was also found in a BMD patient with mild muscle weakness.[126] It is therefore concluded that as in DMD, any BMD patient is at risk to develop DCM, irrespective the mutation site or type.

Therapy for dilated cardiomyopathy

It is important to detect DCM at an early stage because nowadays treatment modalities have become available which have proven to be effective in DCM, including idiopathic DCM (the one which most resembles dystrophinopathic DCM). ACE-inhibitors reduce the incidence of heart failure and the rate of related hospitalizations among patients with asymptomatic chronic heart failure (CHF)[129] and reduce mortality in symptomatic chronic heart failure, irrespective of the cause. It should, therefore, be standard therapy in dystrophinopathy patients and carriers with symptomatic and asymptomatic left ventricular dysfunction. At present, beta-blockers may have a possible but not yet proven effect on mortality in carriers and patients with CHF. Beta-blockers give a reduction of mortality in patients with symptomatic ischemic and non-ischemic CHF (New York Heart Association (NYHA) class II and III)[156-158] but their effectiveness in young patients with asymptomatic (NYHA I) and symptomatic *idiopathic* CHF (NYHA II and III) remains to be solved. Furthermore, the effect of both ACE-inhibitors and beta-blockers on left-ventricle dilation is unknown. One should bear in mind that some drugs which are effective in non-dystrophinopathy patients with CHF might not be effective in DMD and BMD patients. Therefore, therapeutic trials must resolve the effect of beta-blockers (and ACE-inhibitors in the case of left ventricle dilation) in the prevention of progression of DCM in carriers and patients.

Dystrophin analysis in carriers with muscle weakness

DMD carriers may show a mosaic pattern of dystrophin positive and negative fibers on immunohistochemical staining in muscle biopsy.[4,80-83,85,86,88-90,96] Related to the possible muscle weakness which may be present in carriers, dystrophin analysis could answer some important questions. *Is there a minimal amount of dystrophin in muscle to prevent symptoms in carriers? Is there a correlation between the amount of dystrophin and severity of clinical symptoms in carriers?* However, dystrophin analysis turned out to be very disappointing. In only three of 17 carriers (18%) with muscle weakness immunohistochemical abnormalities were found. Therefore, we concluded that there is no association between muscle weakness and dystrophin abnormalities (**chapter 3**). This relatively low proportion might in part be due to the fact that we only biopsied the left vastus lateralis muscle, which was not always affected. Moreover, even when weakness of the left quadriceps muscle was present (5 carriers), in only three instances dystrophin negative fibers were found. This could be explained by local variation of dystrophin abnormalities in one muscle. Otherwise, dystrophin positive myonuclei might compensate for dystrophin negative ones (biochemical normalisation). But it remains enigmatic that muscle weakness may become overt in aging carriers, whereas on the other hand dystrophin abnormalities become less obvious. A study of biopsies from symptomatic carriers showed a correlation between higher degree of dystrophin deficiency, higher CK levels and more severe clinical symptoms in isolated cases (girls without a family history for DMD).[86] All these cases were, however, selected on the basis of a clear mosaic pattern of dystrophin positive and negative fibers in muscle biopsy. No correlation was found in the familial cases, and this was ascribed to ascertainment bias in their study.[86] Indeed, it is likely that in isolated female carriers of DMD symptoms have to be severe before they are recognised, and therefore there is greater chance of having dystrophin abnormalities. Furthermore, isolated cases are usually younger than familial cases. Young carriers (below 10 years) tend to have more dystrophin abnormalities as compared to older carriers.[4,83,85,87,97]
The proportion of dystrophin abnormalities was higher in BMD than in DMD carriers (58% vs. 24%; $p = 0.025$), but this difference was caused by an abnormally sized or reduced intensity of dystrophin protein on Western blot in BMD carriers (**chapter 3**).

Carrier detection

1. SCK-analysis

Muscle involvement in carriers is reflected by serum creatine kinase (SCK) activity elevation. Traditionally, before dystrophin or mutation analysis was feasible, SCK activity has been used to detect DMD and BMD carriers. Earlier studies have found raised CK activity in 60-80% of DMD carriers[1,142-145] and in 42-62% of BMD carriers.[140,146,159] In our study only 53% of DMD carriers and 30% of BMD carriers had elevated SCK (**chapter 5**). Only one other study found similar data in DMD carriers.[141] Perhaps, the difference between our study and those carried out before the nineties, is due to the fact that in most of these investigations repeat measurements have been done, whereas we confined ourselves to one measurement. SCK activity was neither associated with muscle weakness in carriers, nor with dystrophin abnormalities, histopathological abnormalities in muscle tissue or age (**chapters 1 and 5**). However, we did find a decreasing trend of mean CK activity per age group in increasing age groups.

The all-over sensitivity of elevated SCK for carrier detection in our study was 45%. However, minor and major elevations above normal can occur in women with and without muscle diseases, rendering this a non-specific test for a dystrophinopathy. Nowadays, SCK determination alone is not used anymore as a diagnostic test for carrier detection.

2. Dystrophin analysis

In our study in 70% of definite carriers (and in 70 % of families) a mutation was detected by DNA analysis (**chapter 1**). In theory, dystrophin analysis could have been useful for carrier detection in 30% of carriers. Six asymptomatic carriers (26%) had one to several dystrophin negative fibres. This suggests that 26% of carriers in a carrier population could be detected by dystrophin immunohistochemistry of muscle tissue. This raises the issue of the specificity of dystrophin analysis as a diagnostic test. DMD patients have less than 3% of dystrophin protein in muscle tissue and BMD patients have 10-40% of normal dystrophin protein or have an abnormally sized and/or reduced amount of dystrophin on Western blot.[79,160] To date, no dystrophin negative fibers have been found in normal muscle tissue. A small amount of dystrophin negative fibers may be found however, in muscle tissue of patients with congenital muscle dystrophy.[161] In patients with polymyositis, focal interruption of dystrophin staining may be found.[162] In sarcoglycanopathies (autosomal recessive muscular dystrophies caused by mutations in genes encoding for four different glycoproteins of the sarcoglycan complex), patients may show diffuse hyporeaction or focal plasma membrane

interruption of dystrophin in muscle fibers.[163] In patients with congenital muscle dystrophy and sarcoglycanopathy this is thought to be due to muscle immaturity, which is shown by a similar co-expression of beta-spectrin and dystrophin in consecutive cryosections of muscle tissue. Spectrin staining in polymyositis patients can be normal.[162] Beta-spectrin is used to check the muscle membrane integrity. Regenerating fibers in patients with a dystrophinopathy, sarcoglycanopathy patients and inflammatory myopathy express low levels of dystrophin and spectrin and other sarcolemmal proteins.[161,163,164] In 14 BMD patients, 73% of regenerating fibers (detected with fetal myosin) were also negative for anti beta-spectrin staining.[164] Experimental regeneration in muscle tissue of non-dystrophic dogs has shown that the appearance of dystrophin precedes that of spectrin and the normal immunostaining pattern of both is restored by 10-14 days.[165] Thus, a dystrophin negative fibre in a muscle biopsy of a suspected dystrophinopathy carrier in combination with normal spectrin labeling confirms carriership. When a women without a family history for DMD/BMD presents with high SCK or muscle weakness, one should not rely on dystrophin analysis alone, but also investigate other possible myopathies.

Is there still a role for dystrophin analysis in carrier detection? Three of six asymptomatic carriers with abnormal dystrophin immunostaining had normal CK activity, and in one of these three no mutation was found (**chapter 5**). Therefore, in selected cases dystrophin analysis of muscle tissue might be helpful in carrier detection, provided that the processing and judgement of the immunohistochemical dystrophin stains of muscle biopsy is in experienced hands.