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**Title:** Clinical genetic aspects of Duchenne and Becker muscular dystrophy in the Netherlands  
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Duchenne/Becker muscular dystrophy in the family: have potential carriers been tested at a molecular level?
Original Article

Duchenne/Becker muscular dystrophy in the family: have potential carriers been tested at a molecular level?


Duchenne muscular dystrophy (DMD) is the most common inherited neuromuscular disease. After identification of the mutation in the index patient, family members can be reliably investigated. Carriers should be informed about their risk of having offspring with the disease and about their own risk for cardiomyopathy for which regular cardiac surveillance is recommended. In a small country like the Netherlands with well-organized genetic services, one would expect that most DMD families are adequately informed about the above mentioned risks for carriers. We have investigated whether women at risk had been tested at a molecular level. In the national Duchenne/Becker database 311 DMD and 99 Becker muscular dystrophy (BMD) patients had been registered up to 1 July 2009. These patients were asked to give information about the number of sisters and maternal aunts of the DMD/BMD patient and anything that was known about their genetic status and that of the mother. This information was compared with the information known at the genetic laboratory. Thirty-five of 104 adult sisters/maternal aunts of DMD patients with a 50% risk of being a carrier and 45 of 148 adult women with a 4.3% risk because of germ line mosaicism for DMD had not been tested by DNA analysis. Our study indicates that about one third of the potential carriers have not been tested. Given the possible far-reaching clinical consequences of being a carrier, further studies are needed to investigate the reasons why potential female carriers have not been tested.

Conflict of interest

None declared.

Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disease. Most patients become wheelchair bound before the age of 12, and their mean age of survival is 26 (1). DMD has an incidence of roughly 1 in 3500 (28.5 per 100,000) live male births (2). DMD is the most common X-linked recessive muscle disease and is caused by a mutation in the dystrophin-encoding DMD gene on the X chromosome which results in the absence of dystrophin in the muscles of the patient. Becker muscular dystrophy (BMD) is a less common and a milder form of dystrophinopathy. Dystrophin in the BMD muscle is present but in an aberrant and/or a reduced amount.
In two-thirds of the patients DMD is inherited from the mother, implying that the sisters of the patient have a 50% risk of being a carrier. In one third of the patients the mutation has arisen de novo (3). However, in the latter cases the mutation may be present in the mother as a germ line mosaicism, in which case the sisters have an estimated 4.3% risk of being a carrier (4).

Because DMD is a devastating disease for which there is no curative therapy so far, much emphasis has been put on prevention. For known DMD families, offering genetic counseling to women at risk is the first step toward prevention. The women are informed about their risk of being a carrier, the available test, the recurrence risks and their reproductive options.

Another aspect relevant to female carriers is their own health. It is estimated that about 10% of female carriers develop dilated cardiomyopathy (5). A recent study in the United States has shown that only 62.9% of the carriers are aware of their risk for cardiomyopathy (6).

At a consensus conference held in 2002, it was agreed that female carriers should be advised to start cardiological examination at diagnosis or at the age of 16 with follow-up examinations at least every 5 years (7).

In the Netherlands most, and from 1997 onwards all, DMD DNA diagnostics is carried out in the genetic laboratory in Leiden. For most of the children with DMD, a molecular test is performed according to the best practice guidelines (8), and genetic counseling is offered to family members. The Netherlands is a small country with 17 million inhabitants and clinical genetic departments in each of the eight university hospitals. Each DMD family can have counseling within a distance of <100 km from their home. During our work as clinical geneticists (A. H-E, M. H. B) and clinical molecular geneticists (E. B, H. B. G), we have come across several DMD families in which women appear to be unaware of their risk of being a carrier. These families prompted us to carry out this study (see examples of families under the Results section). To the best of our knowledge no studies have been performed on the results of cascade screening (9) in DMD and BMD. A study on childless young women at risk for DMD in Brazil who received genetic counseling showed that the magnitude of the genetic risk did not influence the request for DNA testing (10).

In this study we have investigated whether all women at risk for carrying the familial mutation in the DMD gene have been tested at a molecular level.

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Materials and methods

All patients/families that had been registered up to 1 July 2009, in the Duchenne/Becker database in the Netherlands (www.lumc.nl/duchenne), were asked to fill in a questionnaire about the phenotype of the patient as well as about the family history as a part of the registration procedure. The registration started during the spring of 2008.

Registrees were requested to list the number of brothers, sisters, maternal uncles and maternal aunts of the Duchenne/Becker patient. They were also asked whether each of the male members was healthy or affected with DMD/BMD. The women were also asked whether the mother of the DMD/BMD patients and the other female members had undergone carrier testing and if so, were they carriers.

The information from the questionnaires was compared to the information known at the genetic laboratory in Leiden. If two or more individuals from the same family were registered in the database, they were included as one family. The reasons for not including some of the families are given in Table 1.

In the investigated families where the mother was a carrier we checked whether the maternal grandmother, the sisters and the maternal aunts of the Duchenne/Becker patient had been tested at a molecular level at the genetic laboratory.

Subsequently we calculated the number of women who had a 50% risk of being a carrier. A woman was defined as having a 50% risk if she had not been DNA tested, was not an obligate carrier, had a brother with DMD/BMD and her mother was a proven carrier. We also calculated the number of women at a 4.3% risk because of

<table>
<thead>
<tr>
<th>Reason for excluding</th>
<th>DMD</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family abroad</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>No sample at the Leiden lab&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27</td>
<td>10</td>
</tr>
<tr>
<td>No information on the questionnaire&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Unidentified mutation</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Multiple family members</td>
<td>17 (out of 14 families)</td>
<td>15 (out of 13 families)</td>
</tr>
</tbody>
</table>

<sup>a</sup>No blood sample received by the laboratory in Leiden for a molecular test. Not known whether the families had been tested elsewhere.

<sup>b</sup>Information on the phenotype of the patient given but the section on the family history was left blank on the questionnaire.
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germ line mosaicism in the de novo families (4). Germ line mosaicism is defined as the presence of two or more genetically distinct cell lines confined to the precursor (germ line) cells of the egg or the sperm. In this study, a woman who has a brother with DMD/BMD was considered to have a germ line risk even if her mother did not have a mutation in her lymphocytes because of the possibility of a germ line mosaicism in the mother. Maternal aunts of a DMD/BMD patient were considered to have a germ line risk if the mother of the patient carried the mutation in her lymphocytes and the carrier status of the grandmother was not known. In BMD families, the maternal grandfather was not affected with BMD.

We counted the number of mothers and grandmothers tested for carrier status in families who were sent in before (old family) and after 1 January 1997 (recent family) to see if there was a possible difference in the number of women tested in the two periods.

We chose this date as it was exactly in the middle of the period between 1984, when DNA diagnostics of the DMD gene was started in Leiden, and 2009, the year in which most of this study was carried out.

The women at risk were divided into two groups: younger than and older than 16 years because cardiologic examination is recommended for carrier women from this age onwards (7). If the date of birth of a woman was not known, an estimate was made by considering her position in the pedigree where the ages of other family members were known.

If, in the families that were included in the study, the information on the questionnaire about one or more family members did not match the information present in the laboratory (Table 2), the information from the laboratory was used.

Results

On 1 July 2009, 311 Duchenne and 99 Becker patients had been registered at the national database. Among these, 89 DMD and 36 BMD patients were excluded from the study (Table 1), leaving 222 DMD and 63 BMD patients. The information in the questionnaire and the laboratory was concordant in 186 of 222 (84%, 95% confidence interval: 79–89%) DMD and in 43 of 63 (68%, 95% confidence interval: 57–80%) BMD cases. Table 2 shows the reasons for discordance in 36 of 222 DMD and 20 of 63 BMD families. Table 3 shows the number of women at risk in DMD families that had not had a DNA test. Eighty-six of 155 women with an a priori 50% risk of being a carrier had not been tested. This includes 35 of 104 (34%) women older than 16.

Seventy-seven of 180 women with a germ line risk had not been tested, including 45 of 148 (30%) women older than 16. It is noteworthy that we found germ line mosaicism in five de novo

Table 3. Number of women in DMD and BMD families with an a priori 50% risk of being a carrier and with a 4.3% risk because of a germ line mosaicism tested or not tested in the laboratory

<table>
<thead>
<tr>
<th>Age group</th>
<th>Women with a 50% risk</th>
<th>Women with a 4.3% risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not tested</td>
<td>Tested</td>
</tr>
<tr>
<td>DMD ≥ 16 year</td>
<td>35</td>
<td>69</td>
</tr>
<tr>
<td>DMD &lt; 16 year</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>Total DMD</td>
<td>86</td>
<td>120</td>
</tr>
<tr>
<td>BMD ≥ 16 year</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>BMD &lt; 16 year</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Total BMD</td>
<td>10</td>
<td>32</td>
</tr>
</tbody>
</table>

Table 2. Families included in the study where the information about family members on the questionnaire did not match that present in the laboratory

<table>
<thead>
<tr>
<th>Conflicting data on results obtained from DNA test between laboratory and the form</th>
<th>DMD</th>
<th>BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family member ‘known’ on the form but unknown in the laboratory</td>
<td>5/36 (14%)</td>
<td>3/20 (15%)</td>
</tr>
<tr>
<td>Family member not on the form but known in the laboratory</td>
<td>8/36 (22%)</td>
<td>2/20 (10%)</td>
</tr>
<tr>
<td>No information on female members on the form</td>
<td>8/36 (22%)</td>
<td>7/20 (35%)</td>
</tr>
<tr>
<td>Form confused, no conclusions could be drawn</td>
<td>8/36 (22%)</td>
<td>7/20 (35%)</td>
</tr>
</tbody>
</table>

DMD, Duchenne muscular dystrophy; BMD, Becker muscular dystrophy.

aFemale family member listed as a carrier on the questionnaire but not a carrier according to the DNA test or vice versa.
bFemale family member of an index patient in whom a mutation had been detected in Leiden, listed as a carrier or a non-carrier but never tested in Leiden.
cNo mention of female family members on the questionnaire but female relatives tested in Leiden or had come to light via testing of other relatives.
dFemale family member(s) mentioned on the questionnaire but without the (non)carrier status, known in the laboratory.
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Examples of families

Family A illustrates the use of unreliable tests for carrier detection and failure to test the maternal grandmother

The index (IV:4), born in 1975, was diagnosed with DMD at the age of 3 (Fig. 1 pedigree A). His older brother (IV:3) who had died at the age of 4 during a light narcosis had apparently had the same pattern of walking. Around 1980 the blood (probably creatine kinase (CK)) and muscle biopsies of the maternal grandmother and a maternal aunt were tested. They were not found to be carriers. A mutation was identified in the index (IV:4) at the age of 13. At the time, only a pediatrician was consulted. The sister of the index was tested and was found not to be a carrier. However, the mother and the maternal grandmother were not tested. In 1993 DMD was diagnosed in another 4-year-old child (IV:6) who carried the familial mutation. Subsequently, information from the carrier mothers led to the confirmation of DMD in yet another child (IV:10).

When we contacted the family in 2009 for permission to publish their pedigree, we learnt that a son of a sister of the maternal grandmother (III:1)
of the index patient had probably also had DMD. He was ambulatory when he was young but later became wheelchair bound and developed scoliosis. Old photographs showed enlarged calves. He died in 1966 at the age of 16 and was thought to have been affected with poliomyelitis. We also informed the mother of the index patient that her sister (III:4) had a risk of being a carrier. This was particularly important because her daughter (IV:2) was in her 20s and did not have a family yet.

Family B, an example of germ line mosaicism

Duchenne muscular dystrophy (DMD) was diagnosed and confirmed at the molecular level in the index at the age of 7 (Fig. 1, pedigree B). The family history was negative for DMD. A DNA test on lymphocytes showed that the mother was not a somatic carrier. However, the sister was found to be a carrier of the same mutation as her brother, proving germ line mosaicism in the mother.

Family C illustrates communication problems within a family

A pregnant woman was referred for genetic counseling because she knew that her mother had had three brothers who died at a young age and were possibly affected with DMD. The mother of the pregnant woman did not cooperate, so a prenatal test could not be performed. A boy was born. At the age of 2, he was referred for molecular testing because of suspicion of DMD and a mutation was detected. Subsequent testing of the mother of the DMD patient revealed that she was the carrier.

Discussion

In 1999, Hoogerwaard et al. (5) reported the results of a study performed on 129 Dutch carriers of DMD and BMD. They showed that for these women, being a carrier not only had consequences for their offspring but also for their own health, namely an increased risk for cardiomyopathy. One would expect that in the last 10 years this knowledge would have influenced the uptake of molecular testing of potential carriers. However, our study shows that even in the Netherlands, with its well-organized genetic counseling services, there is still a large number of women in DMD/BMD families who have not been tested at the DNA level.

In the 222 of 311 registered DMD families that could be analyzed, 86 women with a 50% risk of being a carrier and 77 women with 4.3% risk because of germ line mosaicism had not been tested. In general, it is agreed that carrier testing in minors should be deferred until the child can give proper informed consent (11). So, if we consider...
only women older than 16 it emerges that 34% of
dwomen with a 50% risk and 30% of the women
with a germ line risk had not been tested.

In the 63 of the 99 registered BMD families
that could be analyzed, 10 women with a 50% 
risk of being a carrier and 10 women with a germ
line risk had not been tested. The percentages of
women older than 16 who have not been tested
in the BMD families are 11% for women at a 50% 
risk and 22% for the women with a germ line risk.

The above percentages of the women at risk are
probably underestimates because we were able to
look at only the first- and second-degree relatives
of DMD/BMD patients.

There could be a variety of reasons why a
woman has not been tested: she does not wish
to have (more) children, she relies on the result
of an earlier (unreliable) test by serum creatine
kinase measurements or muscle biopsy (family
A), has moral or religious objections to testing,
she is unaware of her risks because of lack of
communication within the family or she is not
able to undergo reliable testing because her family
does not cooperate (family C). Molecular testing
of the maternal grandmother in DMD families,
where the mother is a carrier, is important for
genetic counseling. Two-thirds of these maternal
grandmothers are expected to be carriers (3). In
our study, the genetic status of 48 maternal
grandmothers was unknown in the 114 DMD
families where the mother was a carrier. More
grandmothers were not tested in recent families
(47%) than in old ones (33%). Besides the reasons
given above for sisters and maternal aunts, there
may be additional reasons why grandmothers had
not been tested. These include death, anticipated
guilt feelings should a mutation be detected or
lack of understanding of the inheritance pattern
especially if her healthy sibs have healthy sons.

Our observations illustrate the importance of
drawing attention to the germ line risk of which
many doctors and patients are not aware. In fact
the germ line risk is 4.3% (4) and as mentioned
earlier, we found proven germ line mosaicism in
five DMD families and in one BMD family. In
family B the healthy sister who asked for genetic
counseling prior to starting a family turned out to
be a carrier of the mutation, which was present
in the germ line cells of the mother. With this
knowledge, she and her partner can make informed
choices in planning their family. Furthermore, she
could be referred for periodic examination for
cardiomyopathy.

Agreement between information in the question-
naire and that present in the laboratory was high for
both DMD (84%) and BMD (68%) families, but

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more women from the DMD families were aware
of their risks. This may be because some BMD
patients with a very mild phenotype may not see
the importance of informing their relatives.

In 16% of the DMD and 32% of the BMD
families, there was a discrepancy between the
information obtained via the questionnaire and
that known in the laboratory. Among the reasons
given for the discrepancy in Table 2, the first
two are particularly worrisome. The first reason,
conflicting data on results DNA test between
laboratory and the form, demonstrates that in five
DMD and three BMD families women think they
are carrier/non-carrier whereas the result of the
DNA test showed the opposite. The second reason,
family member known on the form but unknown in
the laboratory, shows that in eight DMD and two
BMD families women think that they have been
tested whereas they have not been tested. Possible
explanations could be that either the respondent
was not well informed or that the women had been
tested elsewhere (DMD diagnosis was offered in
Groningen before 1997), even though the mutation
in the index patient had been detected in Leiden.
The information on the questionnaire will also
have been influenced by the memory and family
ties of the respondents.

Our results show that even in the Netherlands
genetic counseling and follow-up of families of
Duchenne and Becker patients are incomplete.
As a consequence, female family members are
not informed of their risk of being a carrier. In
some cases, this may have led to the birth of an
affected boy. Adequate cascade carrier screening
would have been helpful in these cases. The
results indicate that our standards of care need to
be substantially improved to enable us to offer
a possibility of prevention to all at-risk family
members.

The General Medical Council in the United
Kingdom issued a guidance named ‘Confidentiality’
in October 2009 (12). For the first time, giving
genetic information to protect the family has taken
precedence over protection of the privacy of an
individual (13).

According to the new advice, doctors should be
told explicitly that it may be justified to reveal
information in the interest of relatives even if
the patient objects. Such information could, for
example, alert family members with a genetic pre-
disposition to cancer to the need for surveillance or
treatment, or influence decisions on reproduction.
Both these aspects are valid also for DMD/BMD
families: carrier women need to undergo regular
cardiac surveillance and can use the information to
decide which reproductive options suit them best.
In a recent review on diagnosis and management of DMD (14, 15), genetic counseling is indicated as a part of the diagnostic process. However, so far the question of how the family should be informed has not been addressed. The question why so many women at risk have not been tested at a molecular level will be the subject of further research. For women relying on the result of an earlier unreliable test, for example serum creatine kinase measurements, it is clear that now there is a more reliable molecular test available. An ethical dilemma arises in approaching a woman who has not been tested because she was not informed about the risk of being a carrier, as a result of either lack of communication or lack of cooperation within the family. In the UK guidance of the General Medical Council, it is suggested that the patient’s identity should not be disclosed in contacting and advising the family members about their risks, if practicable. These last two words are crucial for it is often impossible not to disclose the patient’s identity. It is also not always possible to contact family members without the help of the patient. One form of lack of cooperation by the family members can be their refusal to provide a blood sample for identifying the mutation. This problem will be solved in the future with the availability of newer molecular techniques.

At present, in the genetic counseling practice in the Netherlands, the index case is handed a letter with which he or she can inform the family members. In this way the privacy of the index is protected because he or she can choose not to distribute the family letter. In a recent study by Van der Roest et al. (16), 88% of the family letters were distributed in the family and as a result 57% of the family members of patients with a high-risk genetic cardiac disease underwent screening. For diseases such as hereditary cancer with preventive options, on average 50% of family members undergo genetic testing (17, 18).

There is an ongoing debate on whether a more active approach of family members is justified, considering the fact that such a high percentage of family members does not undergo genetic testing. This study should help in bringing this problem under the attention of the doctors and those who care for DMD/BMD families. Thus, more women can be made aware of the implications of being a carrier, both for their offspring and for themselves, and can be offered molecular testing.

Acknowledgements

We thank all the patients, mothers and fathers who filled in the questionnaires. Without them this study could not have been performed. We thank Dr K. Madan for editing the English of this article.

References
