Focus on female carriers of dystrophinopathy: refining recommendations for prevention, diagnosis, surveillance and treatment.

263rd ENMC Workshop: 2nd Virtual meeting: Friday 26 November '21 (2 hours) 3pm-5pm UK time

Workshop Convenors
Alessandra Ferlini (AF), Anna Sarkozy (AS), John Bourke (JB), Ros Quinlivan (RQ)

Participants: Alexandra Breukel. Emily Crossley, Aleks Pietrusz, John Bourke, Lidia Gozalez, Pia Gallano, Jana Haberlova, Ines Barthelemy, Elizabeth Vroom, Linda Cripe, Allesandra Ferlini, Nicole Voermans, Esther Schmit, Louisa Pollitano, Michaela Guglieri.

Welcome to the workshop by the ENMC representative and organisers

Short review of the 1st virtual meeting (9th June '21)

AS began by restating the overarching aims of the workshop, which were to discuss and agree expert recommendations for the investigation and management of female carriers of dystrophin-gene variations (DMD).

Specific aims:

1) Raise priority and need for greater clinical and research focus on female carriers of Becker and Duchenne dystrophy [BDMD],

2) Highlight unmet needs,

3) Propose a clinically useful classification of carrier status

4) Demonstrate how available preventative and therapeutic approaches can improve patient outcomes

Justification for the workshop comes from the recognition that carriers of DMD/BMD gene variations present a complex and traditionally neglected patient group, whose best management remains ill defined. Currently clinical decision making is hampered by: (i) lack of robust definitions of "female carrier status" and "manifesting carrier"; (ii) lack of systematic natural history data on which to base recommendations for surveillance and management and (iv) inadequate validation of clinical outcome measures. These cumulative deficits have prevented a proper understanding of the needs of 'carriers' and, looking ahead, potentially also block access to preventative and novel therapies to those who would benefit from them. In addition and up to now, clinical trial research involving BDMD-gene carriers has been considered to be of "low priority". There is a pressing need, therefore, to prioritise research into the various and currently inadequatedly-understood implications of the 'BDMD-carrier-status' in order to correct the gaps in knowledge.

AS summarised the discussions from the June WS-session. Following that meeting, **a series of questions** were highlighted as needing further consideration. These were were circulated to give time -for participants to consider them ahead of this session and some had already provided brief responses to begin the discussions. The questions were:

Q1: From your data-sets and experience, does knowledge of the extent of X-chromosome inactivation result in differential expressions / manifestations in various tissues and organs?

Q2: When early skeletal muscle symptoms / signs are evident ('muscle manifesting carriers'), does their severity inevitably progress / deteriorate over time?

Q3: What is the prevalence of cognitive impairment, learning difficulties, anxiety / depression in your cohort?

Q4: Do you have experience in using steroids to treat skeletal-muscle weakenss? How acceptable was / is long-term steroid therapy to these patients? What benefits and/or adverse effects did you encounter? It may be possible to draw-up early guidance based on our amalgamated 'anecdotes' about experiences of steroid therapy in 'carriers'(?)

Q5: Some carriers develop a progressive form of dilated cardiomyopathy while others appear to have chronically 'borderline low' measures which remain stable. How robust is the evidence to support the emerging assumption that heart function inevitably deteriorates, particularly once fibrosis is evident on cardiac MR-imaging or early left ventricular dysfunction is seen on echo?

Summary of responses & discussions:

Q1: Does the extent of X-chromosome inactivation result in differential manifestations in the various organs/tissues?

LP reported data from a 50 patients cohort showing that blood and muscle can have same pattern of X Inactivation as they arise from same embryological cell-line. She also emphasied the point that cells in BDMD-carrying females are genetically mosaic for the gene variation. This means that they have both dystrophin negative and positive fibres. This is important because it can make the findings of muscle / organ biopsy, for example, misleading as it is subject to site specific variation.

AF agreed, pointing out that discordant results in the literature on the role / importance of X-chromosome inactivation on the effects of the condition may also be contributed to by the fact that the process occurs early during embryonic development as well as the variations highlighted by **LP** on the findings of tissue sampling. A literature review might be

helpful for the group to summarise current knowledge ahead of the planned face-to-face sessions and as a possible way of identifying how to investigate this topic further.

LP mentioned that she undertook such a review, encorporating a mixture of younger and older carriers of either BMD or DMD gene variations, about two years ago performed review of literature two years ago. This heterogeneous cohort may explain the complexity of her group's finings.

Emanuela Viggiano, Manuela Ergoli , Esther Picillo , Luisa Politano. Determining the role of skewed X-chromosome inactivation in developing muscle symptoms in carriers of Duchenne muscular dystrophy. Hum Genet. 2016 Jul;135(7):685-98.doi: 10.1007/s00439-016-1666-6. Epub 2016 Apr 21.

Viggiano E, Picillo E, Ergoli M, Cirillo A, Del Gaudio S, Politano L. Skewed X-chromosome inactivation plays a crucial role in the onset of symptoms in carriers of Becker muscular dystrophy. J Gene Med. 2017 Apr;19(4). doi: 10.1002/jgm.2952.PMID: 28316128

Viggiano E, Picillo E, Cirillo A, Politano L. Comparison of X-chromosome inactivation in Duchenne muscle/myocardium-manifesting carriers, non-manifesting carriers and related daughters. Clin Genet. 2013 Sep;84(3):265-70. doi: 10.1111/cge.12048. Epub 2012 Dec 20.PMID: 23110537

JB. Raised the question of how differences in the pattern of populations of dystrophin positive or negative cardiac myocytes might change in older carriers? What effect does the 'normal' ageing process in the heart, interacting with the various genetic variations, determine whether older BDMD-carriers might be prone to develop cardiomyopathy in older age (ie: cumulative cardiac effects)?

LC stated that based on the work of her group in ~ 100 BDMD-carriers, women at older ages were more likely to have MRI abnormalities. The schedule of screening, therefore, should – in her view, continue lifelong, since the risk of cardiomyopathy was lifelong. She also highlighted the problem of mothers loosing contact progressively with care teams once their sons have died and so become lost to cardiac follow-up also. with

---

Mah ML, et al..Duchenne and Becker muscular dystrophy carriers: Evidence of cardiomyopathy by exercise and cardiac MRI testing. Int J Cardiol. 2020 Oct 1;316:257-265. doi: 10.1016/j.ijcard.2020.05.052. Epub 2020 May 27

Ishizaki M, et al. Female dystrophinopathy: Review of current literature. Neuromuscul Disord. 2018 Jul;28(7):572-581.doi: 10.1016/j.nmd.2018.04.005. Epub 2018 May 2..

Johnston TP, et al Young Becker muscular dystrophy patients demonstrate fibrosis associated with abnormal LV-ejection fraction on cMRI. Circulation 2019, 12(7): https://doi.org/10.1161/CIRCIMAGING.119.008919

JB mentioned, on a more reassuring note, an Scottish epidemiologics study which seemed to show that survival of BDMD-carriers in Glasgow was better than that of the general population!

Holloway SM, Wilcox DE, Wilcox A, Dean JC, Berg JN, Goodie DR, et al. Life expectancy and death from cardiomyopathy amongst carriers of Duchenne and Becker muscular dystrophy in Scotland. Heart. 2008, 94:633–636.

LC mentioned the need to define what constitutes older age in BDMD-carriers?

JB has a cohort of carriers, without echo-evidence left ventricular dysfunction - some in their 70s, and so has routinely patients at that age from further cardiac follow-up. However, in light of LC's cMRI findings, this may no longer be appropriate

EC mentioned that from the cohort of 293 carriers in the CPRD database, only 3% were identified by code for cardiomyopathy as compared to 49% for anxiety and depression. She agreed to study the data further with regard to cardiac findings, with specific regard to effects in older carriers.

Q2: When early skeletal muscle symptoms are evident, does their severity inevitably progress / deteriorate over time?

LP stated that the -amount of dystrophin in skeletal myocytes will dictate the level of symptoms. Younger carriers with symptoms typically have less than 50% of cells expressing dystrophin.

MG mentioned that since we do not assess all carriers routinely, those reporting symptoms bias our understanding of full spectrum of particularly mild disability. Patients reporting symptoms of muscle weakness or chonic discomfort may also influence the findings even of an objective muscle assessment. Furthermore, it is difficult to differentiate pain, strength and fatigue retrospectively. Assessments are likely to be more accurate in chidlren with more severe myscle manifestations.

LP stated that muscle-manifesting BDMD-carrier tend to manifest comparable symptoms similar to males with BDMD.

RQ has experience of adult patients only in her clinics.

EN asked whether there was consensus or guideline to differentiate BDMD-carrier muscle abnormalities from other conditions.

MG has more experience of paediatric subjects in her clinics than of adult. If their course was unexpectedly severe, they tended to undergo more investigations and testing – which may skew our understanding inadvertently

EN One of the stated aims of the workshop is to develop and propose a consensus definition of carriers

AF Pointed out that there is not a specific phenotype in female carriers and clinical features can be highly variable. It is important, she suggested, to bear this in mind when we come to propose carrier definitions and guidance for their diagnosis, follow-up and therapy.

MG suggested that there was need for carrier-studies of both cross-sectional and longitudinal design. Cross sectional studies should be able to provide characterisation and describe the diversity of the condition. However, we need prospective longer-term studies for information on disease progress and rates of change.

Q3: What is the prevalence of cognitive impairment, learning difficulties, anxiety / depression in your cohort?

MG felt that it was important to distinguish secondary effects and impact of anxiety and depression from primary cognitive and learning difficulties occuring as part of the genetic variations themselves
.
LP felt that a both retrospective and prospective study designs are appropriate to provide a more comprehensive overview of the variability of the BDMD-carrier population. This would also provide useful information in planning health service provision and increasing access for patients to appropriate and timely care (eg: cardiac screening; educational care planning, etc). She recommended that such studies should include all carriers and not just those with symptoms.

JB wondered whether comparing assessment findings in mothers with affected sons in comparison to 1st degree female relatives without the additional social / domestic stressors might help distinguish primary from seconadary cognitive or psychological effects of the condition.

MG pointed out that in BDMD-adult carriers we do not systematically assess cognitive function.

RQ recommended we design a cross sectional study to look specifically at the effect of aging on cognitive function in carriers

AF suggested that it would be important to correlation severity of phenotype with age and degree of X inactivation. Results of that could inform the design of future studies also.

Q4: Do you have experience in using steroids to treat skeletal-muscle weakenss? How acceptable was / is long-term steroid therapy to these patients? What benefits and/or adverse effects did you encounter? It may be possible to draw-up early guidance based on our amalgamated 'anecdotes' about experiences of steroid therapy in 'carriers'(?)

It was agreed that we should try to collect this information also from the patient and care-team survey being conducted as part of the workshop. This might provide information on which to base some initial recommendations for patients and care-teams.

LP described two younger carriers in her cohort under follow-up who are being treated with steroids and benefiting.
Consider collecting data in a more systematic way

AF pointed out that this was not included as an output in our initial ENMC application. She also asked whether the FORDMD study of different steroid regimes in DMD males might provide data that could be extrapolated to carrier-females
MG confirmed that the FORDMD study was completed, has now been submitted for publication and its main findings will be presented at the PPMD meeting in Rome next year (2022?).
AF suggested would be good to have an update of trial findings as part of the workshop
MG agreed it would be interesting to see how many carriers are on steroids, but they are likely to be mostly paediatric rather than adult aged cases

LP stated that her group has data on some 30 BMD-carriers on steroid therapy, including some with longitudinal follow-up data

JH also has prospective data from a cohort study of 40 carriers on steroid therapy, which she is happy to present to the WS.

AS suggested that we generate a patient-reported outcome (PROMM) questionnaire between now and the face-to-face meeting for discussion, refinement and deployment thereafter. Hearing of the studies described by LP / JH would inform the questionnaire development also.

EN, **AS** thought creation of a registry would be the best way to progress this but that would require someone to take the lead(?)
AF pointed out the need to clarify whether Teresinha Evangelista was not already undertaking this before starting something new?

JB suggested that even though the proportion of carriers receiving long-term steroids is small, it would still be worth describing their pre- and on-steroid clinical courses in terms of relative risks-benfits of treatment in a publication.
AS suggested we could decide that when we had a clearer idea of the patient numbers and the quality of the data available.

NV mentioned that some students, supported by a patient organisation they conducted a survey of patients with X linked myotobular myopathy in Holland, and perhaps something similar could be conducted for BDMD with support of UK DMD charities, reaching out to patients and have them complete questionnaires. I think this is feasible
EN suggested that it may be possible to conduct this using existing registries.
EC said that we would need to be clearer about which PROMM instrument to use and wasn't convinced that patient engagement in such an exercise would be sufficiently high.
EV said that the World Duchenne Organisation could help in such a project, if we were clear about what data we wanted to collect.
AS proposed that we return to this topic with more information and consider adding it to the final WS-meeting programme. To help maintain momentum on this, she asked participants to report back on the following as soon as possible:

a) Numbers of your BDMD-carrier patients on steroids
b) What information can you provide on natural history progression on muscle wasting? assess data collection for progression re carriers at meeting
c) Please respond to the on-line survey, which is still open and ongoing,To date there have been only 71 responses from clinicians and 91 from patients and relatives. Please add your data, if you have not already done so. This will allow all data to be presented at face-to-face meeting

AS presented the state of the agenda for the final meeting and all partipating were happy with the latest outline of the programme and the list of participants.

The convenors will review, finalise and circulate the final agenda / programme along with the speaker schedule for comment shortly.

The meeting ended.
