1. Introduction

The 195th ENMC International Workshop on newborn screening for Duchenne muscular dystrophy (DMD) was held in Naarden, The Netherlands, on 14–16th December 2012. Twenty-one participants from seven countries (UK, The Netherlands, Germany, Italy, Canada, USA, and Australia), with expertise in neuropsychiatrics, neurology, genetics, biochemistry, pathology, psychology, ethics, sociology and parent representatives were present. DMD NBS was last discussed at an ENMC workshop in 1992 [1], where it was concluded that the justification to screen for DMD ‘is to provide optimal and specific information to the parents in time, so they can make the decisions which are the most appropriate for their family’. This point was reviewed in the current workshop.

DMD is a progressive muscle wasting disorder, leading to wheelchair confinement in the mid-teens, and development of an associated cardiomyopathy. Death is mainly from respiratory failure [2] although with the implementation of optimal respiratory care, mean age at survival is now well into the late 20s [3–7]. A milder form of the condition, Becker muscular dystrophy (BMD) is also recognised. DMD is an X-linked condition, arising from out-of-frame or frame shift mutations in the DMD gene, encoding for the cytoskeletal protein dystrophin. Two unusual features of this disease are: (i) the large size of the DMD gene and thus susceptibility to de novo mutations; (ii) the absence of specific clinical symptoms sufficient to alert medical professionals to the presence of the disease in neonates. The most commonly recognised first disease sign is ambulatory delay, with ~50% of DMD boys first walking after 18 months [2]. Subtle progression of clinical symptoms is responsible for a prolonged diagnostic process, often lasting ~2.5 years, giving a mean age of diagnosis of ~4.5 years [8,9]; a figure which has changed little over the last 3 decades. This often means that the optimal Standards of Care (SOC) [6,7,10,11] are initiated far later than desired. Despite this, it is clear that the pervasive nature of DMD has much earlier manifestations than the motor ones; global delay of development, feeding difficulties or failure to thrive and the level of social interaction of affected infants, are common early features. These issues cause substantial parental distress as children fail to meet life milestones, but are only rarely identified by medical professionals as early signs of the condition. These parental concerns play a significant impact on family life-quality and on the perceived ability to be good parents. These aspects are important because for many families the time interval free of concerns for their affected children is in reality limited to the first few months of life. This is a considerably shorter time period than commonly acknowledged.

The delay in the diagnostic process, with its associated emotional stress, economic costs, inadequate feelings of parents towards providing optimal care for their ‘problem’ child, have all been considered in planning strategies for diagnosing DMD boys under the age of one and this includes newborn screening. Despite the experience generated from at least 17 pilot newborn screening (NBS) programmes for DMD in 10 countries, spanning three decades, no country nationally screens for DMD at the moment. In view of the evidence [6,7,10,11] of benefit for earlier implementation of optimal SOC and the emerging potential therapies in this field, there is...
increasing pressure to discuss the pros and cons of a neonatal screening programme or other measures to diagnose DMD in infancy. These issues prompted this DMD NBS workshop.

1.1. Aims

The aim of this workshop was to assess the status of NBS programs for DMD around the world, and to assess the technical, ethical, and practical aspects that need addressing, based on our current understanding of treatment options for boys affected by this disorder. Our discussion was informed from multiple parties, including participants representing parent organisations and those from DMD families who have boys either diagnosed by NBS, or from following the traditional route. Our discussion included the following topics:

- To heighten awareness of the revised incidence of DMD.
- Suitability of the Wilson and Jungner 1968 population screening criteria for newborn screening for all rare diseases in the 21st century.
- Reviewing DMD NBS programmes to identify an optimal methodology for such a programme.
- The so-called ‘carefree’ period in DMD.
- Outcome measures needed to assess natural history and potentially effect of intervention in infancy.
- Natural history data on DMD boys up to 4 years old.
- Risks associated with DMD NBS screening versus risks associated with not screening.
- How to improve public education dissemination methods to prospective parents and physicians.
- Feasibility of prenatal and preconception screening programmes.

2. Revised incidence of DMD

DMD (OMIM 310200) is one of the most common fatal early childhood onset conditions known, with a global prevalence, of 250,000, and a reported average incidence of 1:3500 [12]. However, Jerry Mendell recently re-examined these figures [13,14] presenting his findings at this workshop. He demonstrated that the average global DMD incidence is now closer to ~1:5000, with the drop being attributed to both reproductive planning within DMD families and medical advances since the 1990s. Similar figures were reported recently also from a number of screening programmes worldwide. It is therefore important that the updated DMD incidence is quoted in future documents on DMD.

3. Newborn screening criteria for rare diseases

In 1968, the WHO commissioned a report [15] that listed ten criteria to be met to support mandatory, state financed population screening. These criteria were devised for adult screening and did not encompass specialised criteria required for either child and/or rare disease screening. The basic idea was to safeguard the best interests of those individuals included in a mandatory screening programme. Not everybody will benefit. They emphasised those cases in the unclear/false positive/false negative range and wrote: “the ‘border-line’ group in a population may be far greater than the diseased”. Community interests are included, but public health criteria are not prioritized over individual interests. The Wilson and Jungner approach was however meant less as a list of fixed criteria than as an elaborated methodical point of view to evaluate new screening possibilities. Since 1968, these criteria have been implemented and in part amended by various national screening committees across the globe in an attempt to generate new standards in keeping with medical and technological advances. The most publicised and radical changes were proposed in a multi-stakeholder and multi-phased process supported by the Quebec Agence d’évaluation des technologies et des modes d’intervention en santé (AETMIS) [16,17] where the criteria were reviewed in the context of genomic technological advances, so as to expand the criteria to cover newborn and possibly also preconception screening. However, the result, a “decision-guide”, is not yet published. These amendments generated country-to-country variation in the interpretation of the screening criteria, but in all instances a medical treatment needs to be available which improves health outcomes for a disease to be added to a NBS programme. Recent discussions by the USA’s Secretary’s Advisory Committee on Heritable Disorders, and US National Research Council, opted to include a broader concept of benefit for the infant and added the family and society. Benefit beyond a medical treatment might comprise, therefore, early identification to facilitate immediate intervention, even though the latter may not be changing the clinical course of the disease. As a result, each State in USA screens for treatable diseases, looking towards the benefit of the family as well as to the child. However, each State can decide on which diseases to screen for. This broader perspective and stance should be encouraged for other countries to follow.

4. DMD NBS programmes past, present and future

We discussed the screening test technologies and practicalities associated with four DMD NBS past programmes (Germany, Wales (UK); Manitoba (Canada) and Ohio (USA)). Each of these programmes uses serum creatine kinase (CK) elevation as the primary test assay [18] analysed from a bloodspot collected as part of the heel prick test taken shortly after birth. The ideal test should have a high sensitivity and specificity, unequivocal predictive value and a low false positive rate. Unfortunately the CK test assay does not meet all of these criteria. Firstly, the CK test is a secondary marker
for the dystrophic process, i.e. an indirect indicator for DMD. Therefore any test measuring CK will always produce false negatives, due for example, to modifier gene expression influencing serum CK level [19]. The CK test can also produce false positive results, either from birth trauma or due to the presence of other muscular dystrophies (e.g. limb-girdle muscular dystrophy) since the test is non-specific for DMD. Although this latter point may be considered a ‘bonus’ in being able to identify more than one disease from the same test, this raises ethical issues and regulators insist that the test assay should be specific for the tested condition. If we are unable to persuade the regulators otherwise, then the test assay may need to be redesigned.

Currently though, the CK test assay is the assay of choice, confirmed with a following genetic test in the DMD gene. Different programmes use a varying threshold for positivity in order to reduce the frequency of false positive tests. For example in the study in Ohio, the threshold for considering the test abnormal was raised from 600 U/L (used in a previous pilot phase) to 750 U/L when the study was extended to the entire State, reducing the false positive test rare from 1.6% to 0.52%. However, there has been no standardisation of this assay, and other programmes (e.g. the Welsh), have had high false negative rates [20]. In the Welsh program, if the CK level was above the predetermined threshold, a repeat assay was performed 4–6 weeks later to exclude false positives. If this second test was also high, DMD was confirmed by sequencing the DMD gene. This entire screening process can take up to 2 months and lead to significant parent anxiety. The Wales Newborn Screening Programme ran from 1990 to 2011; ending due to the UK Laboratory Accreditation Service being unable to support the accreditation of the DMD testing following the withdrawal of the external quality assurance scheme by the Centres for Disease Control and Prevention in the USA.

In Canada, NBS is a provincial responsibility and a pilot voluntary opt-out DMD NBS programme began in Manitoba in 1986 with the aim of reducing the number of second DMD children born [21]. It became part of the Manitoba in 1986 with the aim of reducing the number of second DMD children born [21]. It became part of the Manitoba programme and its results. In addition, 134 boys (1:4000) were found with a newly detected benign dominantly-inherited blood anomaly with high CKMB isoenzyme in platelets or leukocytes. Therefore, each high-CK blood spot was tested with and without CKMM-antibody to distinguish between the two high-CK situations. Only about 5% of all male newborns were tested in this programme; the price of €15/test paid by the parents themselves was one reason, the absence of an effective early treatment another, but the main reason was the lack of professional promotion which the financial situation did not allow.

Two revised test assay procedures were reported at the workshop. The first is a blood spot CK-MM immunoassay (Stuart Moat, Wales) which measures the muscle isoform of the enzyme and is expected to be more sensitive than total CK. The second is a two-tiered CK/DNA test, where in cases of very high CK, the DMD gene is sequenced on the same heel prick blood spot, confirming the significance of a possible elevation in the first CK (enzymatic) test without the need to proceed with another blood sampling weeks later [13]. This has the advantage of reducing parent anxiety time. It was agreed at the meeting that this two-tiered test was the way forward, and that the specificity and sensitivity of the CK-immunoassay should be investigated further. To ensure quality control and quality assurance it will be necessary to identify at least one international diagnostic
company capable of supplying these test reagents. Similarly, Standard Operation Procedures will need to be developed for this new assay.

Finally, a new preparatory pilot working towards a DMD NBS screening programme is about to begin in Australia, where each State operates under its own regulatory rules. Although originally planned for Western Australia, centred in Perth, this State concluded they would not be able to add DMD to their NBS programme due to it failing to meet the Wilson and Jungner criteria, which they rigorously implement. This pilot programme will now operate from Sydney (New South Wales), where a wider interpretation of the Wilson and Jungner criteria is used. It is planned to begin in 2013. This contrasts with the recently unsuccessful UK (March 2012) National Screening Committee (NSC) application (www.screening.nhs.uk/musculardystrophy) which presented data demonstrating that it was timely to add DMD to the UK NBS programme. The four major failings of this application were: (i) lack of a validated screening test acceptable to the public; (ii) inadequate understanding of the natural history in DMD boys under 4 years old; (iii) no identifiable early symptomatic phase and (iv) absence of accepted treatments for neonatal use. Point (i) has already been discussed above. Each of the remaining points were discussed at the workshop (as detailed below) and illustrate that much evidence is already being collected to address these and that the experience in this field is changing rapidly.

5. Newborn screening criteria specific to DMD

The original Wilson and Jungner criteria for mandatory, state funded screenings acknowledged that ‘there should be a recognisable latent or early symptomatic stage’, which upon revision suggested by Andermann [17], had added to its end ‘or increased level of genetic risk’. One of the perceptions in DMD is an associated long pre-symptomatic or ‘carefree’ period, because affected children are frequently not seen by a healthcare professional until they are at least 2 years old. However, in reality it is only the first few months following birth, that a true pre-symptomatic period exists [25–28]. Pane et al. [26] report that in 45% of boys with a mean age of 27 months were found to have a suboptimal developmental quotient, with the motor function and speech and language domain more frequently affected after the age of one year, compared to their peers. The boys themselves become aware that they are different, becoming weaker, rather than stronger with age. This leads to their social exclusion, and considerable parent anxiety, who feel unable to be ‘good’ parents, as forcefully discussed by some of the members of the advocacy groups at the meeting. While it is true that precise appreciations of the milestones in an entire population of DMD infants is not available, this is obviously related to the fact that while a problem is suspected for years, the definitive diagnosis only occurs after a mean age of 2.5 years from the first medical consultation.

Immediate pharmacological interventions do not currently exist for infants with DMD. We discussed at the workshop the pros and cons of whether this should not necessarily mean we also delay the age of diagnosis, since this in turn will exclude initiation of the agreed optimal SOC at an optimal age. [6,7]. These SOC include the timely intervention of physiotherapy and glucocorticoids, proactive treatment towards psychosocial and behavioural issues and cardiac surveillance. In a recent publication on a large UK population of ~400 DMD boys, the mean age of initiation of corticosteroids was 6.4 years (range 3.4–9.8), confirming that a significant number of DMD boys do not receive treatment until the clinical symptoms are well progressed [11]. Furthermore, there was a clear trend of better motor milestone acquisition and maintenance at higher function for longer in DMD boys treated earlier [11]. This trend was further and forcefully reinforced by another recent publication, where the initiation of corticosteroids at an even younger age (2–4 years) was associated with remarkably good long term outcome after a 14 years observation period [29]. In this study, the majority of the boys remained ambulant between the age of 16 and 18 [29]. Ongoing work to ascertain steroid treatment in 3 year olds, includes an anticipated MDA-funded clinical trial through the Clinical Research Network. The parent organisations agreed in the workshop to collect data on possible additional benefits (or lack of) of steroids on neurodevelopment in this age group. In addition, there are several novel therapies for DMD children in clinical trial, which if applied to infants are likely to be particularly effective in reducing or slowing clinical progression. These all require as much active, intact muscle mass as possible, and include drugs which read through nonsense codons (e.g. ataluren) [30] and RNA therapy technology known as exon-skipping. Both treatments restore dystrophin expression and stability of the 6 min walk time [31,32]. There are thus additional, novel treatments in the pipeline, ready to be administered to an identified neonatal population. Indeed, a meeting between DMD health professionals and the UK regulators, European Medical Agency, in 2009 concluded that since exon-skipping is likely to be beneficial to the neonate population, NBS should be considered for DMD [33]. Outcome measures for assessing clinical improvement of treated infants are being developed as part of a collaborative effort of the MDA-DMD Clinical Research Network (http://mda.org/disease/duchenn-muscular-dystrophy/research/special-programs) [34]. These involve monitoring improvements in gross motor development tests, which are impaired in this age group. This will allow ascertainment of whether a newborn diagnosis, followed by earlier treatment, is of better patient benefit than the current diagnostic pathway.
NBS programme regulators across the world, view the lack of a ‘medical treatment suitable for neonates’ as a major obstacle for adding DMD to their national screening programmes. The workshop members commented that the impression that there is no treatment for DMD frequently comes from scientists or physicians remote from DMD in their professional life. This widely held belief has lead to a classification that recognises conditions such as cystic fibrosis as ‘treatable’, but classifies DMD as an ‘untreatable’ disease, despite there being effective treatment options available for both [35]. In reality, the doubling in mean age of DMD survival in the last 20 years, thanks to the implementation of anticipatory care, amply illustrates how treatable DMD has now become [4–7].

Health economic figures of the cost of long-term care or of current healthcare provision at all stages of care on DMD patients do not exist within Europe. Several European funded projects such as BURQOL-RD (www.burqol-rd.com), a 3 year project to generate a model to quantify socio-economic costs and health related quality of life for up to 10 rare diseases, including DMD within Europe) are currently compiling such data, from a range of sources including the parent organisations and a study is also ongoing with the TREAT-NMD patient registries. Once obtained, the cost of NBS and its associated long-term care can be compared with other diseases diagnosed by NBS, and contrasted with their expenses when the diseases are diagnosed in infancy.

6. Benefit and risk to the patient and families

There is a delicate balance of interest between the parents’ and child’s welfare; the need to avoid potential harms, including psychological, physical and social harms by a general and mandatory screening scheme in the families of all groups, versus the advantage for the children of receiving early SOC, but also for the families in whom a prompt diagnosis allows for planning and better parenting.

In general, NBS programmes for conditions other than DMD operate nationally and are state-run, with recommended participation, but with the option to opt-out of all or any individual test offered. Countries piloting DMD NBS programmes operate by working alongside the state-run universal programme, providing information during pregnancy (see below) for an informed choice, with the option of test-specific opt-out. A number of issues were discussed as to whether DMD NBS should be optional. These included: the right of families to have a choice; the importance of considering both the best interest of the consenting parents but also of the affected children; the benefit for the families and the benefit for the society. In conditions in which there is immediate and life-saving intervention, such as several metabolic diseases for which NBS is mandatory, there is wide acceptance that the state-run opt out programme represents the best option for the children. Similarly, there is no global harmonisation of how detailed the informed consent process should be and it can even be very variable between centres within the same country. Therefore, not unsurprisingly, parents frequently do not fully understand the implications of being included in a NBS programme. However, there is also evidence that the more active the parents’ decision is to test, the lower the participation [1,36,37].

Workshop participants discussed at length whether screening should be performed at birth, with the result communicated in the newborn period or at a later stage, and if screening should occur say 6 months after birth, to ensure parent–child bonding had occurred. Criticisms regarding the “early” newborn period for the communication of the diagnosis typically focused on the disruptions to the bonding process, due to parental stress and anxiety, at a time conventionally thought of as a joyous event with an apparently ‘normal’ baby. However, quantitative and qualitative research suggests possibly harmful psychosocial issues, or lack of bonding and anxiety are not true or valid [27,28,35,38]. Furthermore, there is never a good time for receiving the diagnosis of DMD, but hearing sooner rather than later allows for the appropriate planning of the needed financial, reproductive and social support [39,40].

Given that parents and recently published studies report neurocognitive delays in pre-diagnosed DMD boys (see section 5), who are aged between 6 and 12 months, a consideration that was discussed relates to the possibility of implementing a mass diagnostic campaign at around the age of 1 year, together, for example, the compulsory vaccination programme. The advantage of this approach is that the news regarding the diagnosis may be better received, as the parents may already have some concerns regarding their child’s health, and will be looking for answers. This will ensure optimal parenting and implementation of SOC. This approach has its attractions, but needs to face the practicalities of starting a complex process at a time when no other mass diagnostic program is underway worldwide. But alongside this fact, it should also be born in mind that any delay in the diagnostic process will have resulted in late genetic counselling and possibly the birth of a second DMD child.

7. Education dissemination methods to prospective parents and physicians

Evidence was also presented at the workshop on a survey looking at the way different European countries organise their neonatal screening programmes, including information for families, timing for communication of the test, and even policies for communication of carrier status [41,42]. This survey highlighted a very high lack of global harmonisation. It stressed that too often the
information regarding the aims of the NBS is offered in an incomplete fashion and too late i.e. towards the end of pregnancy, despite recommendations that clearly indicate this information should be provided in the first trimester. More studies are needed to ascertain when a pregnant mother is most receptive to knowledge and whether a video would be more effective than a leaflet [43]. Modern Smart-based technology would also allow information to be conveyed through QR codes and links to Facebook interviews of DMD parents are now possible. Another option would be to follow the practices applied for NBS for cystic fibrosis, where the patient organisations additionally lobby their government to raise the disease profile at both a national and international level. Health care professionals also need to be updated of the early DMD symptoms and one way to do this is through advertising the www.childmuscleweakness.org web site, plus publishing in journals read by primary care physicians (e.g. BMJ, Midwifery Times, Health Visiting Journal). Interestingly, physicians approve of diagnostic genetic testing of at-risk children [44], but are less supportive of expanding newborn screening to diseases that do not satisfy the Wilson and Jungner criteria, unless they also want to screen their own children [45].

8. The future/way forward?

While results from ongoing experimental studies using drugs that induce exon skipping and read-through stop codons appear to be very encouraging, and the outcome of confirmatory phase 3 studies will be available during the course of 2013, at the moment there is no immediate intervention in the neonatal period that directly benefits the affected DMD children. This fact ought to be reflected in the range of options available to families when considering the introduction of NBS. Given the current status, it is likely that a NBS programme for DMD will be introduced as voluntary screening. The format and circumstances of such a voluntary screening scheme need to be devised in detail, evaluated and discussed. It is important that this is addressed sooner rather than later since a new DMD NBS programme recently began in Taiwan, and another is planned in Yucatan, Mexico. It was also clear at the workshop that the recently devised two-tiered CK/DNA NBS test for DMD NBS should be developed for use by other screening centres. As a first step, Jerry Mendell recently presented the data surrounding the two-tiered CK NBS test at the Secretary’s Advisory Committee for Heritable Diseases in Children and Newborns, where it was well received and he was encouraged to submit a formal application in the future when more newborns have been screened (the 40,000 screened in the Ohio programme is too small to ascertain its true effectiveness). Therefore we need to strengthen our efforts in developing a Standard Operating Procedure for this assay, distribute it to other screening sites across the globe, so as to assess its screening efficacy on more children and across populations. The more data we can provide to support the Ohio programme, the better positioned the neuromuscular community will be to successfully add DMD to national NBS programmes.

Finally, the workshop participants recognised that the rapid pace of DNA technologies for instigating affordable and accurate preconception screening (DNA), foetal screening and newborn genome screening may supersede any devised specific enzymatic test assay, for any newborn condition in ~5–10 years time. However, genome-screening will heighten the need for more not less discussions, to ensure the psychological and social welfare aspects associated with genetic screening are able to be met.

9. Workshop participants

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