Characterization of the DMD/BMD patient population in Czech Republic and Slovakia using an innovative registry approach

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Abstract

Effective planning of clinical trials requires an appropriate number of patients who fulfill given inclusion criteria. In the case of so-called “orphan” diseases, such as Duchenne and Becker muscular dystrophy (DMD/BMD), the number of suitable patients within one country is usually limited. We developed a detailed registry of Czech and Slovak DMD/BMD patients which may contribute to cooperation on the European level.

The registry uses internet and database technologies with a multilevel architecture. Patients may view their own data. As of May 2008, 163 patients have been registered in the database. The registry provides a detailed phenotypic and genotypic description of patients.

The main purpose of such a registry is the time-effective recruitment of eligible patients for a clinical trial or therapy and may allow the anticipation of possible future effects of appropriate therapy on individual patients. The importance of the DMD/BMD patient registries has recently also been rising with new clinical trials focused on mutation-specific approaches. Other outputs include assessment of epidemiology, phenotype and genotype relationships, or standards of care.

1. Introduction

Duchenne and Becker muscular dystrophy (DMD/BMD) are X-linked allelic neuromuscular disorders caused by mutations in the dystrophin gene (DMD gene) [1,2]. Duchenne muscular dystrophy is the most frequent lethal X-linked recessive disease, affecting approximately 1 out of 3500 live male newborns. DMD patients usually suffer from a mutation that disrupts the reading frame and leads to the synthesis of prematurely truncated dystrophin protein, which is, therefore, non-functional. The absence of functional dystrophin causes muscle degeneration and weakness and subsequent respiratory or cardiac failure and premature death, usually between the 20th and 30th year of life.

A milder form, BMD, has similar symptoms but its progress is significantly slower. In contrast to DMD, mutations in BMD patients usually maintain an open reading frame. Their dystrophin, although often lacking a part of the central rod domain, contains both N- and C-terminal domains important for the dystrophin function. Thus, the protein is partly or largerly functional. This milder form of DMD is about five times less frequent.

The DMD gene contains 79 exons and is the largest of 30,000 genes in the human genome. Thus, it is very vulnerable to various genetic defects [3]. Deletions of one or more exons are responsible for approximately 70% of DMD/BMD cases. Duplications and point mutations are less frequent (approximately 20% and 10%, respectively) [4].

Therapeutic strategies for DMD/BMD fall into three categories [5]: (1) therapy on the genetic or molecular level (gene delivery with adeno-associated viral vectors, exon-skipping, or read-through of premature stop codons), (2) cell therapy (stem cells, mesoangioblasts), and (3) pharmacological and other interventions to improve the patient’s quality of life (corticosteroids, calpain inhibitors, anti-inflammatory drugs, amino acids, spinal surgery, nocturnal ventilation, anticipating potential heart problems).

All of these approaches have been the subject of intensive research over the last two decades. Promising results and subsequent clinical trials have brought a need for detailed patient registries to allow for an appropriate choice of patients for testing. Such a registry would allow for the possibility of collecting, viewing or searching for data regarding a patient’s phenotype and genotype profile and other medical information for the benefit of future studies and establishment of a therapeutic strategy.
According to our information, several national registries have been developed (in the UK – http://www.dmdregistry.org, in the Netherlands – http://www.lumc.nl/duchenne, in France – http://www.cochin.inserm.fr, in the USA – http://dystrophy.genetics.utah.edu, and in Germany – http://www.dmd-register.de). Current activities within Europe have recently been covered by the TREAT-NMD project (http://www.treat-nmd.eu). This project integrates national activities, researchers, and has set up a clinical trials coordination centre as well as international patient registries. In 2004, work started on the Czech DMD/BMD patient database/registry (http://dystrophy.registry.cz). The original aim of the database was to effectively integrate Czech patients into international clinical studies. International cooperation and an active search for patients on the international level is necessary, since suitable patients matching particular clinical and genetic criteria are not found in adequate numbers in individual countries. As far as identifying an effective therapeutic method, the central database will speed up the selection and designation of appropriate patients. For instance, the importance of the DMD/BMD patients registries has been emphasized due to recent clinical trials in which mutation-specific approaches have been tested [6,7].

In this paper, we would like to introduce the structure and operation of the Czech DMD/BMD registry, the system of data collection and analyses, and the initial results of practical use of the registry in choosing suitable patients for clinical trials.

2. Patients and methods

2.1. Institutions involved in the project

Currently there are seven Czech centres in Prague, Brno and Ostrava participating in this project. The project also includes patients from Slovakia, made possible by successful cooperation with the Organization of Muscular Dystrophy Patients in Slovakia (http://www.omdvsr.sk).

2.2. Technological design

The technological base of the project is provided by the Institute for Biostatistics and Analyses, Masaryk University, Brno. The database system was originally based on a modified version of TrialDB system [8], which is currently customized for the collection of specific clinical data of the DMD/BMD project.

The database is implemented online and uses internet and database technologies featuring a multilevel architecture (client – web server – database server). All submitted data are collected in a central server, where they are safely stored in a database, administered in the ORACLE 9i system. The registry is accessible via internet from any PC equipped with MS Internet Explorer 5.5 or higher, which supports encrypted communication with a 128-bit SSL protocol.

Access to the database is protected by a hierarchical system of access rights, from the database administrator overseeing participating centres to individual patients. All clinical centres participating have equal rights, allowing for the addition of new patients, filling out forms and protocols and modifying their clinical and genetic data. The centres have access to all records in the database.

Patients (or their representatives) may only view their own data. Any changes may be made only by the physician who registered the patient in the database.

Other users interested in detailed information may ask the administrator for access to the database. The administrator registers such users and provides them with a login and password. They may only view the clinical records of individual patients without any personal identification data.

2.3. Data collection and handling

The patients and data related to them are entered in the registry, in keeping with both national and international law (EU Directive 95/46/EC, Czech Act No. 101/2000, etc.). Patient records are entered into the database on the basis of agreement by their legal representatives (informed consent), which informs the patient about the aim and use of the database. Personal data may not be provided to a third party without the patient’s permission. The consent further states that participation is voluntary, implies no obligations for the patient, and that the subject will be removed from the system immediately upon his request without requiring a reason for the decision. Refusal to participate does not affect subsequent medical care for the patient.

2.4. Patients’ follow-up and structure of the form

The follow-up includes clinical symptoms, biochemistry, electromyography, muscle biopsy, and description of the specific mutation. The parameters examined and methods used, as well as the whole structure of the CRF, are shown in Table 1.

3. Results

3.1. Access by patients and/or their representatives to the registry

The registry allows patients and/or their representatives to enter the database and view their own forms and data. Of the 163 registered patients, 22.7% were interested in receiving login and password to allow access to the registry. 86% of these patients or their representatives actually used the option to enter the registry at least once (several accesses on a single day were treated as one access) and 54% of them more than once. The mean number of individual accesses per month was 4.63.

3.2. Patient and symptom structure

As of May 2008, 163 patients were registered in the database. 102 of them were DMD patients, 24 BMD patients, 4 intermediate patients, 31 verified and 2 suspected carriers. 90.2% of DMD and 62.5% of BMD patients were clinically symptomatic. Approximately 5% of DMD patients underwent the steroids therapy. The most frequent genetic mutation found among the DMD/BMD patients was deletion of exons (61.1%), followed by point mutation (14.3%) and duplication (9.5%). No mutation was found in 15.1% of patients (Table 2). These cases have been diagnosed as DMD/BMD patients on the basis of muscle biopsy, which confirmed lack of dystrophin expression. The open reading frame was disrupted in all DMD patients, while it was maintained, with one exception, in all BMD patients. Exon deletions occur in two usual hot spots described in the literature, i.e. between exons 5–25 and 42–54 (Fig. 1A). Exon duplications are less frequent and occur in two regions as well – exons 2–7 and 22–55 (Fig. 1B).

DMD/BMD is usually accompanied by a number of symptoms, such as calf pseudohypertrophy, cardiomyopathy, respiratory insufficiency, etc. The overview of the six most frequent symptoms is given in Table 3.

The patients have varied walking capability. Among the DMD patients, one quarter are capable of normal walking and running; by contrast, more than one third are wheelchair bound. The mean age when the DMD patients lose ambulation is 10.1 years. Fifty percentage of patients suffering from the milder form, BMD, are capable of normal walking and running, while 8.3% are wheelchair bound. The motor functions are summarized in Table 4.
Individual patient status is characterized by the functional score (developed by PV, currently validated in a pilot study), which is calculated from the severity of symptoms mentioned above. The highest value is 100, representing a patient with no symptoms. Fig. 2 shows the relationship between the functional score and age of patients. The score decreases with age and the decrease is more distinct with patients who have the more severe DMD form of the disease.

3.3. Model selection of candidates for the clinical trials

The detailed clinical registry allows for a very accurate selection of patients for clinical trials. The selection can be carried out based upon a very specific and detailed criteria composition, if these are available. This is extremely valuable especially in cases of less frequent diseases with various types of disorders, such as DMD/BMD. Recent trials based on the mutation-specific approach, such as PTC124 or antisense oligonucleotides against exon 51, may serve as illustrative examples of the importance of clinical registries.

Approximately 10–15% of DMD patients have a nonsense point mutation that results in a premature stop codon and synthesis of a shorter protein incapable of fulfilling its function. The drug PTC124 has been reported to promote ribosomal readthrough of mRNA containing a premature stop codon [6]. A preliminary selection of suitable patients from the Czech DMD/BMD registry for the Phase IIB clinical trial was carried out in January 2008. The patients had to meet criteria stated in Table 5. 21 DMD/BMD patients with point mutation were found in the registry. However, only 10 fulfilled the designated criteria and could be selected for the clinical trial.

Other recent clinical trials have been focused on the skipping of exon 51 during the mRNA splicing by means of antisense oligonucleotides (AONs). This method can restore the open reading frame and allow for a synthesis of shorter, but largely functional dystrophin, thus shifting the DMD phenotype to a milder BMD. Such therapy is suitable for approximately 16% of all DMD patients with defined ranges of exon deletions around the exon 51 [7].

4. Discussion

The DMD/BMD registry allows patients and/or their representatives to enter the registry and view their own data. 22.7% of the 163 patients have made use of this opportunity, 2/3 of them than once. The right to view their documentation is granted by Czech Act No. 20/1966. Internet access simplifies this procedure and saves the time of those involved (physicians, personnel, patients). The number of patients availing themselves of Internet access illustrates the relatively strong interest by patients in their documentation with regard to fact that access is fully voluntary. This is something which will likely rise with increasing Internet use.
The structure of mutations found in the registry was different for two forms of the disease. Point mutations, which usually disrupt the reading frame, mostly result in DMD, while there were only three cases of these mutations among the BMD patients. Duplication rates were equal in both groups. Thus, deletions of exons were relatively more frequent in BMD patients. The general ratio of deletions, duplications, and point mutations found in our registry is in agreement with other literature [4,9].

Our results also support findings that the final phenotype depends on the open reading frame preservation rather than on the...
range of the exon duplication or deletion. This could be illustrated by fact that all DMD patients had out-of-frame mutation, while in the BMD patients, with one exception, in-frame mutations were found. Moreover, one of the largest duplications (two patients with duplicated exons 28–55) resulted in a BMD phenotype, while the duplication of a similar range (exons 22–49) was found in a DMD patient.

Distribution of exon deletions in two “hot-spot” regions (exons 5–25 and 42–54) corresponds with literature [4,9]. On the other hand, most duplications were found for exons 25–55, in contrast to data published by Aartsma-Rus et al. [4] or White et al. [10], who reported the highest duplication frequency near the 5’ end of the gene (exons 2–7).

The registry contains a complete phenotype profile of 130 DMD and BMD patients. The symptoms, such as cardiomyopathy, respiratory insufficiency, scoliosis, etc. are generally more frequent and severe in DMD patients. One could note that the percentage of patients treated with steroids is relatively low compared to other (Western Europe, US) countries. This type of therapy had not been widely used in the Czech Republic and Slovakia before the adoption of the TREAT-NMD project guidelines, which started in 2007. The number of patients in the registry who underwent the steroids therapy will surely increase with the ongoing increased use of this treatment.

International cooperation is necessary in case of “orphan” diseases and associated clinical trials, since the number of suitable patients fulfilling genotypic and phenotypic criteria within one country is usually limited. A detailed international database of patients is the first basic step for efficient research and clinical trials, especially in their later phases. Such a database can provide a quick selection of patients based on a priori arranged genotypic and phenotypic criteria. In the case studies mentioned in Section 3.3., only 10 of 130 DMD/BMD male patients were found to be suitable for the PTC124 trial, and only two for the AONs trial. This illustrates and supports the necessity of international databases to reach a sufficient number of patients.

The Czech DMD/BMD registry has been under construction since 2004 and currently contains 130 DMD/BMD patients and 33 female carriers. It is currently one of several databases of its kind in Central and Eastern Europe. In 2007, it was integrated into the TREAT-NMD, which covers a net of cooperating neuromuscular centres and organizations in order to coordinate diagnostic and therapeutic care, as well as research activities in the field of neuromuscular diseases. The registry is still being developed and extended.

Particular attention is paid to actively searching for female carriers among the relatives of DMD/BMD patients. These carriers are provided with full genetic consultation services, including prenatal diagnostics.

DMD affects 1 of 3500 and BMD 1 of 17,000 newborns. In the USA, the estimated prevalence is 15,000 cases. Converted to the Czech and Slovak population, the total number of DMD/BMD patients is expected to be approximate 700, which means that theoretically only 1/5 of the Czech and Slovak DMD/BMD patients are currently registered in the database. Nevertheless, the number of registered patients is still relatively high compared to the other DMD/BMD registries within Europe.

Thus, one of the main general aims for the future should be an active and detailed search for DMD/BMD patients and female carriers, as well as educational issues concerning the advantages of registration in the database.

The main purpose of such a patient registry should be the easy and time-efficient selection and recruitment of patients eligible for a certain clinical trial or therapy and vice versa: it may allow the anticipation of possible future effects of an appropriate therapy on individual patients.

**Conflicts of interest statement**

None declared.

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