Genetic and epidemiologic studies in duchenne muscular dystrophy

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Summary

In the general introduction (chapter 1) to this thesis, the clinical, epidemiological and genetic aspects of Duchenne muscular dystrophy (DMD) have been reviewed.

DMD is the most frequent muscular dystrophy in childhood. It is characterized by progressive muscular weakness. Floppiness, developmental delay, or speech delay may be early features, already noted by the parents well before the age of two years. More than 50% of patients start walking after 18 months. However, DMD is usually diagnosed between 3 and 5 years, after difficulties with walking become apparent. Eventually, walking becomes impossible before the age of 13 years. Death usually ensues before 25 years. Respiratory infections and insufficiency and myocardial insufficiency are the main causes of death. Becker muscular dystrophy (BMD) has a milder but similar phenotype and is allelic to DMD.

DMD has an X-linked recessive inheritance. Microscopically visible deletions in males, X-autosome translocations in affected females, as well as linked DNA-markers mapped the DMD locus to Xp21, the middle of the short arm of the X-chromosome. Subsequently, in 1987 the dystrophin gene was the first human gene found by positional cloning. The dystrophin gene is the largest known human gene. It spans more than 2.3 Mb and contains 79 exons coding for a 14 kb mRNA. Five promoters specify transcription, three for full-sized dystrophin and two distal ones for smaller dystrophin isoforms. Alternative splicing events add up to many more dystrophin isoforms.

A peculiar feature of the dystrophin gene is its high intragenic recombination rate of 12%, which is four times higher than expected for its size. Approximately 65% of DMD and BMD patients have partial dystrophin gene deletions. Partial duplications are found in about 6%-7% of patients. These gross rearrangements can be detected by cDNA blotting, or by multiplex PCR. Deletions are clustered in two so-called hot-spots, one proximal in the 5' end comprising exons 2-20 (30%) and one more distal 3' comprising exons 44-53 (70%). Duplications are more frequently found in the 5' hot-spot. The recombination hot-spots within the dystrophin gene coincide with the deletion prone regions. Point mutations are found in ever increasing numbers all over the gene since 1991. Absence of clustering is one of the reasons why point mutations are more difficult to find in this huge gene.

Dystrophin is a membrane-bound rod-shaped cytoskeletal protein with a molecular weight of 427 kD. The dystrophin protein makes up 0.002% of total muscle protein and 5% of the membrane cytoskeleton. Dystrophin is associated with a complex of membrane-bound proteins and glycoproteins.

The disease spectrum of dystrophinopathies is very broad and encompasses several disorders like idiopathic high creatine kinase levels in asymptomatic individuals, or exercise-induced cramps and myoglobinuria, or isolated cardiomyopathy, or BMD, or DMD, or presentation as a congenital muscular dystrophy. Generally, dystrophin gene mutations that cause DMD disrupt the reading frame, which results in premature termination of translation. This results in absent dystrophin, or a truncated dystrophin protein with severely compromised function and stability, which is probably rapidly degraded. In BMD, dystrophin has an abnormal size and/or quantity. The aberrant dystrophins in BMD are semi-functional.

In the studies comprising this thesis, answers have been sought to the following questions:

What are the birth prevalence and point prevalence of DMD in The Netherlands? (Chapter 2)
Correct estimates of birth and population prevalence of DMD are important for planning medical services, estimates of the DMD mutation rate and further research. Therefore, we made an inventory to estimate birth and population prevalence of DMD in The Netherlands. Seven ways of case finding were used. Data on 496 definite, probable or possible DMD patients born since 1961 or alive on January 1, 1983 were obtained. Several methods gave an estimated ascertainment of more than 95% during 1961-1974 and this period was chosen to determine birth and point prevalence. The birth prevalence of DMD was estimated at 23.7×10^{-5} (1:4,215) male live births (MLB) yearly. DMD prevalence in the male population on January 1, 1983 was 5.4×10^{-5} (1:18,496). About 1% of the males in this study may have (had) autosomal recessive Duchenne-like muscular dystrophy. A literature review showed no convincing evidence for geographic differences in DMD prevalence at birth. The DMD mutation rate calculated by the indirect method was 7.9×10^{-5} genes per generation. However, this may well be an overestimate, as this method does not account for germline and/or somatic mosaicism.

Is there a method to determine the proportion of new mutants in DMD, allowing for the inclusion of data on germline mosaicism? (Chapter 2)
Estimating the proportion of sporadic DMD patients is difficult but important when assessing genetic risks in DMD families. Using a modified sex ratio method we estimated the proportion of sporadic DMD patients among all patients to be around 0.11 (range 0-0.33). The high frequency of germline mosaicism in DMD was considered to be a likely cause for the apparent lack of sporadic cases as found in previous studies, if mutation rates in male and female gametes are equal. Therefore, methods for estimating the proportion of new mutants in DMD should take germline mosaicism into account. The modified sex ratio method allows incorporation of data on germline mosaicism if available. The method should be even further extended, to allow for the inclusion of the proportion of patients and carriers who are somatic mosaics.

What are the results of a bias-free method to determine the mean and median ages at onset, first walking, diagnosis and death in DMD patients in de Dutch population? (Chapter 3)
Accurate estimates of life-time events give us the possibility to evaluate possible trends of these parameters over the years. Life time events of Dutch DMD patients were analysed for birth years 1961-1974, to avoid possible effects of downward bias of age at diagnosis resulting from inclusion of birth years 1975-1982. No significant difference was found in the distribution of life-time events between 376 patients classified as certain DMD and 97 patients classified as probable or possible DMD. Mean and median in certain DMD patients were calculated for, age at onset: 2.4 and 2.0 years (range 0.5-7 years), age at first walking: 1.8 and 1.7 years (range 0.8-4.5 years), age at diagnosis: 5.3 and 5.0 years (range 0-10 years), diagnostic delay: 3.1 and 2.7 years (range 0-9.5 years), chairbound age: 9.5 and 9.0 years (range 6-12 years) and age at death: 16.7 and 16.8 years (range 3.1-21.3 years). There was a significant ($P<0.001$) downward trend in age at diagnosis and diagnostic delay during 1961-1974. Age at diagnosis is significantly ($P<0.01$) lower in boys

tal presymptomatic diagnosis was found in 3 cases (1.1%) by elevated creatine kinase or transaminase activities. Presymptomatic diagnosis because of family history was made in 1.5% of cases. Respiratory insufficiency (25.6%), pulmonary infections (18.6%) and cardiac complications (30.2%) were the main causes of death. Median survival was 19.4 years (95%CI 19.0-19.8 years; follow-up 1961-1985) for patients who had reached an age of 10 years or more and 23.6% of patients lived at least 23.3 years. Age of loss at ambulation was correlated positively with survival. On average deceased patients died 7.9 years after becoming chair ridden (range 2.6-12.4 years). In 23 unrelated sib pairs median age at diagnosis in the second affected boy was significantly (P=0.006) lower (5.0 years) than in the first affected boy (6.0 years).
Previous reports on life-time events in DMD did not correct their results for incomplete ascertainment of cases in recent birth years and for limited follow-up of the patients. This may have lowered the estimates of age related events. Our observation of a fall in age at diagnosis and diagnostic delay in a cohort with high ascertainment is noteworthy. Suggestions that a standing regimen, scoliosis surgery and treatment of pulmonary and cardiac complications prolong the life-span of DMD patients need further evaluation.

Is there a difference in the frequency of new dystrophin deletions and duplications in male and females germ cells? (Chapter 4)
Knowledge about the parental origin of new mutations and the occurrence of germline mosaicism is important for estimating recurrence risks in Duchenne (DMD) and Becker muscular dystrophy (BMD). However, there are problems in resolving these issues as not all mutations can be directly detected yet, and as genetic ratios are very sensitive to ascertainment bias. Analysis was therefore restricted to currently detectable mutations (deletions and duplications) in particular types of families which tend to be rare. In order to obtain sufficient data we pooled results from 25 European centres.
In women (mothers of affected patients) who were the first in their family with a dystrophin gene deletion or duplication the ratio between paternal and maternal origin of this new mutation was 32:49 (binomial test P=0.075) for DMD. In 5 BMD families the ratio between paternal and maternal origin of new mutations was 3:2.

Is there a bias-free method to determine the recurrence risk in families with isolated DMD with an apparent new mutation? (Chapter 4)
We studied pooled data concerning 152 selected European DMD patients with apparently new mutations. Haplotype analysis and mutation detection were done in subsequent children of families with only an affected son and no further children, when the family was first referred for genetic analysis. Also families with only girls with an isolated DMD brother were analysed. The sisters did not undergo previous indirect carrier testing and did not have sons yet. In 12 out of 59 (0.20; 95%CI 0.10-0.31) transmissions of the risk haplotype the DMD mutation was transmitted as well. No recurrences were found in 9 BMD families.

What should be the protocol for genetic analysis of DMD and BMD families based on the present state of knowledge and technology? (Chapter 5)

Female family members of DMD and BMD patients are often concerned about their genetic risks. Accurate genetic tests are very important for identifying the mutation in the patients and carriers and for prenatal diagnosis. The methods of direct mutation detection of dystrophin gene mutations are rapidly growing. After a large rearrangement in the dystrophin gene has been found in a DMD or BMD patient, subsequent carrier detection is possible by assessing intensity of the relevant bands, but preferably by a qualitative test method. Detection of microlesions in DMD and BMD is currently underway. Single strand conformational analysis, heteroduplex analysis and the protein truncation test are mostly used for this purpose, but are not routine yet. In families where the mutation cannot be found, haplotype analysis and serum creatine kinase determinations in female family members close to the patient, enable fairly accurate carrier detection in most of these families. We reviewed the available methods for detection of large and small mutations in patients and in carriers and propose a systematic approach for genetic analysis and genetic counselling of DMD and BMD families, including prenatal and preimplantation diagnosis.

Considering the foregoing, there is clearly a need for development of new mutation scanning techniques. For this purpose, we will develop together with the Department of Human Genetics in Leiden, two-dimensional DNA electrophoresis for comprehensive mutation scanning of the dystrophin gene.

As the majority of DMD and BMD cases cannot be prevented, hopefully gene therapy will provide young DMD and BMD patients a way for stopping disease progression. However, there are still major obstacles to be taken. A possibly very promising approach has been developed recently. Efficient long term *in vivo* gene transfer was established in young *mdx* mice muscles after intravenous injection of a recombinant adenovirus. The highest expression of transferred genes was observed in the muscles of neonatal mice. These encouraging results will stimulate further development of vectors directed towards muscle cells, which can be injected intravenously. Alternatively upregulation of utrophin expression in patients could compensate dystrophin deficiency. Any real therapeutic option in the near future will an extra argument for considering neonatal screening for DMD, besides providing parents the opportunity to make optimal choices for their specific situation.