Duchenne muscular dystrophy

Summary

The X-linked Duchenne muscular dystrophy (DMD) occurs due to mutations in the Xp21 gene, which consists of 79 exons encoding the dystrophin protein. Children with DMD typically manifest between the ages of 2 and 5 years with symptoms of delayed attainment of motor milestones. Universally they have highly elevated serum creatine kinase levels and liver enzymes. Progressive proximal weakness with calf hypertrophy occurs in all. DMD children generally lose independent ambulation by 12 years of age. Prevalence of cardiomyopathy increases with age, and without ventilatory intervention, death typically occurs by 20 years of age. Non-motor manifestations include cognitive impairment, delayed language development, diminished intelligence quotient (IQ), autism, attention deficit hyperactivity disorder, and obsessive-compulsive disorder.

Until molecular diagnosis became available, muscle biopsy with immunohistochemistry and western blotting was the GOLD standard procedure. Current genomic analysis detects around 93% to 96% of mutations. The remainder consists of mRNA rearrangements that require analysis of muscle-derived mRNA. Complete mutational analysis of the DMD gene is now a readily available and affordable genetic test and is considered the standard of care. Further-more, mutation specific molecular therapies are in or are nearing clinical trials, and it is imperative to consider the suitability of the patient for these.

Deletions account for approximately 65% of DMD. The simple multiplex polymerase chain reaction (mPCR) test was widely used but is not useful for detection of carriers or patients with duplications or point mutations. Multiplex ligation-dependent probe amplification is the test of choice, which identifies exon duplication mutations, defines the extent of contiguous exon deletions and detects carriers. Currently, next-generation sequencing approaches are the best. Management is ideally provided at a multidisciplinary care setting and Glucocorticoids remain the mainstay treatment.

This thesis comprises of a compilation of articles reporting on the experience of progress in research on DMD over the last 15 years at a multidisciplinary Neuromuscular Disorders (NMD) clinic at the National Institute of Mental Health and Neurosciences (NIMHANS) in Bangalore, Karnataka, India. From a clinical and biochemical diagnosis with histopathological confirmation, we have evolved through simple genetic to complex genetic analysis, genotype-phenotype correlation, cognitive assessment, muscle MR imaging, mutations in familial forms of DMD to the latest Quantitative Brain Imaging and Next Generation Sequencing in DMD.

The first progress at NIMHANS in the genetic diagnosis of DMD was in the year 2002 with mPCR testing for DMD. In this prospective study (chapter 2), 112 DMD patients had muscle biopsy and were tested for exon deletions. The diagnosis of DMD was confirmed by histopathology and/or genetics in 101 patients. Here, we concluded that this high deletion rate by mPCR (90%) was possibly due to good phenotyping.

For the purpose of understanding the natural history in DMD, confirmed cases seen between 1998 and 2014 were contacted through letter / telephone / follow-up visit (chapter 3). Around 275 cases out of 500 families participated, 31% by completing the questionnaire and 69% by completing the questionnaire and attending the clinic.

Wheelchair status was attained in 124 (45.1%) boys at a mean age of 10.4 years and bedbound state in 24 at a mean age of 11.8 years. Seven patients (2.6%) had died at a mean age of 15.2 year. This is the first study from India describing the disease progression of DMD. The patterns of major DMD milestones, including the age at onset, age at loss of ambulation and death in our cohort is comparable to that of the Western cohorts.

In the subsequent study (chapter 4), we compared the sensitivity of mPCR and MLPA testing in 83 DMD cases. MLPA-negative cases underwent muscle immunohistochemistry (IHC). mPCR detected deletions in 60 (72.3%), while MLPA detected deletions in an additional six cases (7.2%) and duplications in 5 (6.5%). IHC confirmed dystrophinopathy in 30 (36.1%), sarcoglycanopathy in 30 (36.3%), and no deficiency in 23 (27.7%). This is the first comparative study of its kind in India (and possibly in the world) to study the sensitivity and pattern of mutations by both mPCR and MLPA in the same cohort.

Later on we studied the genotype-phenotype pattern in 317 patients with dystrophinopathy confirmed by MLPA method (chapter 5). There were 279 cases of DMD, 32 of BMD and 6 of intermediate phenotype. In this cohort 88% of the rearrangements in the dystrophin gene caused DMD.The reading-frame rule was present in 90% of DMD and in 94% of BMD cases. A tendency toward a lower IQ and earlier wheelchair dependence was observed with distal exon deletions.

It is well known that DMD children have a high incidence of neuropsychiatric manifestations. In chapter 6 we describe our study in which a total of 22 school-going DMD boys aged between 6 to 10 years old were neuropsychologically assessed by means of Wechsler Intelligence Scale for Children-Third Edition, Rey's Auditory Verbal Learning Test, and the Memory for Designs Test. They had a significantly lower IQ (88.5). Verbal IQ (86.59) was found to be lower than Performance IQ (92.64). The neuropsychological findings supported the hypothesis that these children have specific cognitive deficits as opposed to a global intellectual deficit. Based on these results we offer advanced neuropsychological testing as well as cognitive retraining for DMD cases at NIMHANS.

Clinicallly DMD children have a particular pattern of muscle weakness and to correlate this we performed lower limb muscle MRI and identified a distinct pattern in all cases irrespective of the age at evaluation (chapter 7). Muscle MRI could help as an imaging biomarker for directing genetic testing and, if serially performed, could help in assessing drug response.

In our latest report (chapter 8) we have studied 606 cases of DMD and compared various parmeters between familial and non-familial forms of DMD. The mutations comprised of exonic deletions in 81.2%, duplications in 5.4% and small mutations (point mutations and INDELs) in 11.5% of cases. Families with two or more affected males contributed to 12% of the entire cohort. MLPA identified the larger mutations more often in sporadic (88.2%) than in familial cases (75.3%), while nonsense mutations were more common in familial (17.8%) than in sporadic cases (10.7%). The familial group reported an earlier onset of disease (2.8 ± 1.7 years) as compared to sporadic cases (3.8 ± 1.6 years). MLPA could identify mutations in a high percentage of our DMD children. The preponderance

of small mutations was noted to be distinctly higher in the familial group. Intriguingly, the familial form of DMD formed a small percentage of the entire cohort. The reasons could be increasing awareness among parents and physicians with early identification of DMD cases, genetic counseling and prenatal testing.

The above studies on DMD depict the journey we have experienced and the outcome of the research over the last many years at NIMHANS. In Chapter 10, all findings of the various chapters were put into broader perspective. Moving from clinical and biochemical diagnosis in the 1980's we now have reached the status of center of excellence and advanced care for DMD at par with leading international centers. However, we still lack in several palliative care aspects and end-of-life care for DMD children. Also, more serious attempts to identify all DMD carriers followed by genetic counseling are required.

The roughly 4000 newly diagnosed DMD patients each year in the Indian subcontinent have a tremendous societal and economic burden. Every attempt is necessary to diagnose DMD at an early stage and offer genetic counseling and pre-natal testing.

Mutation-negative cases should be subjected to novel NGS or Whole Genome Sequencing (WGS) in case of high clinical suspicion and positive family history. NGS has the added advantage of detecting complex rearrangements and large-scale intronic alterations, thus offering a higher mutation detection rate than MLPA and other exon-based tests. A small number will require mRNA studies.

Having a genetic diagnosis in 100% of suspected DMD cases is imperative to offer the important genetic counseling and prenatal testing. Finally, because at NIMHANS we currently can go through all stages of genetic testing, we can compete with most international advanced standards of genetic testing and care in DMD.