Duchenne muscular dystrophy

Valorisation

In this thesis we have investigated various aspects of Duchenne Muscular Dystrophy (DMD).

Here we would like to indicate the impact of our research on the standard of diagnosis and management of children with DMD, their family members and care takers as well as future directions.

The Dystrophin (DMD) gene is the largest gene described in human beings. It is made up of more than 2.5 million base pairs (bp), which comprises about 0.1% of the total human genome and 1.5% of the entire X chromosome (Kunkel LM et al., 1989). The properties of the Dystrophin (DMD) gene offer several challenges that include the large size of the gene, frequent new mutations and de novo mutations and problems in identifying its optimal expression level and the target tissue. Due to the huge size of the DMD gene, vector and vector delivery programs have met with hurdles in effective gene therapy. Despite these difficulties, new potential forms of genetics-driven therapeutic strategies are being tested and implemented, e.g. stem cell therapy, virus-based gene therapy, and exon skipping by antisense oligonucleotides or morpholinos. DMD is a rapidly progressive debilitating disease with skeletal and cardiac muscles being the most commonly affected tissues in DMD. However, brain is also a major site of dystrophin expression with several neuropsychiatric manifestations. Presently there is no specific cure for DMD and the lack of any effective treatment has emphasized the need for prenatal diagnosis and carrier detection. Genetic testing for dystrophinopathy is highly sensitive and specific, however identifying a proband often leads to implications for several relatives at risk for cardiomyopathy, weakness, or anesthetic reactions which is a huge ethical challenge. There is currently no genetic screening program implemented in India. The findings of this thesis can be used to design genetic testing which must be sideguarded by genetic counseling before and after the genetic testing. The translation of our research work into standard of care for all patients with DMD including the latest availability of disease modifying agents is the next application of this thesis.

Currently, several genetics-driven therapeutic approaches to cure DMD are being investigated, which can be categorized into two groups: therapies that aim to restore dystrophin expression, and those that aim to compensate for the lack of dystrophin. Therapies that restore dystrophin expression include read-through therapy, exon skipping, vector-mediated gene therapy, and cell therapy. Of these approaches, the most advanced are the read-through and exon skipping therapies. In 2014, Ataluren, a drug that can promote ribosomal read-through of mRNA containing a premature stop codon, was conditionally approved in Europe. Presently, we are part of the global Ataluren trial in DMD cases with nonsense mutation. This is an example of the genetic-pharmacology that is possible through the stringent geno-phenotype analysis that is presented in this thesis.

Improving care through knowledge sharing

The data in this thesis has not only been presented at national and international meetings and conferences, but also in several continuing medical education and muscular dystrophy awareness programs. We also participated in patient – parents programs and shared our research data with peers, students and teachers. All previous data has been published

in peer-reviewed journals and are accessible for reading. Without knowledge sharing through publications as well as conference deliberations on DMD, the less common phenotypic presentations of DMD (predominant cognitive form, cardiomyopathy form) would only be appreciated by neuromuscular specialists and not by general physicians and pediatricians. Every year, we host rotations at NIMHANS for medical personnel to improve their knowledge and understanding in DMD and all other neuromuscular disorders. The collected and acquired data is shared with the public and particularly the parents and relatives of DMD children. We also have prepared dedicated manuals on DMD for the patient and caregivers in English, Hindi, and Kannada. All patients registered at NIMHANS receive a copy of this.

The role of muscle MR imaging and its potential capacity to act as a non-invasive biomarker is emphasized as a highly interesting application of this thesis. Children with high clinical suspicion of DMD but negative by MLPA testing could undergo muscle MRI to look for the characteristic findings and, if positive, then proceed with more expensive genetic testing for DMD. Thus, this could lead to avoiding the painful invasive muscle biopsy in some cases. Moreover, in clinically challenging cases with childhood muscular dystrophies such as sarcoglycanopathy and Fukutin related protein (FKRP) disorder with Duchenne like phenotype, the DMD-specific pattern of muscle involvement on MRI could aid in proceeding for advanced targeted genetic testing, further reducing the need for muscle biopsy. Thus, our findings of the distinct MRI pattern in DMD will aid in this decision. Furthermore, the degree of fibro-fatty infiltration could be a useful marker for assessing the disease severity and progression as well as the response to treatment. Thus, muscle MRI could be utilized as an "adjunct and localized biomarker" for DMD.

Patterns of symptom clustering with Attention Deficit Hyperactivity Disorder, Autistic Spectrum Disorder, and mental subnormality as identified in this thesis suggest that DMD is a major neuropsychiatric syndrome that requires prompt evaluation and early intervention by child psychiatrist / psychologist. In this thesis we used a battery of intelligence, learning, and memory tests to identify the neuropsychological profile in boys with DMD. We identified specific cognitive deficits like significantly lower IQ (88.5) with verbal IQ (86.59) lower than performance IQ (92.64). We also found impaired performance on the processing speed, freedom from distractibility, and verbal comprehension indexes. Specific deficits in information processing, complex attention, immediate verbal memory span, verbal working memory, verbal comprehension, vocabulary, visuoconstruction ability, and verbal learning and encoding were also observed. However, perceptional organization, general fund of information, abstract reasoning, visual discrimination and acuity, visual learning and memory, and verbal memory were adequate. Our findings were opposed to the more frequently reported global intellectual deficit in DMD. Cognitive disturbances have been identified even in infants and children less than 5 years of age with DMD. Enhanced psychology testing to include both cognitive and neurobehavioral disorders is nowadays recommended for all children with DMD. The results of this thesis can be used to develop a program to help DMD-patients with cognitive disorders.

Special cognitive training modules could be developed fot the DMD children based on the impairment as identified at school or in special education centers during the elementary school years itself.

We suggest that the standard evaluation for young boys with global developmental delay includes an inexpensive but sensitive serum creatine kinase test to capture undiagnosed cases of DMD at an early stage. Early diagnosis and genetic counseling can prevent mothers from having a second child with DMD. The program to address the non-motor cognitive deficits in DMD should include early identification of the various disturbances as well as appropriate measures including behavioural therapy, special school admission and cognitive training.

Neurologists and child psychiatrists should evaluate for ADHD in DMD children with behavioral concerns (e.g., inattention, hyperactivity, impulsivity) or poor academic progress using validated assessment tools with observers from several settings (home, school, community) and self-observation. Behavioral treatments are recommended for preschool-aged children and may be helpful at older ages. Effective behavioral therapies include parent training, classroom management, and peer interventions. While there is no cure for specific learning disorders in DMD, we can help them to improve their reading, writing, and mathematics skills. They would require both strengthening the skills and developing a learning strategy tailored to take advantage of the child's strengths. Treatment for specific learning disorder often also involves multimodal teaching. A learning specialist can identify the learning disabilities in the DMD child and help determine the special services a child might benefit from at school. Psychotherapy, cognitive behavior therapy in particular, may also be helpful in treating the emotional and behavioral problems in DMD. Ultimately, the goal of treatment is to improve symptoms, optimize functional performance, and remove behavioral obstacles. Finally, the aim is that all these interventions would lead to the improvement in the quality of life for DMD patients and their families. We at NIMHANS are now in a strong position to identify all forms of mutations, to perfom genotype-phenotype correlation and to offer early diagnosis. This will result in early intervention with respect to cognitive disturbances and will provide a base for appropriate counseling for schooling and vocational training.

Major components of the thesis work presented here were directly and clinically applicable and resulted in a successful standard of care for all cases of DMD, which was agonizingly lacking in our country. A confirmatory and reliable genetic diagnosis of DMD children was not available till a decade ago, leading to lack of genetic counseling and prenatal testing. There were no means to advise / test regarding carrier status and mothers took a risk with future male births. Further, as a result of the continued knowledge deficit among the illiterate / literate families and physicians there are families with multiple members affected in multiple generations leading to profound emotional and economic burden on the already poor families.

Therefore, the long term goals of this research data is to collaborate with the knowledge users such as the government, philanthropists, social and humanity related individuals. Financial support and commitment of governmental and non-governmental agencies is of utmost importance to continue with clinical and genetic work. National policies need to be framed that will have resource allocation for research and improvement of clinical care on such rare diseases because families with DMD children silently suffer.

India needs more specialists in neuromuscular disorders to take care of the large numbers of these patients although they are rare disorders. Informal collaborations that are common among scientists need to be made more formal between institutions. Our research consists of expertise, experience and knowledge gathered over several decades of research on DMD, beginning with humble clinical studies to the latest and high technology genetic studies.

Future perspectives

DMD research needs "transdisciplinary research" and management strategies. Multidisciplinary specialists comprising of pediatricians, neurologists, neuromuscular specialists, clinical geneticist, neuropsychologists, rehabilitation specialists, counselors, special teachers and social workers, need to come together to provide the best care for all patients and their families. In our research work we have involved different specialities in the armamentarium of DMD management which can be translated to other neurology centers in the country. In addition, we have learnt that there is a need for participatory transdisciplinary research in DMD. The fact that the knowledge gained through our DMD research enabled us to be part of an "advisory transdisciplinary" group for other neurodegenerative diseases may be the utmost application of the work presented in this thesis.