SUMMARY

Muscular dystrophy is a general term that describes a group of inherited and progressively debilitating myogenic disorders. They are broadly divided on the basis of onset and pattern of weakness into those with onset of weakness at birth (Congenital muscular dystrophy) and those with later onset limb-girdle weakness, the X-linked dystrophinopathies (Duchenne, Becker, and female carriers of a mutated dystrophin gene), and the limb-girdle muscular dystrophies (LGMD).

The aim of the study was to characterise differential diagnosis of dystrophinopathies, sarcoglycanopathies and congenital muscular dystrophy for correspondence between phenotype, genotype with protein abnormalities in 29 Indian muscular dystrophy patients. Of these, 19 were diagnosed as suffering from DMD, 2 BMD, 4 female carriers, 3 LGMD and 1 CMD. The dystrophin gene was analysed in 18 DMD/BMD families for the presence of deletion by mPCR for 27 exons and cDNA probes for the entire gene. Fourteen independent deletions were identified in 21 DMD/BMD patients. The concordance between the clinical phenotype and ‘reading frame’ hypothesis was observed in 10 (71%) cases. Of these, 3 DMD patients were wheelchair bound between 6-10.6 years of age and the remaining DMD patients (age range 2.6-12 years) showed progressive difficulty in walking. Four DMD patients had in-frame deletion and although ambulatory had difficulty in walking. There were 3 DMD patients who showed no deletion and were ambulatory. Severe dystrophin deficiency in DMD phenotype was demonstrated by either a complete absence or by presence of isolated dystrophin positive fibres. However, truncated dystrophin was detectable in 2 patients while a complete absence was observed in rest. Dystrophin deficiency in DMD patients was also associated with deficiency of the
components of the dystrophin-associated proteins (DAPs; β-dystroglycan and α-sarcoglycan) complex. Two BMD males (SN and RS) (age range 10-12 years) presented to the clinic with symptoms suggestive of progressive proximal muscle weakness and enlargement of calf muscles. Physical examination revealed muscle hypertrophy, positive Gower’s sign and waddling gait. In-frame deletion was identified in patient SN. Reduced levels of altered size dystrophin were observed in western blots. RS with no detectable deletion had reduced levels of normal-sized dystrophin. Both of these patients produced a patchy and discontinuous pattern of labelling for dystrophin on tissue sections. The reduction in the DAPs staining in these patients was milder compared with DMD patients. The relative quantity of β-dystroglycan in patients RS and SN was 86% and 100%, respectively, while amount of α-sarcoglycan was similar to that of normal control.

Correlation of phenotype, genotype with protein abnormalities in DMD/BMD patients showed that relative levels of muscle dystrophin not only correlated with immunocytochemical patterns of subsarcolemma staining but also with the clinical severity exhibited by these patients.

Four female dystrophinopathy patients presented to clinic between age 7-24 years with difficulties in climbing the stairs and rising from the floor. Dystrophin immunostaining in three carriers (SD, SA, and ST) occurred in a mosaic pattern. All the three female patients showed dystrophin of normal molecular weight but reduced amounts by immunoblotting. DMD carrier NT showed no detectable dystrophin either by immunostaining or immunoblotting. The immunocytochemical studies of β-dystroglycan and α-sarcoglycan in SD, SA and ST revealed a mosaic pattern, labelling at near normal intensity and patchy pattern, respectively. A complete absence of labelling for both β-dystroglycan and α-sarcoglycan was observed in NT. Immunoblot for β-dystroglycan and α-sarcoglycan in SD and ST showed 43 kDa and 50 kDa bands of reduced intensity while no reduction in intensity of
either of the bands was observed in SA. In NT a complete deficiency of β-dystroglycan and α-sarcoglycan was observed. It has been observed that as patients get older, both the preferential loss of negative fibres and diffusion of dystrophin within the cytoplasm of single fibres serve to cloud correlation between the muscle dystrophin expression and clinical phenotype. This was true in case of SA (24 years) as the disease severity did not match the high dystrophin content.

Three LGMD patients belonging to two families were studied. One family consisting of five sibs of which two brothers (BJ, 16 and BJB, 14 years of age) mentally normal, uneducated products of healthy parents presented to neurology clinic with progressive difficulty in walking and frequent falls for last 5 years. They could not walk vertically but both were moving on their rump at the time of presentation. The third LGMD patient PT presented to the clinic at age 10 years with history of progressive muscular weakness since last 6 months. He had normal mental and motor milestones. Physical examination revealed weakness of the trunk muscles and Gower's sign was positive. In patient BJ, there was a complete lack of α-sarcoglycan associated with reduced staining of β-, γ-, and δ-sarcoglycans while, in PT a complete lack of β- and γ-sarcoglycans was associated with reduced staining of α-sarcoglycan and δ-sarcoglycan. Muscle biopsy from BJ and PT showed dystrophic changes and reduced, patchy binding of dystrophin. Immunoblot analysis revealed dystrophin of normal molecular weight but of decreased quantity in both the patients.

CMD Patient PK presented to the clinic at age 1.5 years with marked hypotonia and weakness in all limbs since the age of 3 months. A complete absence of merosin (laminin α2) was observed in this patient while immunocytochemical staining with antibodies against dystrophin revealed interfibre and intrafibre variability.
Secondary abnormalities of dystrophin staining were observed in both LGMD patients BJ, PT and also in CMD patient PK. The pattern of dystrophin abnormalities found were similar to that seen in the milder primary dystrophinopathies (BMD). It seems that the diagnosis of a primary dystrophinopathy cannot be based exclusively on decreased or patchy staining with dystrophin antibodies and abnormalities on immunoblot.

A variation in the HindIII restriction pattern of the dystrophin gene with cDMD probe 11-14 was observed. A strongly hybridising fragment of 2.8 kb was present in each of the Indian subjects. The 1.9 and 1.8 kb bands appeared as a single faint co-migrating fragment in all these subjects. X-chromosomal origin for all the fragments was confirmed by dosage, with female DNA showing two copy intensity and male DNA showing single copy intensity. The observations in Indian subjects with respect to other ethnic groups have shown similar results indicating that the Indian population in fact does not differ from other populations. The slightly different standard pattern of restriction fragments detected in the present study when human DNA, normal or disease-risk was cleaved with HindIII and hybridised with probe 11-14 is important since a few deletions have been mapped to this region with probe 11-14 in DMD patients.