INTRODUCTION

The muscular dystrophies are defined as hereditary disorders characterised by progressive muscle weakness and wasting. The discovery and cloning of the gene, responsible for Duchenne muscular dystrophy (DMD) (Monaco et al., 1986; and Koenig et al., 1987), and its protein product, dystrophin which is absent in the muscle of Duchenne muscular dystrophy patients (Hoffman et al., 1987), has ushered the muscular dystrophies into the era of molecular genetics. Since then, most of the genes and the protein defects that are involved in the pathogenesis of muscular dystrophy have been or are being defined at a breathtaking pace.

Duchenne de boulogne (1868) made a detailed description of a group of 28 patients and established diagnostic criteria for the Duchenne muscular dystrophy. Gowers, (1879) noted the predilection of the disease for males and emphasised its exclusively maternal inheritance pattern in familial cases. The existence of an X-linked muscular dystrophy with later onset and slower progression than in DMD was noted by Becker (Becker and Keiner, 1955), who proposed that the disease was a benign variant of DMD, known as Becker muscular dystrophy (BMD). In 1980s, cytogenetic studies and reverse genetics methodology demonstrated the position of DMD locus at Xp21 (Verellen-Dumoulin et al., 1984; Francke et al., 1985; Ray et al., 1985; and Kunkel et al., 1985). Similar studies in BMD indicated that DMD and BMD map to same locus and that they are allelic disorders (Kingston et al., 1984). In 1987, the DMD and BMD gene was cloned and sequenced (Monaco et al., 1986; and Koenig et al., 1987) and its 427 kDa protein product identified and labelled as dystrophin (Hoffman et al., 1987). The mutations affecting the dystrophin gene are due to large rearrangements of the gene, consisting of deletions and occasionally duplications of DNA portions, in approximately
70% of DMD and 85% of BMD patients. In remaining patients, mutations are small and consist of point mutations and microdeletions (Engel et al., 1994; and Nigro et al., 1995).

There is no consistent relationship between the size and location of the dystrophin gene defect and the severity of the clinical phenotype, ranging from severe DMD to mild BMD. Most of the differences in severity are explained by the translational frame rule, proposed by Monaco et al. (1988). If a mutation produces a shift in the nucleotide sequence of the codons (frame-shifting mutation), the open reading frame of the mRNA is not maintained, which leads to premature termination of translation due to reading of an eventual stop codon. If a mutation maintains the open reading frame, the mRNA is translated into a protein with an internal deletion of amino acids, corresponding to the deleted part of the gene. Frame-shifting mutations of the dystrophin gene typically lead to synthesis of truncated dystrophin molecule without cysteine rich and C-terminal domains. This causes severe dystrophin deficiency with a DMD phenotype as demonstrated by absent sarcolemmal dystrophin immunostaining and absence of dystrophin on immunoblotting of muscle homogenates when using antibodies against C-terminal domain (Hoffman et al., 1988; Beggs et al., 1991; and Nicholson et al., 1993a, b, c). Most in-frame mutations produce a smaller size dystrophin molecule, often in reduced amounts, which is associated with a BMD phenotype. Immunostaining with different dystrophin antibodies shows reduced sarcolemmal dystrophin expression, which is completely absent when antibodies against a deleted dystrophin epitope is used. Immunoblot analysis of BMD muscle homogenates with a panel of dystrophin antibodies provides quantitative information on the degree of dystrophin deficiency, the size of the residual dystrophin molecule, and the regions of the molecule that are deleted and preserved. In general, the severity of the clinical phenotype correlates well with the relative abundance of the muscle dystrophin, which
depends on the functional significance of the deleted region of the gene (Beggs et al., 1991; and Nicholson et al., 1993b). Dystrophin deficiency is associated with deficiency of components of the dystrophin-associated-protein (DAP) complex, and the degree of DAP deficiency correlates with the paucity of residual dystrophin and loss of DAP binding regions of dystrophin (Matsumura and Campbell, 1994). Severe DAP complex deficiency has been reported in DMD patients in whom truncated dystrophin with selective lack of the cysteine rich and C-terminal domains is properly localised to the sarcolemma while in BMD patients DAP complex is well preserved (Matsumura et al., 1993a, b, 1994). These finding indicate that dystrophin is necessary for the DAP complex to be properly organised in the sarcolemma.

Women with an abnormal dystrophin gene on one X chromosome are DMD/BMD carriers. They have a mixture of dystrophin-positive and dystrophin negative myonuclei in the multinucleated skeletal muscle fibres, as a consequence of inactivation of one of the X-chromosomes during embryogenesis according to the Lyon hypothesis. This mosaic pattern of dystrophin expression probably explains why a vast majority of carriers are asymptomatic. Clinical expression of the gene defect is thought to be precluded in asymptomatic carriers because dystrophin, produced by dystrophin positive nuclei, diffuse to areas of the sarcolemma, controlled by dystrophin negative myonuclei (Pegoraro et al., 1995). Indeed, sarcolemmal dystrophin immunostaining is normal in most muscle fibres, only occasional muscle fibre being unstained in asymptomatic carriers (Clerk et al., 1991). However, muscle weakness is clinically evident in 2.5% to 10% of carriers, who are therefore called manifesting carriers (Moser and Emery, 1974). The symptoms vary from mild proximal weakness to severe disability, similar to that of DMD (Sewery et al., 1993; and Bushby et al., 1993b). The main mechanism for disease expression in manifesting carriers appears to be non random, preferential inactivation of healthy X chromosome during
embryogenesis (Pegoraro et al., 1995). Dystrophin immunostaining in muscle from manifesting carriers occurs in a mosaic pattern with normal, reduced, and absent staining of muscle fibres (Clerk et al., 1991). The percentage of dystrophin-negative muscle fibres appears to correlate with the severity of the clinical expression.

The genetic heterogeneity of the limb-girdle muscular dystrophies (LGMD) has become better understood, thus leading to a new classification of these disorders (Bushby and Beckmann, 1995). Eight genes have been already mapped for AR-LGMDs which are: LGMD2A at 15q (Beckmann et al., 1991), LGMD2B at 2p (Bashir et al., 1994), LGMD2C at 13q (Noguchi et al., 1995), LGMD2D at 17q (Roberds et al., 1994), LGMD2E at 4q (Lim et al., 1995; Bönnemann et al., 1995), LGMD2F at 5q (Passos-Bueno et al., 1996), LGMD2G at 17q (Moreira et al., 1997) and LGMD2H at 9q (Weiler et al., 1998).

Among these AR-LGMDs: LGMD2D, LGMD2E, LGMD2C, and LGMD2F arise from mutations in genes encoding four different dystrophin-associated glycoproteins [DAG/dystrophin-associated proteins (DAP)], α- (adhalin), β-, γ-, and δ-sarcoglycans respectively (Noguchi et al., 1995; Roberds et al., 1994; Lim et al., 1995; Bönnemann et al., 1995; Passos-Bueno et al., 1996; McNally et al., 1994; and Nigro et al., 1996a, b). Mutations in any of the four known members lead to more or less pronounced secondary deficiencies of the other components of the complex, indicating the importance of the integrity of the entire complex for the prevention of muscle cell degeneration (Mizuno et al., 1994b). The results of extensive genotype-phenotype correlation studies, indicate that patients with LGMD2E and LGMD2F (β- and δ-sarcoglycan) are always severely affected, whereas considerable inter-intra familial clinical variability may be observed among patients with LGMD2C and LGMD2D (Angelini et al., 1999; McNally et al., 1996; and Passos-Bueno et al., 1999). It was proposed that the residual amount of sarcoglycans correlate with the severity of the disease (Carrie et al., 1997; and Eymard et al., 1997). The
clinical severity of primary $\alpha$-sarcoglycanopathy (LGMD2D) varies strikingly. The most severe course has been observed in whom $\alpha$-sarcoglycan was completely absent. A pronounced but variable decrease in $\alpha$-sarcoglycan, were usually observed in milder forms of variable severity (Piccolo et al., 1995; and Jeanpierre et al., 1996).

The congenital muscular dystrophies (CMD) are a heterogeneous group of disorders, transmitted by an autosomal recessive inheritance pattern. Four major forms of congenital muscular dystrophy have been identified: classic (occidental) or 'pure' form of CMD (Banker et al., 1994); Fukuyama type CMD (F-CMD) (Fukuyama et al., 1960, 1981); Muscle-eye-brain disease (MEB) (Santavuori et al., 1977, 1989); and Walker-Warburg syndrome (Dobyns et al., 1989). Analysis of the components of the DAP complex has revealed that there is a selective absence of the M (laminin $\alpha_2$) chain of merosin in muscle of significant number of patients with occidental CMD (Tomé et al., 1994; Dubowitz and Fardeau, 1995). In these patients the genetic defect is localised to the M (laminin $\alpha_2$) chain on the long arm of chromosome 6 (6q2) (Hillaire et al., 1994). Absence of merosin may disrupt the linkage between sarcolemma and the basal lamina, thereby leading to muscle changes according to a mechanism, which is similar in occidental CMD, DMD, and SCARMD (Severe childhood autosomal recessive muscular dystrophy) (Tomé et al., 1994; and Campbell, 1995). In other patients with occidental CMD, the expression of merosin is either normal or reduced, and there is no linkage to the M (laminin $\alpha_2$) chain (Hillaire et al., 1994; and Dubowitz and Fardeau, 1995).

Abnormalities of dystrophin, the sarcoglycans, and laminin $\alpha_2$ are responsible for a subset of the muscular dystrophies. The present study has been undertaken with the following objectives. An attempt has been made to study:

1. The differential diagnosis of dystrophinopathies, sarcoglycanopathies and congenital muscular dystrophy
(a) To analyse DMD/BMD patients for intragenic deletions using multiplex polymerase chain reaction and by cDMD probes.

(b) To characterise dystrophin and dystrophin-associated proteins in muscle biopsy specimens from patients with dystrophinopathies, limb-girdle and congenital muscular dystrophy.

(c) To correlate phenotype, genotype with protein abnormalities.

2. To assess the variation in HindIII restriction pattern of the dystrophin gene with cDMD probe 11-14.

3. To study autosomal recessive limb-girdle muscular dystrophy (AR-LGMD): immunocytochemical analysis and clinical aspects.