DISCUSSION

Sarcoglycanopathies (SGs) are muscular dystrophies due to disruption of the dystrophin-associated sarcoglycan complex caused by mutations in any of the four already identified sarcoglycan genes (α-, β-, γ-, and δ-SG) (Noguchi et al., 1995; Roberds et al., 1994; Lim et al., 1995; and Bönnemann et al., 1995). Mutations in any one of these sarcoglycans leads 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 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). The clinical severity of primary α-sarcoglycanopathy (LGMD2D) varies strikingly. The most severe course has been observed in those where α-SG was completely absent. A pronounced but variable decrease in α-SG, were usually observed in milder forms of variable severity (Piccolo et al., 1995; and Jeanpierre et al., 1996). Patients BJ and BJB were severely affected sibs with contractures in the hip, knee, and the ankle joints but clinically they could not be classified as DMD as age of onset was in the early part of the 2nd decade and late part of 1st decade respectively and neither could be classified as BMD as their disease progression was very fast. On the whole, the clinical characteristic of the BJ and BJB are remarkable by: (1) the peculiar clinical presentation in being able to slide on their buttocks; (2): with late onset but rapid progression resulting in contractures of hip, knee and ankle joints. Patient PT was more or less normal till the age of 9.6 years. Provisional clinical diagnosis in case of PT was BMD as he had presented to the clinic at 10 years of age with progressive trunk muscle weakness for last 6
months. Analysis of sarcoglycans and dystrophin confirmed that BJ, BJB and PT were cases of primary sarcoglycanopathy and not dystrophinopathy.

In agreement with Beckmann and Bushby, (1997) to discriminate between different LGMD2 entities, both immunocytochemistry and western blot are helpful for the initial screening. α-SG immunocytochemistry can be taken as the first step to screen all the cases for sarcoglycanopathy and to separate these cases from others. Mutations of any component of the sarcoglycan complex result in the secondary loss of the other components of the complex. In many cases, the defective sarcoglycan protein was not detected by immunocytochemical analysis, whereas other sarcoglycans were weakly or strongly stained (Oxelé et al., 1996; Sewry et al., 1996; Vainzof et al., 1996; and Eymard et al., 1997). In patients with α-sarcoglycan deficiency on immunostaining, a complete deficiency (absence of staining) was found to be more specific for mutations in the α-sarcoglycan gene than for mutations in β- or γ-sarcoglycan gene. On the other hand, a partial deficiency of α-sarcoglycan on immunostaining was not specific for mutations in any of the sarcoglycan genes (Duggan et al., 1997a). It is most likely that patient BJ suffers from primary adhalinopathy, since immunoreactivity for α-sarcoglycan was completely absent while β-, γ-, and δ-sarcoglycans were highly reduced. However, in patient PT, β- and γ-sarcoglycan were absent while α- and δ-sarcoglycan were reduced. All the sarcoglycan genes need to be investigated in this patient.

Immunocytochemistry revealed a patchy pattern with antibodies against dystrophin in BJ and PT. Western blot of BJ revealed slightly reduced amount of dystrophin (90% of normal with rod-domain and 89% of the normal with C-terminal domain). Immunoblot analysis of dystrophin in PT revealed moderate reduction by rod-domain antibody (34% of the normal) but no dystrophin could be observed by C-terminal antibody. Normal amounts of dystrophin have been observed in SCARMD (Jelloun-Dellagi et al., 1990; Fardeau et al., 1993; and Piccolo et al., 1995). In contrast, studies by Duggan et al. (1996) in LGMD2D
patients showed reduced to variable staining with antibodies directed against dystrophin while muscle protein studies by Passos-Bueno et al. (1999) in Brazilian population for seven autosomal recessive LGMDs revealed that dystrophin was reduced in quantity in all LGMD2C, 2E, and 2F patients. However among the seven LGMD2D patients only one severely affected showed a reduction in dystrophin quantity by western blot. Jones et al. (1998) described three patients with primary sarcoglycanopathy; two could be proven γ-sarcoglycanopathy. Dystrophin in these two patients was abnormal on immunocytochemistry and immunoblot. In contrast, the third patient had absent staining with β-sarcoglycan, patchy staining with α- and γ-sarcoglycan but relatively normal dystrophin staining, a pattern suggesting a primary abnormality of another component of the sarcoglycan complex. Vainzof et al. (1999) described an LGMD2C patient showing deficiency in dystrophin by means of western blot and immunofluorescence, comparable with an DMD manifesting carrier.

Staining for β-dystroglycan in BJ and PT was found to be patchy while by immunoblot a moderate reduction of β-dystroglycan (63% of the normal and 34% of the normal respectively) was observed. Duggan et al. (1996) observed only a single of 30 childhood onset muscular dystrophy patients who had partial deficiency of α-sarcoglycan and showed variable staining with β-dystroglycan. β-dystroglycan was found to be preserved in SCARMD patients from North-Africa, Europe, and Japan (Matsumura et al., 1992; Fardeau et al., 1993; and Hayashi et al., 1995).

In conclusion, abnormalities of dystrophin should not be used to exclude patients from sarcoglycan analysis without confirmation of primary dystrophinopathy, as dystrophin abnormalities may be secondary.