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Probable high prevalence of limb-girdle muscular dystrophy type 2D in Taiwan

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ABSTRACT

Limb-girdle muscular dystrophy type 2D (LGMD2D), an autosomal-recessive inherited LGMD, is caused by the mutations in *SGCA*. *SGCA* encodes alpha-sarcoglycan (SG) that forms a heterotetramer with other SGs in the sarcolemma, and comprises part of the dystrophin-glycoprotein complex. The frequency of LGMD2D is variable among different ethnic backgrounds, and so far only a few patients have been reported in Asia. We identified five patients with a novel homozygous mutation of c.101G>T (p.Arg34Leu) in *SGCA* from a big aboriginal family ethnically consisting of two tribes in Taiwan. Patient 3 is the maternal uncle of patients 1 and 2. All their parents, heterozygous for c.101G>T, denied consanguineous marriages although they were from the same tribe. The heterozygous parents of patients 4 and 5 were from two different tribes, originally residing in different geographic regions in Taiwan. Haplotype analysis showed that all five patients shared the same mutation-associated haplotype, indicating the probability of a founder effect and consanguinity. The results suggest that the carrier rate of c.101G>T in *SGCA* may be high in Taiwan, especially in the aboriginal population regardless of the tribes. It is important to investigate the prevalence of LGMD2D in Taiwan for early diagnosis and treatment.

1. Introduction

Limb-girdle muscular dystrophy (LGMD) is a group of clinically and pathologically similar but genetically heterogeneous diseases. It is clinically characterized by progressive muscle weakness predominantly in shoulder and pelvic girdles with sparing of the facial muscles, and pathologically necrotic and regenerating process of muscle fibers with variable extent of fibrosis. To date, more than 20 causative genes have been reported for LGMD2, the recessive form of LGMD, which is more common than the dominant form of LGMD (LGMD1). The prevalence of each LGMD2 is variable among different ethnic populations and geographic areas probably due to the effect of the founder mutations [7].

Sarcoglycanopathy (LGMD2C–2F) is caused by mutations in the *SGCG*, *SGCA*, *SGCB* and *SGCD* genes, encoding gamma-, alpha-, beta- and delta-sarcoglycans (SG), respectively. Sarcoglycanopathy comprises about 10–15% of LGMD2 in Western countries, and is even more common in North Africa, India and Brazil [14,16,20]. In contrast, it is rarely reported from the Asian countries. Among all sarcoglycanopathy, LGMD2D is the most prevalent form and the patients with LGMD2D usually show milder clinical phenotypes, compared with LGMD2C, 2E and 2F [17]. In the skeletal muscles, these four SGs form a heterotetramer in sarcolemma, composing the dystrophin-glycoprotein complex (DGC) together with other proteins. The DGC links cytoskeleton and extracellular matrix, and therefore participates in not only mechanical force transmission but cell signaling.

Abbreviations: LGMD, limb-girdle muscular dystrophy; SG, sarcoglycan; DGC, dystrophin-glycoprotein complex; IHC, immunohistochemistry; CK, creatine kinase.

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The clinical phenotype of sarcoglycanopathies overlaps with those of the muscular dystrophies with the defects in other DGC proteins. Similar to Duchenne muscular dystrophy, the onset age of the patients with sarcoglycanopathy is often in early childhood and the disease progression is rapid. However, milder cases with slow progression also exist. Cardiac and respiratory involvement is commonly seen in the advanced stage, but seems to be relatively rare in LGMD2D.

We herein report five patients carrying a homozygous c.101G>T in *SGCA* from a big aboriginal family. This novel sequence variant may be a founder mutation among different aboriginal tribes in Taiwan.

2. Patients and methods

2.1. Patients

A total of 18 family members from a big aboriginal family were enrolled for mutation analysis and genetic consultation (Fig. 1). Physical examination was done and recorded. Informed consents for blood drawing and mutation analysis for diagnostic purpose were obtained from all 18 individuals or their guardians. This study was approved by the institutional review board of the Kaohsiung Medical University Hospital.

2.2. Histochemistry

Biopsied muscle specimens were frozen in isopentane cooled in liquid nitrogen. Serial frozen sections were stained by a battery of histochemical methods including hematoxylin and eosin, modified Gomori-trichrome and NADH-tetrazolium reductase.

2.3. Immunohistochemistry (IHC)

Frozen sections of 6 μm thickness were used for IHC according to the standard protocols with a Ventana Benchmark automated stainer [8]. Primary antibodies used in this study were monoclonal anti alpha-, beta-, gamma-, and delta-SG antibodies (Leica).

2.4. Mini-multiplex immunoblotting

The detailed techniques of immunoblotting have been described previously [19]. The cocktail of primary antibodies was prepared with 5% milk in TBST including anti-dystrophin, 1:50; dysferlin, 1:1000; p94/Calpain 3, 1:20; alpha-SG, 1:100; caveolin-3, 1:2500. The antibodies were available from Novocastra Laboratories, and Transduction Laboratories (caveolin-3).

2.5. Mutation analysis

Genomic DNA was extracted from blood according to standard protocols. All exons and their flanking intronic regions of *SGCA*, *SGCB*, *SGCG* and *SGCD* were amplified and sequenced using an automated 3100 DNA sequencer (Applied Biosystems, Foster, CA).

2.6. Haplotype analysis

Haplotype analysis was performed by using fifteen microsatellite markers from the National Center for Biotechnology Information (NCBI) on chromosome 17q21.33: D17S2180, D17S1868, D17S797, D17S943, D17S1795, AF028234, D17S588, D17S1319, D17S1869, D17S1815, D17S747, D17S1820, D17S941, D17S956 and D17S809. The physical location and genetic distance of the microsatellite markers are shown in Fig. 4. Genotyping was done by performing PCR amplification with fluorescent-labeled primers followed by capillary electrophoresis on a Beckman CEQ-8000 genetic analysis system.

3. Results

3.1. Patient phenotypes (Table 1)

Patient 1 (V-2 in Fig. 1) is a 17-year-old male. His mother is from Bunun, an aboriginal tribe in Taiwan and his father is half-blooded of Bunun and Tsou, another aboriginal tribe. Patient 1 claimed intermittent myalgia over bilateral lower extremities after exercise from 3 years of age. At the age of 4, difficulty in climbing stairs was noticed and he was brought to our outpatient clinic. At the first visit, he showed bilateral proximal leg muscle weakness and hyporeflexia. Serum creatine kinase (CK) level was up to 39,072 IU/L (normal range < 175). The intelligence test showed normal. Muscle biopsy revealed dystrophic changes with normal dystrophin staining. The disease course was progressive and his muscle weakness spread to upper extremities since the age of 7. He became wheelchair-bound while 11-years-old. Neither cardiac nor respiratory involvement has been found so far.

Patient 2 (V-1 in Fig. 1), an elder sister of patient 1, is a 19-year-old girl. She was diagnosed with idiopathic hepatitis since birth because of persisted hypertransaminasemia, but no symptom or sign of hepatopathy was ever noticed. Since the age of 5, she complained of intermittent muscle cramps after exercise. Proximal muscle weakness and hyporeflexia were observed in the hospital at that time. Blood test showed hyperCKemia (CK = 21,332 IU/L). At age 6, muscle biopsy was performed and showed dystrophic changes with preserved dystrophin staining. Upper extremities were affected since 10 years of age. At age 12, her muscle CT revealed relative muscular atrophy with fatty

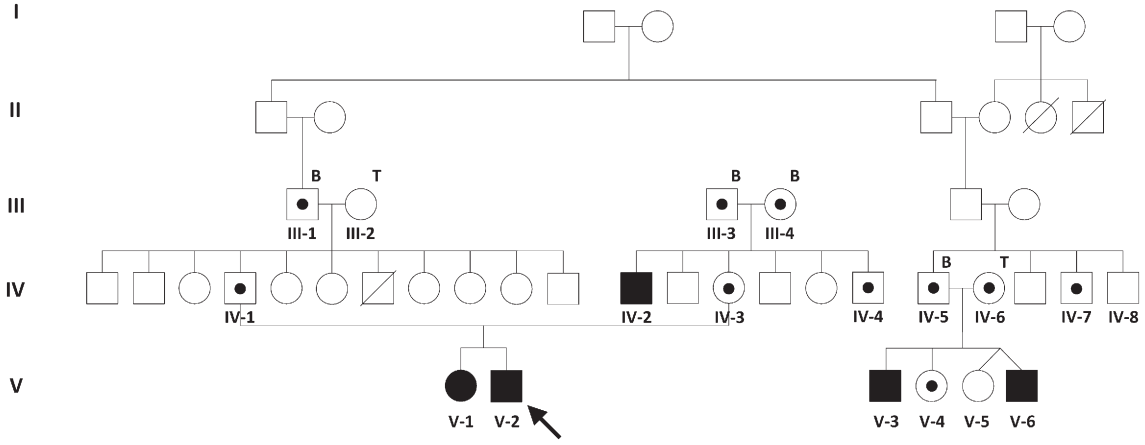

Fig. 1. Family pedigree. V-2 is the index patient, patient 1. V-1, IV-2, V-3 and V-6 represent patient 2, 3, 4 and 5, respectively. Thirteen other family members had mutation analysis (numbered individuals) for c.101G>T in *SGCA* and 10 in 13 were found to be heterozygous carriers (the individuals with dot). B indicates Bunun tribe and T represents Tsou tribe.