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Clinical and Genetic Analysis of Korean Patients with Facioscapulohumeral Muscular Dystrophy

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominantly inherited muscular disorder, which is characterized by weakness of facial, shoulder and hip girdle, humeral, and anterior distal leg muscles. The *FSHD* gene has been mapped to 4q35 and a deletion of integral copies of a 3.3-kb DNA repeat motif named D4Z4 was known to be the genetic background of the disorder. Although FSHD is the second most common muscular dystrophy in adulthood, there were few reports on the genetically confirmed patients in Korea. Recently, we experienced four Korean patients with clinical features resembling FSHD. In order to confirm the diagnosis, conventional Southern blot (SB) analysis by using double digestion with *EcoR*I and *Bln*I and hybridization with p13E-11 probe was performed in three patients and newly developed long polymerase chain reaction (PCR) method was used for one patient because genomic DNA was not enough for conventional SB for this patient. All patients were demonstrated to have shortened D4Z4 repeats that were consistent with FSHD. Therefore, we could confirm the diagnosis of FSHD in four Korean patients and appropriate genetic counseling was done for the patients and their families. It is of note that long-PCR method could be a good alternative for conventional SB when D4Z4 repeats were less than 5.

Key Words : *Facioscapulohumeral Muscular Dystrophy; FSHD; D4Z4; Korean*

Chang-Seok Ki, Seung-Tae Lee, Kyung-Sook Kim, Jong-Won Kim, Yoon-Ho Hong*, Jung-Joon Sung*, Kyung Seok Park*, and Kwang-Woo Lee*

Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; Department of Neurology*, Seoul National University College of Medicine, Seoul, Korea

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Address for correspondence
Kyung Seok Park, M.D.
Department of Neurology, Seoul National University Bundang Hospital, 300 Gumi-dong, Bundang-gu, Seongnam 463-707, Korea
Tel : +82.31-787-7469, Fax : +82.31-718-9327
E-mail : pks1126@chol.com

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INTRODUCTION

Facioscapulohumeral muscular dystrophy (FSHD; MIM 158900) is a neuromuscular disorder with an autosomal dominant pattern of inheritance and the incidence of this disease is estimated to be 1/20,000 in the European population (1). In most cases, the clinical phenotype has been known to be distinctive. Facial weakness, winged scapulae, horizontally placed clavicles, humeral and brachioradialis muscle atrophy, proximal limb weakness, and asymmetry in muscle involvement are found to some degree in most affected individuals (1).

In 1992, Wijmenga et al. revealed that FSHD is due to deletions of integral copies of a 3.2 kb tandemly repeated D4Z4 units at the 4q35 locus (2). In 95% of patients with FSHD, the D4Z4 repeat is contracted to an array of 1-10 units, and, apparently, at least one unit of D4Z4 is required to develop FSHD (2, 3). There is a rough and inverse relationship between clinical severity and the residual repeat size, with the smallest repeats causing the most severe phenotypes (4, 5).

With the aid of genetic testing, the clinical phenotype has expanded to include asymptomatic gene carriers, facial-sparing scapular myopathy, proximal weakness without scapular winging, and distal myopathy (6-11). Intrafamilial clinical variability is a common feature in FSHD (12). Symptoms usually commence in the second or third decade for most affected patients (13).

Although FSHD is the second most common muscular dystrophy after myotonic dystrophy 1 in adulthood and the incidence of this disease in Korean population might not be different from Europeans, there were only few reports on Korean patients with FSHD (13-15). In the present study, we performed a genetic study of Korean patients with clinical features resembling FSHD. In addition, we investigated the correlation between the severity of clinical features and that of genetic abnormalities.

MATERIALS AND METHODS

Four unrelated patients with clinical features of FSHD were clinically and genetically evaluated. To assess the clinical severity, we adopted Clinical Severity Scale based upon the extent of weakness in various muscular parts (16). With informed consent, genomic DNA was extracted from peripheral blood leukocytes. In order to determine the number of D4Z4 repeats on 4q35, we performed conventional South-

ern blot analysis or long-polymerase chain reaction (PCR) as described previously (17). For Southern blot analysis, *Eco*RI or *Eco*RI/*Bln*I digested genomic DNA was separated by gel electrophoresis and then the DNA was transferred to Hybond XL (Amersham Biosciences, Piscataway, NJ, U.S.A.). After hybridization with radioisotope labeled p13E-11 equivalent PCR probe, the *Eco*RI and *Eco*RI/*Bln*I allele sizes were analyzed (18). Long-PCR detection of shortened D4Z4 repeats was performed for patient 3 because the genomic DNA was not enough for conventional Southern blot analysis. All conditions and primer sequences for long-PCR was described previously and a positive control with 2 D4Z4 repeats and a negative control were tested in parallel (19).

RESULTS

Clinical findings

Patient 1 was a 30-yr-old woman complaining of progressive limb muscle weakness. She was in good health until age 12 yr, at which time she began to have a difficulty in raising her right arm above her head. Later, she developed increasing difficulties climbing stairs. By report, her mother had similar symptoms from the age of 40. His 35-yr-old brother was not affected clinically (Fig. 1A). The neurological examination was remarkable for moderate facial weakness; moderate (Medical Research Council [MRC] grade 3) and mild (MRC grade 4) weakness of proximal upper and lower limb muscles, respectively; humeral atrophy; anterior axillary folds; winged scapulae; waddling gait; and hypoactive deep tendon reflexes. The serum creatine kinase (CK) was 233 IU/L (normal, <270 IU/L). Other routine laboratory tests including thyroid and parathyroid function were normal. Audiogram, electrocardiogram, echocardiogram, nerve conduction studies were unremarkable. Needle electromyography showed chronic myopathic changes in the left deltoid, biceps brachii, and vastus lateralis muscles. A muscle biopsy from the right vastus lateralis revealed marked fiber size variations, increased internalized nuclei, endomyseal fibrosis and fat ingrowth, and frequent lobulated myofibers.

Patient 2 was a 36-yr-old man presenting with progressive limb muscle weakness, which developed at the age of 20. Initially, he began to have a difficulty in raising his arm above his head and climbing stairs. The weakness was slowly progressive. His mother was noted to have similar symptoms (Fig. 1B). His 34-yr-old brother and 39-yr-old sister were not affected clinically. The neurological examination was remarkable for moderate facial weakness; mild (MRC grade 4) and minimal weakness of proximal and distal extremity muscles, respectively; and humeral and quadriceps atrophy. Other remarkable findings included winged scapulae, anterior axillary folds, waddling gait, and hypoactive deep tendon reflexes. The serum creatinine kinase (CK) was 610 IU/L. The forced vital capacity (FVC) was 3.2 L (71% of predicted). Electrocardiogram, echocardiogram, audiogram, and nerve conduction studies were normal. Needle electromyography demonstrated chronic myopathic changes.

Patient 3 was an 18-yr-old man complaining of progressive right upper limb weakness over the prior 3 yr. The initial diagnosis was idiopathic right brachial plexopathy. He was a tennis player in his early teens. There was no family history of neuromuscular disease (Fig. 1C). Clinical examination of his parents was normal. Remarkable neurological findings included mild bifacial weakness; mild (MRC grade 4) and minimal weakness of the right and left proximal upper

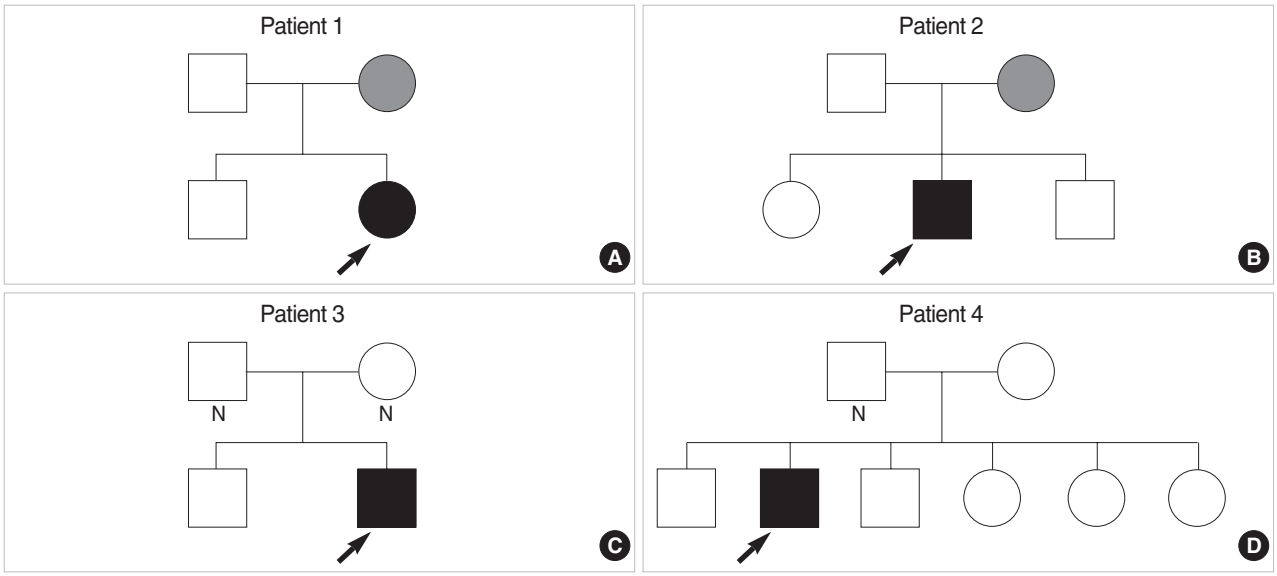

Fig. 1. Pedigrees of 4 patients with FSHD. Circle, female; square, male; black symbol, affected with shortened D4Z4 repeats; gray symbol, possibly affected by history; arrow, proband; N, tested for D4Z4 repeats and found normal.

limb muscles, respectively; winged scapulae in the right side; and hypoactive deep tendon reflexes. The serum CK was 750 IU/L. Other routine laboratory tests including thyroid function were normal. Audiogram, electrocardiogram, echocardiogram, and nerve conduction studies were unremarkable. The FVC was 4.2 L (98% of predicted). Needle electromyography revealed chronic myopathic changes.

Patient 4 was a 46-yr-old man presenting with progressive limb muscle weakness developed in his early twenties. He first noticed a difficulty in raising his arm above his head. There was no known family history of neuromuscular disease in his parents, five sibs, and two daughters (Fig. 1D). The neurological examination was remarkable for mild (MRC grade 4) weakness of proximal upper limb muscles and humeral atrophy. Other remarkable findings included anterior axillary folds, asymmetric winged scapulae, trapezius hump sign, Beevor's sign, and hypoactive deep tendon reflexes. There was no facial weakness. The serum CK was 170 IU/L. The FVC was 4.19 L (87% of predicted). Electrocardiogram, echocardiogram, and nerve conduction studies were normal. Needle electromyography showed chronic myopathic changes.

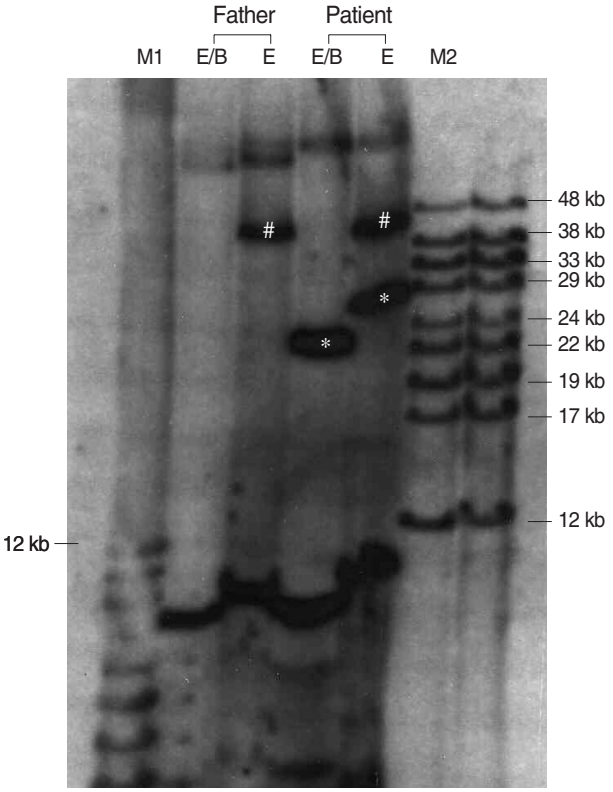

Fig. 2. Representative results of Southern blot analysis. Convential electrophoresis and Southern blot analysis of *EcoR*I (E) and *EcoR*I/*Bln*I (E/B) digested DNA from patient 4 and his father revealed a 28-kb sized *EcoR*I fragment (*) resistant to *Bln*I digestion only in patient 4. Another *EcoR*I framgenet between 38 kb and 48 kb disappeared after *Bln*I digestion (#).
M1, 1-kb size marker; M2, high molecular weight size marker.

Genetic analysis and genotype-phenotype correlations

All four patients were demonstrated to have shortened D4Z4 repeats. Patients 1, 3, and 4 had 4, 6, and 7 repeats of D4Z4 segment confirmed by conventional Southern blot, respectively (Fig. 2). Patient 2 was tested by long-PCR that he had 3 D4Z4 repeats (Fig. 3). When we tested both parents of the patient 3, they did not have shortened D4Z4 repeats suggesting a de novo mutation occurred in patient 3. Since the mothers of patients 1 and 2 were not tested genetically, we could not confirm the inheritance of pathogenic D4Z4 repeats in these families.

In the four patients in our study, the tendency of inverse

Table 1. Clinical features of the patients with FSHD in relation to D4Z4 repeat number

Patient	Onset age (yr)	Clinical severity scale*	D4Z4 repeat number
1	12	3.5	4
2	20	3.0	3
3	15	1.5	6
4	20	1.5	7

*, Clinical severity scale by Ricci et al. 1999.
FSHD, facioscapulohumeral muscular dystrophy.

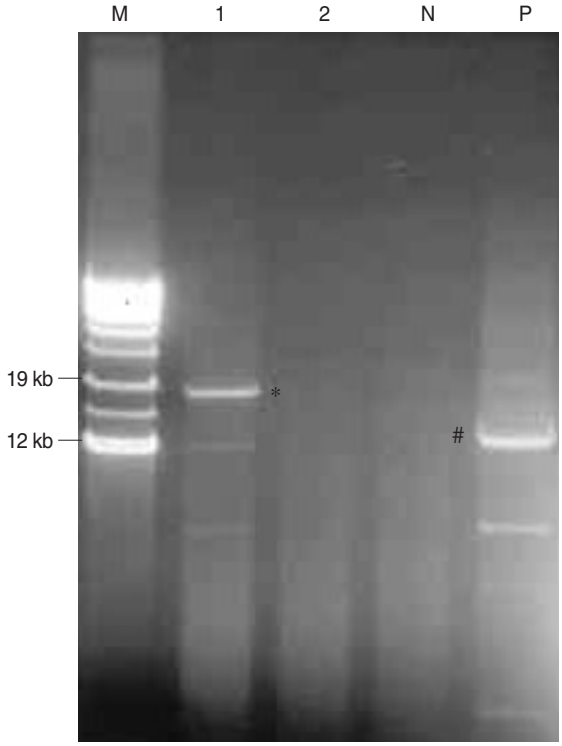

Fig. 3. Result of long-PCR amplification in patient 2. A 18.4 kb PCR product (*) corresponding to 3 D4Z4 repeats was detected in patient 2 (lane 1) while a 11.8 kb sized band (#) was noticed in positive control (P) with 2 D4Z4 repeats. No band was observed in the patient's brother (lane 2) and in negative control (N).
M, high molecular weight size marker.

correlation between clinical severity and D4Z4 repeat number was suggested. However, similar tendency was not found between age of onset and D4Z4 repeat number (Table 1).

DISCUSSION

To date, the diagnosis of FSHD was usually made on the basis of clinical findings in Korea. Although individual patients with FSHD may vary to some extent, most of the patients fit into a clinically typical feature. However, with the availability of genetic testing, it has been recently reported that the clinical manifestations of FSHD are much broader than previously known (6-11). Therefore, it can be misleading to make a diagnosis solely based on the clinical findings especially in the patient with atypical presentations. In our study, two patients showed unusual clinical features. The initial diagnosis of patient 3 was idiopathic brachial plexopathy due to right arm weakness, asymmetric winged scapulae, and a negative family history. In patient 4, facial weakness and family history were absent. Furthermore, other tests including serum CK, needle electromyography, and muscle biopsy often do not helpful in the diagnosis of FSHD, as in our patients. Therefore, genetic testing is essential to confirm the correct diagnosis of FSHD. And it will be also helpful to facilitate the diagnosis and avoid unnecessary tests, especially in the patient with atypical presentations.

Molecular diagnosis for FSHD is used for diagnostic confirmation, pre-symptomatic testing, and for prenatal diagnosis. FSHD diagnosis relies on the detection of a D4Z4 repeat array less than 35 kb (<10 units) (20). However, a number of factors have complicated the molecular diagnosis of FSHD (13). First, it was reported that a highly homologous and equally polymorphic repeat resides on chromosome 10. Second, in nearly one-fifth of all individuals, exchanges of repeat units have been observed between chromosomes 4 and 10. A third complication comes from the observation of a biallelic variation of chromosome 4qter, designated 4qA and 4qB. It is reported that FSHD is associated solely with the 4qA allele and contractions of D4Z4 on 4qB subtelomeres do not cause FSHD (21, 22). Furthermore, a minority of patients carries a contraction of D4Z4 that extends in a proximal direction. The disease allele in these patients often goes undetected, as the probe region is also missing from the disease allele. Lastly, locus heterogeneity still remains. Nonetheless, Southern blot analysis using two restriction enzymes discriminating 4-type and 10-type units are very useful that FSHD can be diagnosed with up to 98% accuracy (23).

Recently, a new diagnostic method for rapid and specific diagnosis of FSHD by a long PCR has been introduced (19). This long PCR method can specifically amplify the repeated region from chromosome 4q up to 18.4 Kb in size and countable from one to five D4Z4 repeated units. By using this new technique, we can confirm a diagnosis of FSHD in one patient in whom we could not perform conventional Southern blot analysis due to insufficient DNA. Although Lemmers et al. (24) argued that at least half of the Caucasian FSHD patients could not be diagnosed by the long PCR method because of the different distribution of D4Z4 repeats alleles in Japanese and in Caucasians, this method might be useful when the conventional Southern blot analysis is unavailable. Nevertheless, careful attention should be made to prevent false-negative results by long PCR method because of amplification failure due to 5 or more D4Z4 repeats.

Penetrance of FSHD was found variable by age and gender that it was 83% by age 30 yr and males were reported to be more severely and more frequently affected than females (95% vs. 69%, respectively) (25). Anticipation in FSHD remains controversial, but an inverse correlation between D4Z4 repeat size and the clinical severity and onset age was reported (4, 5). A similar result was suggested only in the clinical severity in our study. However, the number of the patients in our study was too limited to clearly verify a relationship between the clinical features and D4Z4 repeat size. Further study in a larger number of the FSHD patients is needed.

This study reports four patients with genetically established FSHD. Two of them had family histories while the others did not. Considering that genomic structure of D4Z4 repeat in chromosomes 4 and 10 in Koreans is similar to that in Japanese, the frequency of FSHD might not be different from that in Japan (26). However, there are only limited reports on the FSHD in Korea (14, 15). Therefore, more efforts should be made to identify and to diagnose Korean patients with FSHD by genetic analysis.

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