

# Fukuyama 선천성 근이영양증에서의 분자유전학적 분석

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## Molecular Genetic Analysis in Dystroglycanopathy with the Fukuyama Congenital Muscular Dystrophy Phenotype

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**Purpose:** Fukuyama congenital muscular dystrophy (FCMD) is a rare, autosomal-recessive disorder characterized by early-onset hypotonia associated with brain malformations in dystroglycanopathy. Although the wide spectrum of congenital muscular dystrophies causes difficulty in diagnosis, correlating the genotype with the clinical phenotype can help diagnose FCMD. Here, we evaluated the correlation of targeted molecular genetic analysis of *FKTN* gene mutation with the FCMD phenotype.

**Methods:** This study was conducted retrospectively with 9 subjects. Inclusion criteria included clinical symptoms characterized by early-onset hypotonia with magnetic resonance imaging (MRI) featuring brain malformations. *FKTN* gene-alteration analysis was performed using various *FKTN* gene-analysis methods, including sequencing.

**Results:** Among the 9 subjects studied, 4 (44.4%) were male and 5 (55.6%) were female. The median age of onset of the first symptom was 3.1 months. The first symptom was a delayed milestone in 6 cases (66.7%). All 9 subjects (100%) presented with early-onset hypotonia and global delayed development. All subjects presented with cortical malformation in their brain MRIs. Of the 9 subjects, 6 subjects had previously undergone muscle biopsy and 4 cases (4/6; 66.7%) showed dystrophic or myopathic features. Pathogenic mutations causing FCMD were identified in 3 cases.

**Conclusions:** In this study, all 3 subjects with *FKTN* mutations showed important MRI findings (pachygyria and cerebellar dysplasia). These data suggest that patients with characteristic phenotypes who show pachygyria and cerebellar abnormalities in brain MRIs may have a high probability of being diagnosed with FCMD.

**Key words:** Fukuyama congenital muscular dystrophy, Dystroglycanopathy, *FKTN* gene, Fukutin, Muscle pathology, Brain MRI

### Introduction

Fukuyama congenital muscular dystrophy (FCMD) is a rare, autosomal-recessive disorder characterized by early-onset hypotonia associated with

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brain malformations<sup>1</sup>). First described in 1960, it is the second most common muscular dystrophy in Japan, with an incidence of approximately 2.1–11.9 in 100,000<sup>1,2</sup>). The diagnosis is generally made based upon clinical features, along with laboratory results, muscle histology, and brain imaging<sup>3</sup>). The gene responsible for FCMD, identified as *FKTN*, is located at chromosome 9q31 and encodes the fukutin protein<sup>4</sup>). It has been discovered that FCMD is caused by a 3–kb retrotransposal insertion in the 3′–untranslated region of the *FKTN* gene<sup>5</sup>).

Dystroglycanopathy includes a group of muscular dystrophies caused by defective glycosylation of  $\alpha$ –dystroglycan, due to a reduction in its ligand–binding capacity<sup>6</sup>). Mutations in 6 different genes are known to cause congenital dystroglycanopathy: *FKTN*, *FKRP*, *POMT1*, *POMT2*, *POMGNT1*, and *LARGE*<sup>4,7–11</sup>). Recently, analysis of large cohorts revealed that instead of one gene causing a phenotype, a complex overlapping relationship exists between the underlying gene defects and the resulting clinical phenotypes<sup>12</sup>). This phenomenon makes it difficult to identify the causative gene; thus, molecular genetics testing is currently under development to yield confirmative results<sup>13</sup>).

According to previous studies, the carrier frequency of the *FKTN* gene mutation in the Korean population is approximately 1 in 935<sup>14</sup>). The first clinically and genetically confirmed Korean FCMD patient was reported in 2009<sup>15</sup>). Considering the geographic proximity and ethnic similarity between Korean and Japanese populations, it is probable that FCMD is also relatively prevalent in Korea<sup>16</sup>). Although the wide spectrum of congenital muscular dystrophies causes difficulty in diagnosis, correlating patients' genotypes and clinical phenotypes can be informative. In this study, we evaluated the correlation of targeted molecular genetic an-

alysis of *FKTN* gene mutation with the FCMD phenotype.

## Materials and Methods

### 1. Subjects

The study was conducted retrospectively with 9 subjects who were followed at Gangnam Severance Hospital. Inclusion criteria included clinical symptoms characterized by early–onset hypotonia with a MRI featuring brain malformation<sup>12</sup>). Relevant clinical symptoms included muscle weakness and delayed motor development before 6 months of age. Other clinical features, such as personal history, organ involvement, and muscle biopsy results were also reviewed<sup>1</sup>). MRI findings were categorized into 4 subgroups: cerebral abnormalities (polymicrogyria and pachygyria), white matter involvement, cerebellar abnormalities (dysplasia and cysts), and brainstem involvement<sup>12</sup>). Exclusion criteria included retinal abnormalities and MRI findings with severe brain malformation, such as lissencephaly<sup>17</sup>). The study protocol was approved by the Institutional Review Board of the Yonsei University Gangnam Severance Hospital, and written informed consent was obtained from the parents or legal guardians of all patients (4–2011–0285).

### 2. Genetic analysis

*FKTN* gene–alteration analysis based on the GenBank reference sequence NM\_001079802.1 ([www.ncbi.nlm.nih.gov/GenBank](http://www.ncbi.nlm.nih.gov/GenBank)) was performed using various *FKTN* gene–screening methodologies including sequencing the entire coding region and the flanking 20–bp introns, and PCR (polymerase chain reaction). The deep intronic hotspot, c.647+

2084G>T, which is well known to cause FCMD, was also analyzed by Sanger sequencing. The 3'-untranslated region (UTR) hotspot, a 3,062-bp insertion, was also analyzed by the polymerase chain reaction (PCR).

## Results

### 1. Clinical characteristics

The clinical characteristics of the 9 subjects who presented with the FCMD phenotype are shown in Tables 1 and 2. Among these 9 cases, 4 (44.4%) were male and 5 (55.6%) were female. None of the patients showed a family history of muscular dystrophy. Two subjects (22.2%) presented with perinatal asphyxia, both with respiratory difficulty at birth. The median age of onset

**Table 1. General Characteristics of Patients with the FCMD Phenotype (n=9)**

Male:female ratio	4:5
Perinatal asphyxia	2 (22.2%)
Family history	0 (0.0%)
Onset of symptoms (months)	3.1±2.3
First symptom	
Delayed milestone	6 (66.7%)
Respiratory difficulty	2 (22.2%)
Hypotonia	1 (11.1%)

of the first symptom was 3.1 months. The first symptom was a delayed milestone in 6 cases (66.7%), respiratory difficulty was observed in 2 cases (22.2%), and hypotonia was observed in 1 case (11.1%). All 9 subjects (100%) presented with early-onset hypotonia and globally delayed development.

### 2. Organ involvement

Subjects showed clinical symptoms involving various organs, as shown in Table 2. Six patients (66.7%) developed epilepsy, showing a neurological involvement. None of the subjects showed electrocardiogram abnormalities, however; 1 case (11.1%) developed hypertrophic cardiomyopathy as revealed by an echocardiographic abnormality. Five patients (55.6%) showed pulmonary involvement, of which 3 (33.3%) eventually required a tracheostomy. Four cases (44.4%) showed gastrointestinal involvement, of which 3 (33.3%) eventually required enteral feeding.

### 3. Brain MRI findings

All subjects presented with cortical malformation: 6 cases (66.7%) had pachygyria, 6 cases

**Table 2. Symptoms, Diagnostic Evaluation, and Clinical Outcome of Patients with the FCMD Phenotype**

	Hypotonia	Global delayed development	Epilepsy	Cardiologic		Pulmonary		Gastrointestinal		MRI finding				Muscle biopsy	Last evaluation (year)	Maximal motor ability		
				Echocardiographic abnormality	Respiratory symptoms*	Tracheostomy	Dysphagia	Enteral feeding	Cerebrum		White matter involvement	Cerebellum	Brainstem			Bed-ridden	Head control	Sitting
									Pachygyria	Polymicrogyria								
1	+	+	+	-	+	-	+	-	+	-	Focal	Dysplasia and cysts	Atrophy	Dystrophic	6.3	→		
2	+	+	+	HCMP	+	+	+	+	+	+	Diffuse	Dysplasia and cysts	Atrophy	Dystrophic	13.6	→		
3	+	+	-	-	+	+	+	+	+	+	Diffuse	Dysplasia	Normal	Not done	15.3 <sup>†</sup>	→		
4	+	+	-	-	-	-	-	-	-	-	Diffuse	Normal	Normal	Dystrophic	5.0	→		
5	+	+	+	-	+	-	-	-	+	-	Focal	Normal	Normal	Dystrophic	3.3	→		
6	+	+	+	-	-	-	-	-	+	+	Diffuse	Normal	Normal	Non-specific	7.7	→		
7	+	+	+	-	-	-	-	-	+	+	Focal	Cysts	Atrophy	Not done	11.7	→		
8	+	+	-	-	+	+	+	+	+	-	Diffuse	Dysplasia and cysts	Normal	Unavailable <sup>‡</sup>	16.0	→		
9	+	+	+	-	-	-	-	-	-	+	Diffuse	Normal	Normal	Not done	8.9	→		

Abbreviations: HCMP, Hypertrophic cardiomyopathy

\*Respiratory symptoms including frequent aspiration and/or pneumonia; <sup>†</sup>expired; <sup>‡</sup>muscle biopsy was performed, but the results are unavailable due to an insufficient specimen

(66.7%) had polymicrogyria, and 3 cases (33.3%) had both polymicrogyria and pachygyria (Table 2). Abnormal white matter–signal changes were diffuse in the MRIs of 6 subjects (66.7%) and focal in 3 cases (33.3%). Five patients (55.6%) showed cerebellar involvement, of which 3 (33.3%) showed cerebellar dysplasia and cysts, and 1 (11.1%) showed only cysts. Three patients (33.3%) showed brainstem involvement, all of which featured brainstem atrophy.

#### 4. Muscle biopsy findings

Of the 9 subjects, 6 subjects underwent muscle biopsy (Table 2). Four subjects (66.7%) showed dystrophic or myopathic features, including muscle fiber size variability and necrotic or regenerative muscle fibers (Table 2). One subject (16.7%) showed non–specific findings. One subject who underwent a muscle biopsy showed unclear results due to an insufficient muscle content in the specimen.

#### 5. *FKTN* genetic analysis

Among the 9 subjects who presented with the FCMD phenotype, molecular genetic analysis revealed that 3 subjects (1–3) were heterozygous for the 3,062–bp insertion within the 3'–UTR (Fig. 1). In subject 1, a G to T heterozygous substitution at nucleotide position –1,243 of intron 6 was detected. In subject 2, a novel heterozygous missense mutation (p.Ser17Arg, c.49A>C) was found. In subject 3, the previously reported p.Arg47Ter *FKTN* missense variant (which is a pathogenic mutation causing FCMD) was found.

#### 6. Clinical outcomes

Subjects showed various clinical outcomes, as described in Table 2. The mean age of the last evaluation that took place was 9.8 years old, and the maximal motor ability that was evaluated was as follows: 5 subjects (55.6%) were bed–ridden, 2 subjects (22.2%) showed head control, and 2



**Fig. 1.** The 3 cases that revealed mutation in the *FKTN* gene through molecular genetic analysis.

subjects (22.2%) could sit with assistance. No subjects showed motor development beyond sitting with assistance. In addition, subject 3 expired at the age of 15.3 years old.

## Discussion

Over the past 50 years, muscle biopsies have been essential in diagnosing muscular disorders<sup>18)</sup>. Although dystrophic or myopathic patterns with fiber-size variability combined with necrotic or regenerative fibers is observed commonly in congenital muscular dystrophy<sup>19)</sup>, muscle-biopsy results cannot confirm the subtype. In this study, 6 subjects underwent muscle biopsy. Of the 4 subjects that showed dystrophic or myopathic features, 2 subjects (50%) were subsequently found to have an *FKTN* mutation through genetic analysis. Because muscle biopsy alone cannot classify congenital muscular dystrophy, advances in molecular diagnostics are under continuous development to address this unmet need.

Currently, molecular genetic analysis (such as next-generation sequencing) can be readily applied to diagnose muscular dystrophies<sup>20)</sup>. However, next-generation sequencing is time-consuming and expensive; thus, greater efficiency is critical. Dystroglycanopathies are caused by mutations in genes encoding glycosyltransferases that cause a broad spectrum of clinical severity<sup>21)</sup>. The complex genotype-phenotype correlation causes difficulty in establishing criteria for undergoing genetic study, based solely on clinical features. In this study, the clinical characteristics of 9 subjects with the FCMD phenotype were examined, and *FKTN* mutations were detected in 3 of the 9 subjects (33.3%). Genetic analysis of all 3 subjects showed a compound heterozygous mutation in the coding region and a retrotransposal insertion in

the 3'-UTR of the *FKTN* gene. Evidence presented in an earlier study performed by Lim et al. suggested that the c.648-1243G>T mutation was highly prevalent in the Korean population<sup>16)</sup>. Of the 3 subjects with an *FKTN* mutation, subject 3 was found to have the c.648-1243G>T mutation. This variation has not yet been reported according to the mutation database and has not been observed in a control population database (dbSNP, 1000 genomes, ExAC). Therefore, we performed an in silico analysis to evaluate a possible effect of the variation. Bioinformatic analyses revealed that the affected residue is strictly conserved and predicted that it would be deleterious by means of SIFT (<http://sift.bii.a-star.edu.sg/index.html>) and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>). We regarded that as pathogenic variant according to the standards and guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology<sup>22)</sup>.

In previous studies, FCMD patients showed brain abnormalities such as cerebral dysplasia, white matter abnormality, and cerebellar and brainstem abnormalities<sup>23)</sup>. In this study, we analyzed the MRI findings of patients with the FCMD phenotype. Of the 6 subjects with pachygyria, 3 cases (50%) had an *FKTN* mutation. Of the 5 subjects with polymicrogyria, 1 (20%) had an *FKTN* mutation, and this patient showed both polymicrogyria and pachygyria. Of the 5 patients with cerebellar abnormality, 3 (60%) had an *FKTN* mutation, while of the 2 patients with brainstem abnormality, 1 (50%) showed an *FKTN* mutation. Important MRI findings that were consistent in all 3 subjects with an *FKTN* mutation were pachygyria and cerebellar dysplasia. Collectively, these data suggest that patients who show pachygyria and cerebellar abnormalities in brain MRI are

highly likely to be diagnosed with FCMD.

Molecular genetic analysis can be used to confirm a diagnosis of congenital muscular dystrophy. Next-generation sequencing involves whole-exome sequencing, which requires much time and expense; a targeted gene analysis can maximize the efficiency of diagnosis. In this study, patients presenting with early-onset hypotonia and MRI abnormalities of pachygyria and cerebellar dysplasia were found to also have an *FKTN* mutation. Based on this evidence, indicative phenotypic criteria for FCMD can be established to assess candidates that should undergo *FKTN*-targeted molecular genetic analysis. This study has a limitation in that the rarity of congenital muscular dystrophies resulted in a small patient data set, which makes it difficult to provide a generalized prospective regarding FCMD diagnostics. Genetic study of muscular disorders is a field that has yet to mature and requires ongoing investigation.

## 요 약

**목적:** Fukuyama 선천성 근이영양증은 희귀한 열성 유전질환으로 영아 시기에 발병하는 근긴장 저하, 뇌 기형 및 dystroglycanopathy 특징들을 보인다. 선천성 근육병의 넓은 스펙트럼에 여러 질환들이 존재하여 Fukuyama 선천성 근이영양증 진단을 어렵게 하지만, 유전형과 표현형 상관관계를 파악하면 진단을 도울 수 있다. 이 연구에서는 분자유전학 분석을 통해 선정한 *FKTN* 유전자와 Fukuyama 선천성 근이영양의 표현형의 연관성에 대해 알아보았다.

**방법:** 이 연구는 후향적으로 9명의 대상자들로 진행하였다. 영아 시기에 발병하는 근긴장 저하의 증상 및 뇌 자기공명영상에서 기형 소견을 보인 환자들을 대상으로 선정하였다. 그리고 *FKTN* 유전자를 이용한 염기서열 검사를 통해 유전자를 분석하였다.

**결과:** 9명의 대상자들 중 남성이 4명(44.4%), 여성이 5명(55.5%) 였다. 첫 증상이 발병한 나이의 중간값

은 3.1개월였다. 6명(66.7%) 에서 첫 증상이 발달 지연으로 나타났다. 모든 환자들은 영아 시기에 근긴장 저하 및 전반적 발달 지연 소견을 보였다. 또한, 모든 환자들은 뇌 자기공명영상에서 뇌 피질 기형 소견을 보였다. 9명의 환자들 중 6명이 근육생검 검사를 실시하였고 그 중 4명(4/6; 66.7%)이 특이 소견을 보였다. Fukuyama 선천성 근이영양증을 일으키는 *FKTN* 유전자 돌연변이는 3명에서 발견되었다.

**결론:** 이 연구에서 *FKTN* 유전자 변이를 보인 3명의 대상자들은 모두 뇌 자기공명영상에서 큰뇌이랑증 및 소뇌 형성장애 소견들을 보였다. 이것을 통해 근육병 증상을 보이면서 뇌 자기공명영상에서 특징적인 소견들을 보일 시 Fukuyama 선천성 근이영양증을 진단할 가능성을 높일 수 있다는 것을 확인하였다.

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