Mutational Analysis of Fukutin Gene in Dilated Cardiomyopathy and Hypertrophic Cardiomyopathy

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Background Mutations in FKTN encoding for fukutin cause Fukuyama-type congenital muscular dystrophy characterized by severe muscle wasting and hypotonia with mental retardation. Fukuyama-type congenital muscular dystrophy is a recessive genetic trait. FKTN mutations in patients with dilated cardiomyopathy (DCM) have been investigated by our research group. The patients showed hyper-CKemia with mild or no muscle weakness and without mental retardation, suggesting that the clinical spectrum of FKTN mutations are wider than previously thought. The current study was designed to further explore the association of FKTN mutations with DCM or hypertrophic cardiomyopathy (HCM).

Methods and Results A total of 172 patients with DCM, 144 patients with familial HCM and 384 control individuals were analyzed for FKTN mutations. There was a DCM patient who was a compound heterozygote of a 3-kb insertion mutation and a missense mutation Cys101Phe. The patient showed hyper-CKemia with mild muscle involvement and no brain involvement. In contrast, 2 other DCM patients and 3 controls were heterozygous for the insertion mutation and normal allele, showing that the heterozygous insertion mutation itself was not associated with DCM. No mutation was found in the HCM patients.

Conclusions These observations indicated that the compound heterozygous FKTN mutation was a rare cause of DCM. Hyper-CKemia might be indicative of FKTN mutation in DCM. (Circ J 2009; 73: 158 – 161)

Key Words: Cardiomyopathy; Genes; Genetics; Muscles

Idiopathic cardiomyopathy (ICM), which is mainly classified into 2 clinical phenotypes: hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), is a primary heart muscle disorder caused by functional abnormalities in the cardiomyocytes and a major cause of sudden cardiac death and progressive heart failure! Although the etiology of ICM has not been completely elucidated, recent molecular genetic studies have shown that ICM can be caused by a variety of genetic abnormalities! Inheritance of familial HCM is usually autosomal dominant, whereas that of familial DCM is autosomal dominant, autosomal recessive, or X-linked recessive, ie, various type of disease inheritance can be found in DCM cases! It also should be noted that causative gene mutations could be found not only in familial cases but also in sporadic cases, indicating that the absence of family history cannot exclude a possibility of causative gene mutation in ICM cases! In addition, mutations in muscular dystrophy-causing genes might also lead to ICM phenotype, as exemplified that titin/connectin gene (TTN) mutations were found in patients with HCM! DCM! or tibial muscular dystrophy and limb-girdle type muscular dystrophy (LGMD) and that Tcap gene (TCAp) mutations were found in HCM and DCM, as well as in LGMD! These observations indicate that there is an etiological overlap between ICM, and the skeletal muscle disorders!

Mutations in FKTN encoding for fukutin cause Fukuyama-type congenital muscular dystrophy (FCMD; MIM253800), the second most common muscular dystrophy in Japan after Duchenne muscular dystrophy. FCMD is an autosomal recessive disease manifested with severe muscle wasting and mental retardation)! The majority of the FCMD patients were homozygous for a 3-kb insertion in the 3’ non-coding region of FKTN, whereas a small population of FCMD patients were compound heterozygotes of the 3-kb insertion and a missense mutation in 2 sibling cases and 2 sporadic cases of DCM, who manifested with minimal muscle weakness and elevated serum creatine kinase (CK) concentration, hyper-CKemia, but not mental retardation! However, it remains unknown whether FKTN mutation can be associated with ICM not accompanied by signs of muscular dystrophy and in which type of ICM patients who should be examined for FKTN mutations as a disease-causing gene.

In the present study, we searched for FKTN mutations in a large panel of patients with DCM or HCM. We found a compound heterozygote of FKTN mutations in 1 out of 172 DCM patients, who also had mild muscular dystrophy and hyper-CKemia.
Methods

Study Population
We studied 172 genetically unrelated Japanese patients with DCM and 144 patients with familial HCM. Among the DCM patients, family history was not found in 100 patients (sporadic cases), whereas apparent family history was found in 72 patients; 4 were probands of sibling cases (possible autosomal recessive cases) and 68 patients were probands of DCM families, in which disease was inherited as a autosomal dominant genetic trait. In addition, family history consistent with autosomal dominant inheritance was found in all HCM patients. The patients were diagnosed based on medical history, physical examination, 12-lead electrocardiogram (ECG), echocardiography, and other special tests if necessary. Diagnostic criteria for DCM and HCM were described previously. These patients had been investigated for mutations in the known disease genes for ICM, such as sarcomere genes and Z-disc component genes, and no disease-causing mutations were identified. All patients showed no sign of brain involvement, ie, typical FCMD cases were clinically excluded. Control subjects were 384 unrelated healthy Japanese individuals selected at random. After acquiring informed consent, blood samples were obtained from each participant. The research protocol was approved by the Ethics Review Committee of Medical Research Institute, Tokyo Medical and Dental University and that of National Institute of Neuroscience, National Center of Neurology and Psychiatry.

Mutational Analysis of FKTN in ICM
Genomic DNA extracted from peripheral blood was subjected to polymerase chain reaction (PCR). To detect the 3-kb insertion in FKTN, we carried out PCR in all participants using 2 primer sets as described previously. Entire exons and their flanking regions of FKTN were directly sequenced on both strands by using an ABI PRISM 3100 automated sequencer (PE Applied Biosystems Foster City, CA, USA) as reported previously.

Immunohistochemical Analysis
Monoclonal anti-α-DG (VIA4-1, Upstate Biotechnology, Lake Placid, NY, USA) and monoclonal anti-β-DG (43DAG1/8D5, Novocastra Laboratories, Newcastle upon Tyne, UK) were used for immunostaining of biopsied skeletal muscle samples as described previously.

Results
The 3-kb insertion mutation was searched in 172 DCM patients and 144 HCM patients. We found that 3 patients (all were sporadic DCM cases) carried the insertion mutation in the heterozygous state. This 3-kb insertion was not detected in other patients, but was identified in 3 out of 384 controls. We then sequenced all exons and adjacent introns of FKTN in the 3 sporadic DCM patients carrying the 3-kb insertion (Fig 1A) and found a missense mutation (c.302G>T, p.Cys101Phe) in one case (Fig 1B), suggesting that this patient was a compound heterozygote of FKTN mutations.

The patient was a 19-year-old female who manifested with exertional dyspnea and mild muscular weakness at neck and proximal extremities along with bilateral calf hypertrophy. She had shown hyper-CKemia (6,570 IU/L) without any muscle symptoms from the age of 17 years. Since then, she was followed up by physicians as a result of

Fig 1. Gene and histochemical analyses of the dilated cardiomyopathy (DCM) patient carrying the FKTN mutation. (A) Detection of 3-kb insertion. Left, normal individual without mutation; middle, Fukuyama-type congenital muscular dystrophy patient carrying homozygous 3-kb insertion; right, the DCM patient with FKTN mutation. The patient showed both normal and insertion bands. (B) Direct sequencing data from the DCM patient. Polymerase chain reaction products containing exon 4 of FKTN gene from the patient were directly sequenced. Nucleotide sequences are shown along with predicted amino acid sequences. An arrowhead indicates the mutation resulting in TGC (Cys) to TTC (Phe) change. (C) Echocardiography of the patient. Enddiastolic (a, b) and endosystolic (c, d) data for sagital (a, c) and vertical (b, d) views showing left ventricular dilation. (D) Hematoxylin and eosin staining (HE) and immunohistochemical analysis. On HE, only mild variation in fiber size was found. Immunohistochemical analysis using a monoclonal antibody VIA4-1 that recognizes heavily-glycosylated form of α-dystroglycan (αDG), showed reduced sarcolemmal staining, whereas the staining of β-dystroglycan (βDG) using monoclonal antibody 43DAG1/8D5 showed no abnormality. Bar=50 μm.
the hyper-CKemia of unknown etiology. Diffuse left ventricular hypokinesis with left ventricular ejection fraction (LVEF) of 38% was observed at the age of 18 years, along with diffuse muscle atrophy and mild necrosis-regeneration process in biceps brachii muscle biopsy. She felt exertional dyspnea from the age of 18 years and when she was 19 years old, her ECG showed incomplete right bundle branch block, and her echocardiogram showed systolic dysfunction with ventricular dilatation (LVEF, 41%; left ventricular end-diastolic diameter, 53 mm; left ventricular end-systolic diameter, 43 mm; fractional shortening, 20%), whereas no ventricular hypertrophy was observed (inter ventricular septum, 6 mm; posterior wall, 7 mm). Biochemical analysis showed that she had hyper-CKemia (2,485 IU/L). Immuno-histochemical analysis of biopsied muscle sample showed marked decrease of α-dystroglycan staining, whereas distribution and expression of β-dystroglycan was not changed (Fig 1B). This finding was consistent with FKTN mutations albeit that no family history of DCM or muscle disease was evident with her. From these observations, she was finally diagnosed as LGMD manifested with mild DCM phenotype.

In addition, we sequenced the entire coding regions and adjacent introns of FKTN from 72 patients with familial DCM (4 consistent with recessive inheritance and 68 with dominant inheritance). The sequencing analyses showed 2 variations, 1 non-synonymous change in exon 5 (c.608G > A, p.Arg203Gln) and 1 synonymous change in exon 8 (c.1026C > A, p.Leu342Leu), in several patients. However, both variations were reported to be polymorphisms in the SNP database (rs34787999 and rs17309806, respectively), suggesting that these were polymorphisms not related with DCM.

Discussion

The 3-kb insertion into the 3’-untranslated region of the FKTN, which has been derived from a single ancestral founder and causes a significant reduction of FKTN mRNA, could cause FCMD in homozygous states or in compound heterozygous states with another point mutation. FCMD is one of the most severe congenital muscular dystrophy in combination with brain malformation, principally cerebral and cerebellar cortical dysplasia. In contrast to the severely affected skeletal muscle, cardiac muscle involvement is quite rare in FCMD patients. However, we recently showed that the compound heterozygous mutations could also be associated with DCM accompanied by minimal limb girdle muscle involvement and normal intelligence. These observations implied the wide phenotypic spectrum of the FKTN mutations. In the current study, we identified a patient carrying the 3-kb insertion and a missense mutation, who manifested with DCM and mild skeletal muscle phenotype. Clinical phenotype of the patient in this study was similar to those reported previously further supporting that the compound heterozygous mutation was associated with DCM. Because we have not examined her parents for the FNTN mutations, we could not formally exclude a possibility that these 2 mutations were in trans and not in cis. However, if the mutations were in cis, this patient should have one normal allele and the other non-expressing allele due to the 3-kb insertion, which is in a similar situation as the heterozygote of the 3-kb mutation; the situation not causing any disease phenotypes as discussed below.

The 3-kb insertion was also found in 2 other sporadic DCM cases, but these patients did not carry any additional FKTN mutations nor did they show hyper-CKemia, indicating that heterozygote of the insertion mutation and normal allele did not manifest with cardiomypathy or muscle diseases. In addition, we identified 3 heterozygous carriers of the 3-kb insertion in 384 Japanese controls (0.78%), and this carrier frequency was similar to those previously reported by 2 other groups (6 in 676; 0.89% and 15 in 2,814; 0.53%). In this study, we investigated familial HCM patients for FNTN mutations even though the disease was inherited as an autosomal dominant trait as in the most cases of familial DCM. Because mutations in the muscular dystrophy genes such as TTN and TCAP cause skeletal muscle disease as the autosomal recessive trait and cardiomypathy (HCM or DCM) as the autosomal dominant trait, we had not been able to exclude a possibility of FNTN mutations in autosomal dominant cases. However, no FNTN mutation was found in the patients with familial HCM as in familial DCM, examined in this study. These observations suggest that FNTN mutations should be considered as a cause of DCM, albeit not a major cause, especially in the sporadic cases or sibling cases.

What was the characteristic feature of DCM caused by FNTN mutations? The patient carrying the causative FNTN mutations showed hyper-CKemia before manifesting with cardiomypathy and skeletal muscle symptoms. All the patients carrying the compound heterozygous mutations in the previous study had elevated serum CK concentrations, although they showed no or minimal skeletal muscle phenotypes. The hyper-CKemia can also be found in the patients carrying FNTN mutations affected with FCMD or LGMD. These observations are in good agreement with the association between the FNTN mutations and hyper-CKemia. In our cohort of DCM patients, we identified disease-causing mutations in 4 sporadic DCM patients who showed continuously hyper-CKemia. One was the patient carrying the FNTN mutations reported here, whereas the other 3 patients had abnormalities in the dystrophin gene (DMD) with a deletion of exon 3, exon 44, or exons 45–51. The DCM patients with DMD mutations showed elevated serum CK concentrations of approximately 500–1,000 IU/L. The finding was in part consistent with that DCM patients carrying DMD mutations were reported to show hyper-CKemia even though they had no or minimal symptoms of muscle involvement. These observations suggested that hyper-CKemia in patients with DCM might be an indicative sign of FNTN or DMD mutations.

In summary, we have investigated FNTN mutations in a large panel of patients with DCM or HCM and found that a sporadic DCM case with hyper-CKemia was a compound heterozygote of FNTN mutations.

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FKTN Mutation in Cardiomyopathy

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References


