A Japanese child with asymptomatic elevation of serum creatine kinase shows PTRF-CAVIN mutation matching with congenital generalized lipodystrophy type 4

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学位論文の内容要旨

A Japanese child with asymptomatic elevation of serum creatine kinase shows *PTRF-CAVIN* mutation matching with congenital generalized lipodystrophy type 4

無症候性高 CK 血症を呈した日本人小児において先天性全身性脂肪萎縮症 4 型の責任遺伝子である *PTRF-CAVIN* 遺伝子の変異を同定した

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小児科学分野
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Summary

Congenital generalized lipodystrophy (CGL) is an autosomal recessive disorder in which near total absence of subcutaneous adipose tissue since birth. Muscular hypertrophy in CGL is suggested to be to the result of the absence of adipose tissue. On the other hand, muscle dystrophy is a group of inherited disorders characterized by muscle weakness, wasting and degeneration. An elevation of serum creatine kinase (CK) concentration is one of the hallmarks for the identification of muscular dystrophy. Recently, PTRF-CAVIN (polymerase I and transcript release factor/Cavin), has been reported as a new molecule linked to a condition that presents with muscular dystrophy and generalized lipodystrophy and PTRF-CAVIN deficiency was categorized as CGL4 (OMIM #613327). So far, four CGL loci have been identified: 1-acylglycerol- 3-phosphate-O-acyltransferase 2 (*AGPAT2*), Berardinelli-Seip Congenital Lipodystrophy 2 (*BSCL2*), caveolin-1 (*CAV1*) and *PTRF-CAVIN*. CGL4 was first characterized among five non-consanguineous Japanese patients. Their common clinical findings were muscle weakness, elevated serum CK and absence of adipose tissue when patients were older than 8 years of age. But their clinical findings in their younger period were largely unknown.

A Japanese girl (KUCG 748) was referred to Kobe University Hospital at the age of 5 months because of elevated serum CK levels. She was normally born from a non-consanguineous healthy Japanese couple. No family history of lipodystrophy or muscular dystrophy was found. At 1 month, congenital hip dislocation was observed. At 5 months old, lipoatrophy over both extremities and prominent umbilicus were noticed. At 2 years and 5 months of age, facial lipoatrophy became marked and CGL was diagnosed. At 3 years of age, her weight was 13 kg and height was 96 cm (BMI 14.1). Acanthosis nigricans, hepatosplenomegaly, cardiac abnormalities, muscle weakness, other muscle symptoms and mental retardation were not

observed in patient. The biochemical investigation revealed that serum insulin was 28 IU/l and blood glucose was 90 mg/dl. Calculated insulin resistance index (HOMA-R) was elevated to 6.2. Leptin and adiponectin were low, 0.8 ng/ml (normal, 10–15 ng/ml) and 2.1 μg/ml (normal, 5–10 μg/ml), respectively. The serum CK concentration remained high at 1729 IU/L. Blood triglyceride or cholesterol levels were 171 (normal, 28–149 mg/dl) and 158 mg/dl (normal, 146–219 mg/dl), respectively. Because of the asymptomatic elevation of serum CK levels, a carrier status for dystrophinopathy was strongly suggested. But analysis of the *DMD* gene using multiplex ligation-dependant probe amplification (MLPA) showed no abnormality. On the diagnosis of CGL, analysis of *AGPAT2*, *BSCL2* and *CAV1* genes was done but no mutation was found. In addition, *LMNA*, responsible gene for familial partial lipodystrophy, was analyzed but no mutation was observed.

Hayashi et al. disclosed five muscular dystrophy cases complicated with lipodystrophy by identifying mutations in *PTRF-CAVIN* gene, therefore we performed analysis on the *PTRF-CAVIN* gene. The exon 2 encompassing region was amplified as two separated fragments. In the fragment of the 3' region of exon 2, the sequences was blurred because it was rearranged by two sequences; one sequence completely matched with the wild sequence, but the other was a single nucleotide insertion of a C between c.696 and c.697 (c.696-697insC). This mutation matched with that of the common mutation in a previous study. This insertion shifted the translational reading frame, replacing the last 158 amino acids to an unrelated 191-amino acid sequence. An ambiguous peak was also revealed at c.512 where the peak of wild C overlapped with A. The wild c.512C encoded a TCG codon for serine but c.512A (c.512C>A) encoded for a TAG stop codon (p.S171X). The inheritance patterns of these mutations were examined. c.696-697insC and c.512C>A were inherited from the patient's father and mother, respectively. In addition, a 9-bp insertion (c.1235_1236InsTCTCGGCTC), a known polymorphism, was identified in the 3' untranslated region in one allele. This 9-bp insertion was also found in one allele of the patient's mother. In this family, the 9-bp insertion segregated with c.512C>A. Taking into consideration that the 9-bp insertion was observed heterozygously in 26% and homozygously in 2% of 200 Japanese control individuals, c.512C>A segregating with the 9-bp insertion was considered a relatively new event confined to this family.

Immunohistochemical examination of skeletal muscle biopsied muscle showed moderate variation in fiber size. No lipid droplets were observed. Immunohistochemistry using antibodies against dystrophin (DYS1, DYS2, and DYS3), α- and β-dystroglycan, α-, β-, γ-, and δ-sarcoglycans, merosin, syntrophin and emerin showed no abnormalities. Caveolin-3 was feathery expressed in the cytoplasm and slightly decreased in the sarcolemma. Immunoreactivity for dysferlin was markedly decreased in the cell membrane. The expression of PTRF-CAVIN detected by anti-PTRF-CAVIN antibodies (A301-269A and A301-271A) was significantly reduced in the sarcolemma and the stromal blood vessels as compared with control muscle.

This is the sixth Japanese case of CGL4 as far as we know. This case had a novel nonsense mutation of c.512C>A. In five reported cases, four homozygous mutations (c.696_697insC) and one compound heterozygous mutation (c.525delG and c.696_697insC) were identified, disclosing two mutations in the *PTRF-CAVIN* gene. The presently reported mutation is the third mutation but the first nonsense mutation. To date, the three discovered Japanese mutations are all located in exon 2 of the *PTRF-CAVIN* gene. This clustering of mutations may be explained by further studies on the structure and function of PTRF-CAVIN.

Muscular dystrophy with congenital generalized lipodystrophy is a new category of muscular dystrophy. In our case, muscle abnormality was first suggested at 3 months of age by

accidental identification of elevated serum CK levels. This asymptomatic elevation of serum CK was suggested to be because of the slight dystrophic changes in skeletal muscle at age 3.

To characterize the young Japanese CGL4 case, clinical findings were compared with that of the second youngest case (8 years old) of CGL4 with homozygous c.696_697insC mutation. Both cases showed similar clinical findings of low BMI, and generalized lipodystrophy without complications of hypertriglyceridemia or hypercholesterolemia. They did not show mental retardation or acanthosis nigricans. These two cases had differing skeletal abnormalities: congenital hip dislocation in our case but lordosis and contracture in the other case. High serum CK levels were common to both cases. Pathological findings including lipid droplets in skeletal muscle were more severe in the 8-year-old patient case, indicating progressive muscle wasting and weakness. In addition, it is suggested that cardiac muscle is also affected by CGL4, as the 8-year old patient showed cardiac arrhythmia and life-threatening arrhythmia had occurred in older CGL4 cases. In humans, lacking PTRF-CAVIN causes secondary deficiency of caveolins, leading to malformation of caveolae in cell membranes. This could have consequences for the regulation of insulin signaling in adipocytes, as caveolae are known to concentrate insulin receptors along their margins. Insulin resistance is suggested to be the result of caveolae abnormalities caused by CGL4. In the absence of adipocytes designed for synthesis and storage of neutral lipids, dietary lipids accumulate in non-adipose tissues such as the liver and muscle. In contrary, our youngest case did not show hepatomegaly or lipid drops in skeletal muscle. Therefore, it appears that lipid accumulation occurs over long periods of time in these tissues. Our case showed low levels of leptin and adiponectin. Redefining lipodystrophy as an adipokine deficiency syndrome leads to the hypothesis that supplementation of one or more adipokines may improve metabolic outcomes in lipodystrophy.

神戸大学大学院医学系研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲 第2320号	氏名	ERY KUS DWIANINGSIH
論文題目 Title of Dissertation	A Japanese child with asymptomatic elevation of serum creatine kinase shows PTRF-CAVIN mutation matching with congenital generalized lipodystrophy type 4 無症候性高CK血症を呈した日本人小児において先天性全身性脂肪萎縮症4型の責任遺伝子であるPTRF-CAVIN遺伝子の変異を同定した		
審査委員 Examiner	主査 Chief Examiner 西尾久英 副査 Vice-examiner 林祥剛 副査 Vice-examiner 立田悦生		

（要旨は1，000字～2，000字程度）

Introduction

Congenital generalized lipodystrophy (CGL) is an autosomal recessive disorder in which near total absence of subcutaneous adipose tissue since birth. Muscular hypertrophy in CGL is suggested to be to the result of the absence of adipose tissue. On the other hand, muscle dystrophy is a group of inherited disorders characterized by muscle weakness, wasting and degeneration. An elevation of serum creatine kinase (CK) concentration is one of the hallmarks for the identification of muscular dystrophy. Recently, PTRF-CAVIN (polymerase I and transcript release factor/Cavin), has been reported as a new molecule linked to a condition that presents with muscular dystrophy and generalized lipodystrophy and PTRF-CAVIN deficiency was categorized as CGL4 (OMIM #613327). So far, four CGL loci have been identified: 1-acylglycerol- 3-phosphate-O-acyltransferase 2 (*AGPAT2*), Berardinelli-Seip Congenital Lipodystrophy 2 (*BSCL2*), caveolin-1 (*CAV1*) and *PTRF-CAVIN*. CGL4 was first characterized among five non-consanguineous Japanese patients. Their common clinical findings were muscle weakness, elevated serum CK and absence of adipose tissue. In this study, the candidate described a Japanese child with generalized lipodystrophy and asymptomatic elevation in serum CK levels and disclosed compound heterozygous mutations of c.696-697insC and a novel nonsense mutation (c.512cNA) in the *PTRF-CAVIN* gene, leading to a diagnosis of CGL4.

Patient

A Japanese girl (KUCG 748) was referred to Kobe University Hospital at the age of 5 months because of elevated serum CK levels. She was normally born from a non-consanguineous healthy Japanese couple. No family history of lipodystrophy or muscular dystrophy was found. At 1 month, congenital hip dislocation was observed. At 5 months old, lipoatrophy over both extremities and prominent umbilicus were noticed. At 2 years and 5 months of age, facial lipoatrophy became marked and CGL was diagnosed. At 3 years of age, her weight was 13 kg and height was 96 cm (BMI 14.1). Acanthosis nigricans, hepatosplenomegaly, cardiac abnormalities, muscle weakness, other muscle symptoms and mental retardation were not observed in patient. The biochemical investigation revealed that serum insulin was 28 IU/l and blood glucose was 90 mg/dl. Calculated insulin resistance index (HOMA-R) was elevated to 6.2. Leptin and adiponectin were low, 0.8 ng/ml (normal, 10–15 ng/ml) and 2.1 μg/ml (normal, 5–10 μg/ml), respectively. The serum CK concentration remained high at 1729 IU/L. Blood triglyceride or cholesterol levels were 171 (normal, 28–149 mg/dl) and 158 mg/dl (normal, 146–219 mg/dl), respectively. Because of the asymptomatic elevation of serum CK levels, a carrier status for dystrophinopathy was strongly suggested. But analysis of the *DMD* gene using multiplex ligation-dependant probe amplification (MLPA) showed no abnormality. On the diagnosis of CGL, analysis of *AGPAT2*, *BSCL2* and *CAV1* genes was done but no mutation was found. In addition, *LMNA*, responsible gene for familial partial lipodystrophy, was analyzed but no mutation was observed.

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Immunohistochemical examination of skeletal muscle biopsied muscle showed moderate variation in fiber size. No lipid droplets were observed. Immunohistochemistry using antibodies against dystrophin (DYS1, DYS2, and DYS3), α- and β-dystroglycan, α-, β-, γ-, and δ-sarcoglycans, merosin, syntrophin and emerin showed no abnormalities. Caveolin-3 was feathery expressed in the cytoplasm and slightly decreased in the sarcolemma. Immunoreactivity for dysferlin was markedly decreased in the cell membrane. The expression of PTRF-CAVIN detected by anti-PTRF-CAVIN antibodies (A301-269A and A301-271A) was significantly reduced in the sarcolemma and the stromal blood vessels as compared with control muscle.

Gene Analysis

The candidate analyzed the *PTRF-CAVIN* gene of the patient. The exon 2 was amplified as two separated fragments. In the fragment of the 3' region of exon 2, the sequences was blurred because it was rearranged by two sequences; one sequence completely matched with the wild sequence, but the other was a single nucleotide insertion of a C between c.696 and c.697 (c.696-697insC). This insertion shifted the translational reading frame, replacing the last 158 amino acids to an unrelated 191-amino acid sequence. An ambiguous peak was also revealed at c.512 where the peak of wild C overlapped with A. The wild c.512C encoded a TCG codon for serine but c.512A (c.512C>A) encoded for a TAG stop codon (p.S171X). The inheritance patterns of these mutations were examined. c.696-697insC and c.512C>A were inherited from the patient's father and mother, respectively.

Discussion

Muscular dystrophy with congenital generalized lipodystrophy is a new category of muscular dystrophy. In humans, lacking PTRF-CAVIN causes secondary deficiency of caveolins, leading to malformation of caveolae in cell membranes. This could have consequences for the regulation of insulin signaling in adipocytes. Caveolae are known to concentrate insulin receptors along their margins. Insulin resistance is suggested to be the result of caveolae abnormalities caused by CGL4.

Hayashi et al. have already disclosed five Japanese CGL4 patients. According to Hayashi et al., four out of five patients carried homozygous mutations for c.696_697insC in the *PTRF-CAVIN* gene, and one carried compound heterozygous mutations for c.525delG and c.696_697insC. The patient in the present study was the sixth Japanese case, who had a novel nonsense mutation of c.512C>A. The mutation identified in this study was the third mutation but the first nonsense mutation. All three mutations identified in Japanese patients are located in exon 2 of the *PTRF-CAVIN* gene. This clustering of mutations may be explained by further studies on the structure of *PTRF-CAVIN*.

The candidate, having completed studies on congenital generalized lipodystrophy 4, with a specialty in molecular genetics, and having advanced the field of knowledge in the area of disease-causing mutations in the *PTRF-CAVIN* gene, is hereby recognized as having qualified for the degree of Ph.D. (Medicine).