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【 CASE REPORT 】

A Healthy Family of Familial Hypobetalipoproteinemia Caused by a Protein-truncating Variant in the PCSK9 Gene

Hayato Tada, Hirofumi Okada, Akihiro Nomura, Atsushi Nohara, Masayuki Takamura and Masa-aki Kawashiri

Abstract:

We present the first case of a Japanese patient with familial hypobetalipoproteinemia (FHBL) caused by a protein-truncating variant in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene. A 34-year-old woman was referred to our hospital due to her low low-density lipoprotein (LDL)-cholesterolemia (34 mg/dL). She did not have any secondary causes of hypobetalipoproteinemia. Her father and her younger sister also exhibited low LDL cholesterol levels. We identified a protein-truncating variant in the *PCSK9* gene (c.1090_1091del/p.Pro364ArgfsTer62) among them. None of them exhibited atherosclerotic cardiovascular diseases nor any other complications associated with low LDL cholesterol, including fatty liver, neurocognitive disorders, or cerebral hemorrhaging.

Key words: FHBL, PCSK9, LDL cholesterol

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Introduction

Familial hypobetalipoproteinemia (FHBL) type 1 (OMIM 615558) has been described as a codominant disorder mainly caused by a protein-truncating variants (PTVs) in the apolipoprotein B (*APOB*) gene (1). Individuals with this condition typically exhibit low low-density lipoprotein (LDL) cholesterol associated with low APOB levels. We have shown that individuals with a single PTV in the *APOB* gene are quite cardioprotective (2), although some of them present with hepatic steatosis (3). In contrast, individuals with a double PTV in the *APOB* gene exhibit more severe phenotypes, resembling those of patients with abetalipoproteinemia (ABL) caused by microsomal triglyceride transfer protein (*MTTP*) gene mutations (4). In addition to a PTV in the *APOB* gene, a PTV in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene, which encodes a protein that targets the LDL receptor for lysosomal degradation, has also been shown to cause FHBL1. Of note, particular types of genetic mutations in *APOB* and *PCSK9* can cause the opposite phenotype [familial hypercholesterolemia (FH)].

These observations motivated us to develop medications mimicking these mutations in order to reduce the LDL cholesterol levels (5-8). In addition, we can predict the "side-effects" of those medications through observations, such as Mendelian disorders (9). In that sense, the development of a fatty liver through *APOB* or *MTTP* inhibitors is predictable given that FHBL is caused by *APOB* mutations, and ABL is caused by *MTTP* mutations exhibiting fatty liver symptoms (10, 11). However, few data are available regarding this issue in cases with a PTV in the *PCSK9* gene, especially in the Asian population, because of the rarity of such mutations (12, 13). We herein report a family with FHBL caused by a PTV in the *PCSK9* gene, among whom we found no adverse effects associated with this mutation, thus providing supporting evidence for the safe use of *PCSK9* inhibitors.

Case Report

Study subjects

A 34-year-old Japanese woman was referred to our lipid clinic due to her low level of LDL cholesterol without any apparent secondary causes. Her initial LDL cholesterol level

Department of Cardiology, Kanazawa University Graduate School of Medicine, Japan
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Correspondence to Dr. Hayato Tada, ht240z@sa3.so-net.ne.jp

Table. Characteristics of the Family.

Subject (gender)	I.1 (female)*	II.1 (male)	II.2 (female)	III.1 (female)	III.2 (female)
Genotype	W/W	W/M	W/W	W/M	W/M
Age (years)	87	62	60	35	29
Total cholesterol (mg/dL)	180	163	220	126	115
Triglyceride (mg/dL)	39	42	51	30	31
HDL cholesterol (mg/dL)	56	60	72	79	66
LDL cholesterol (mg/dL)	116	91	138	34	43
ApoA-I (mg/dL)	132	150	173	170	144
ApoB (mg/dL)	98	67	94	30	33
ApoE (mg/dL)	4.1	3.1	7.0	4.6	5.2
ApoE phenotype	E3/E3	E3/E3	E3/E3	E3/E3	E3/E3
Hetero-dimer PCSK9 (ng/mL)	250	97	235	96	82
Furin-cleaved PCSK9 (ng/mL)	41	20	38	12	12

Genotype: M=*PCSK9* gene (c.1090_1091del, or p.Pro364ArgfsTer62)

was 34 mg/dL. Both of her parents showed no evidence of consanguineous marriage, and her grandmother, parents, and younger sister were also included in this study. The characteristics of the study subjects are listed in Table.

Biochemical analyses

Fasting blood samples were drawn for assays without any lipid-modifying treatments, except for her grandmother, who had been under statin therapy. The serum concentrations of total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, and LDL cholesterol were determined enzymatically (Qualigent; Sekisui Medical, Tokyo, Japan). The ApoE phenotype was separated by isoelectric focusing and detected by Western blotting with ApoE polyclonal antibody (phenotyping ApoE IEF system; JOKOH, Tokyo, Japan). The serum PCSK9 concentrations were determined using an enzyme-linked immunosorbent assay (14).

Genetic analyses

We isolated the genomic DNA for each participant from peripheral white blood cells using a standard DNA extraction protocol. DNA was pooled, selected for size, ligated to sequencing adapters, and amplified to enrich for targets that were sequenced using the Kapa DNA Library Preparation. A custom NimbleGen in-solution DNA capture library (Roche NimbleGen, Madison, USA) was designed to capture all coding exons in 21 dyslipidemia-related Mendelian genes, including 4 associated with primary hypobetalipoproteinemia (*ANGPTL3*, *APOB*, *MTTP*, and *PCSK9*). The details have been described previously (15). Target-enriched products were sequenced using the Illumina MiSeq. The target coverage for each subject was ≥20-fold in ≥98% of all targeted exons.

Ethical considerations

Genetic analyses were approved by the Ethics Committee at Kanazawa University. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 1975 Declaration of Helsinki, as revised in 2008. Informed consent for genetic analyses was obtained from all of the subjects for inclusion in the study.

Determination of causative variants

We defined a variant as causative if it met any of the following criteria: a) rare (minor allele frequency <1% among the East Asian population) protein-truncating variant (premature stop, insertions or deletions that shift frames, or canonical splice-sites); b) rare damaging missense variant, defined as those predicted as damaging by all five *in silico* software programs (SIFT, Polyphen2-HDIV, Polyphen2-HVAR, MutationTaster-2, and LRT); and c) ClinVar-registered pathogenic or likely pathogenic variants that cause primary hypobetalipoproteinemia.

Characteristics of study subjects

The initial LDL cholesterol level of the proband was 34 mg/dL. She did not exhibit any secondary causes for hypobetalipoproteinemia, including hyperthyroidism, bleeding, or any malignancies. She did not exhibit fatty liver on computed tomography or echo imaging. Our cascade screening revealed that her father and her younger sister also had hypobetalipoproteinemia, suggesting its dominant pattern of inheritance (Table). In addition to low levels of APOB-containing lipoprotein, these subjects' PCSK9 mass levels were markedly lower than those of other family members. None of the family members exhibited atherosclerotic cardiovascular disease (ASCVD) nor any other complications typically considered to be associated with low LDL cholesterol levels, including fatty liver, neurocognitive disorder, or cerebral hemorrhaging.

Genetic analyses

There were no apparent deleterious mutations in the *APOB* gene, which is the most frequent cause of this situation in the proband. However, one protein-truncating variant was found in her *PCSK9* gene (c.1090_1091del/p.Pro364ArgfsTer62). This particular mutation was inherited from

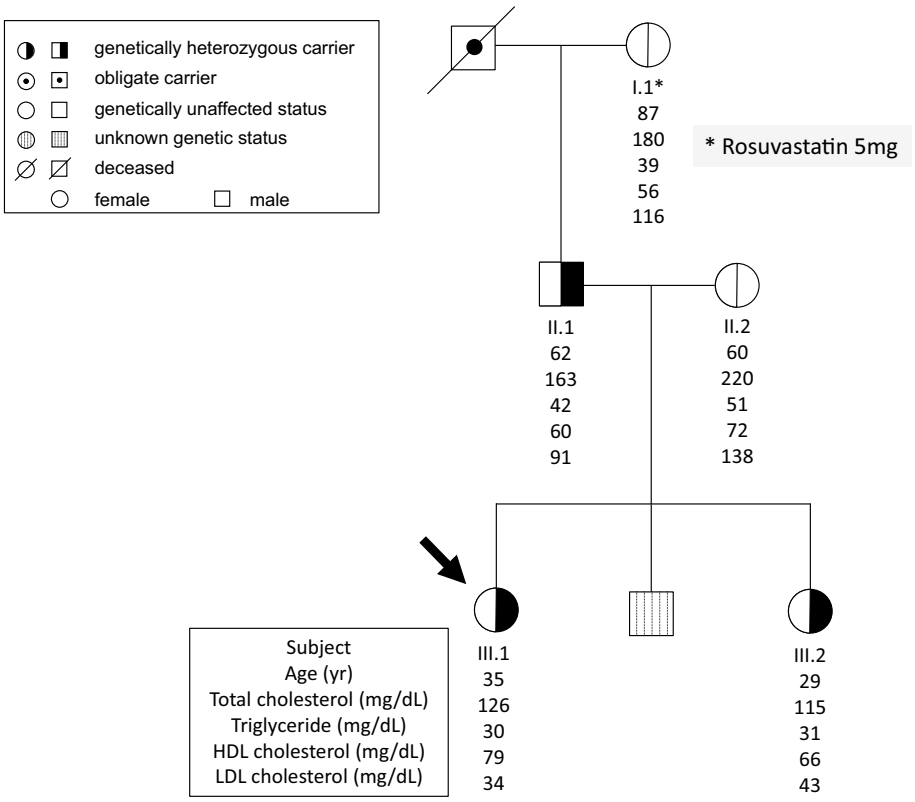

Figure 1. Family tree. Arrow indicates the proband. Black color indicates a carrier of a *PCSK9* mutation (c.1090_1091del, or p.Pro364ArgfsTer62). *under rosuvastatin 5 mg/daily.

her father, whose LDL cholesterol level was relatively low compared with the average levels expected for his age (Fig. 1). In addition, this variant was inherited by her younger sister as well, whose LDL cholesterol was extremely low as well.

Assessments of systemic atherosclerosis and other complications

We assessed systemic atherosclerosis in the proband and her father using carotid ultrasonography and brachial-ankle pulse wave velocity. Neither of them had any plaque in their carotid arteries, and their arterial stiffness was within the normal ranges for their ages.

Discussion

Several different types of mutations associated with a reduced LDL cholesterol level as well a reduced risk of ASCVD in the *PCSK9* gene have been described (16, 17); however, few reports have described "familial" hypobetalipoproteinemia caused by a *PCSK9* genetic mutation where the segregation pattern was well-confirmed. We herein report the first Japanese case of FHBL caused by a PTV in the *PCSK9* gene using comprehensive genetic analyses.

While the proband's father's LDL cholesterol was not very low, a survey by the National Institute of Health and Nutrition (18) indicated that the mean LDL cholesterol among men in their 60s is roughly 120 mg/dL, which was much higher than the father's value. In contrast, the average LDL cholesterol level among women in their 30s is 107 mg/dL. In addition to the absolute difference, a substantial proportion of men in their 60s are treated, while only a few women in their 30s are treated. Accordingly, the LDL cholesterol level of 91 mg/dL in the father without any treatment could be considered to be a low LDL cholesterol level. The novel mutation identified in this family has not been reported before and is believed to produce protein truncation in a catalytic domain of the PCSK9 protein (Fig. 2).

Interestingly, both the hetero-dimer and furin-cleaved PCSK9 levels were decreased in the affected members. In that sense, another nonsense mutation (Cys670Ter) has been associated with an inability of PCSK9 to exit the endoplasmic reticulum, leading to a reduction in both hetero-dimer PCSK9 and furin-cleaved PCSK9 (19). However, another loss-of-function mutation (Ala443Thr) has been shown to increase furin cleavage, leading to elevated levels of furin-cleaved PCSK9 (19). Accordingly, we speculate that the current frameshift mutation is likely to be classified into the former in this context. In addition, other loss-of-function mutations in the *PCSK9* gene, such as Tyr142Ter, Cys679Ter, and Arg46Leu, have been shown to be associated with reduced LDL cholesterol levels and a reduced risk for ASCVD (20). Those mutations have frequencies of approximately 3% and are associated with a reduction in the LDL cholesterol level of 13-35 mg/dL, depending on the ethnicity. It has also been shown that rarer genetic variations tend to have larger effect sizes, based on purifying selection.

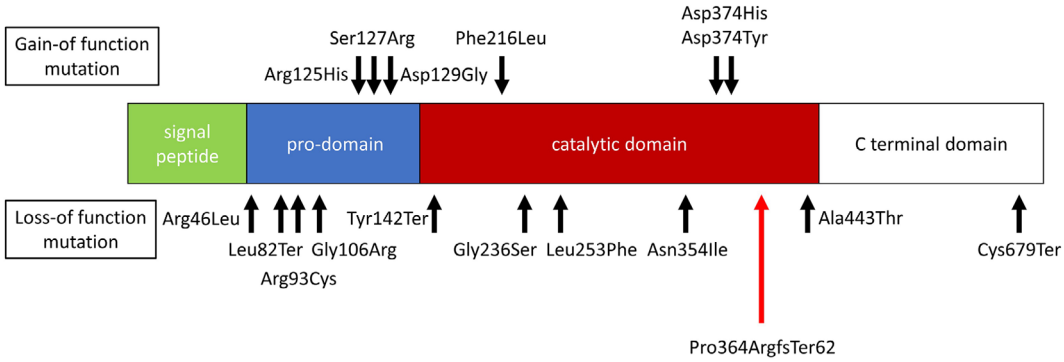

Figure 2. Domain of the *PCSK9* gene. *PCSK9* encodes a 692-amino-acid protein composed of a signal peptide, a pro-domain, catalytic, and C-terminal domains. Black arrows indicate the mutations reported thus far. Red arrows indicate a mutation identified in this study.

Accordingly, it is not surprising that the mutation identified in this study had a rather marked effect on reducing the LDL cholesterol level.

The frequency of this condition in the general population has been estimated to be 1 in 1,000-3,000. Among cases of FHBL, the *APOB* gene seems to be the major cause of this condition, and it is estimated that almost half of such cases may be caused by a PTV in the *APOB* gene. We previously showed that a rare PTV in the *APOB* gene was associated with a reduced LDL cholesterol level and protection against ASCVD (2). However, a PTV in the *PCSK9* gene has also been shown to cause FHBL, although few data on this point exist except for studies in an African population where a particular PTV in the *PCSK9* gene is relatively common.

We herein reported a rare family with FHBL caused by a PTV in the *PCSK9* gene, where apparently "healthy" phenotypes were observed. The accumulation of data from such rare individuals may help us better understand the roles of *PCSK9* in human.

The authors state that they have no Conflict of Interest (COI).

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