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Proprotein convertase subtilisin/kexin 9 V4I variant with *LDLR* mutations modifies the phenotype of familial hypercholesterolemia

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BACKGROUND: Familial hypercholesterolemia (FH) is caused by mutations in the genes encoding low-density lipoprotein receptor (*LDLR*), apolipoprotein B, or proprotein convertase subtilisin/kexin 9 *(PCSK9)*. However, FH shows variability of the clinical phenotype modified by other genetic variants or environmental factors.

OBJECTIVE: Our objective was to determine the distribution of *PCSK9* variants in Japanese FH heterozygotes and to clarify whether those variants and the combination of those variants and *LDLR* mutations modify the clinical phenotypes.

METHODS: A direct sequence analysis was performed for all 18 exons of *LDLR* gene and 12 exons of *PCSK9* gene in 269 clinically diagnosed FH heterozygotes. The serum lipid levels of the carriers of each variant were compared to those of noncarriers. We also assessed Achilles tendon xanthoma and the prevalence of coronary artery disease (CAD) in the patients aged ≥30 years.

RESULTS: Eleven *PCSK9* variants were detected. There were 4 frequent *PCSK9* variants: L21_22insL, A53 V, V4I, and E32 K. The *PCSK9* L21_22insL and A53 V were in linkage disequilibrium with each other. There were no significant differences in serum lipids levels and the prevalence of CAD at the age of ≥ 30 years between *PCSK9* V4I, L21_22insL/A53 V, or E32 K variant carriers and noncarriers without *LDLR* mutations. In the patients carrying *LDLR* mutations and aged ≥ 30 years, the additional *PCSK9* V4I variant was linked to a significantly increased prevalence of CAD in accord with the elevation of the LDL-cholesterol level.

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CONCLUSIONS: The addition of the *PCSK9* V4I was suggested to modify the phenotype of patients carrying *LDLR* mutations by affecting their LDLR metabolism.

Introduction

Familial hypercholesterolemia (FH) is one of the most common inherited disorders, with a prevalence of 1 FH heterozygous patient per 200–500 persons in the general population.[1,2] FH is characterized by hypercholesterolemia, skin, and tendon xanthomas and premature coronary artery disease (CAD). As FH is accompanied by an extremely high risk of CAD, it is very important to make an accurate diagnosis of FH as early as possible and to start treatment. In addition to the clinical diagnosis, genetic testing is necessary for a definite diagnosis of FH.

Low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) were identified as causative genes of FH,[3] whereas autosomal recessive hypercholesterolemia was reported to have a mutation in LDLR adaptor protein 1.[4] Although mutations in *APOB* were reported to cause FH in a Western population,[5] no such mutation has been found in Japanese FH patients.[6] PCSK9 has been reported to have 2 types of mutation, that is, loss and gain of the function.[7,8] Gain-of-function (GOF) mutations of *PCSK9* induce a further degradation of LDLR, followed by an increase in serum LDL-cholesterol (LDL-C) levels, whereas loss-of-function (LOF) mutations cause low LDL-C levels by increasing LDL clearance and were associated with a reduction in the risk of CAD.[9,10]

The phenotype of heterozygous FH shows highly variability.[11] The phenotypic variation is induced not only by gene mutations but also by age, gender, diet, and other genetic variants.[12] Thus, it is necessary to clarify the factors that influence the prognosis of FH including modifier genes.

PCSK9 encodes a 692-amino-acid protein composed of a signal peptide, prodomain, catalytic domain, and C-terminal domain. It has also been reported that there are GOF and LOF mutations of *PCSK9*, mainly in the prodomain, catalytic domain, or C-terminal domain.[13] Many *PCSK9* mutations or polymorphisms have been reported to be associated with cholesterol levels.[14] In Japanese FH patients, it has been reported that a *PCSK9* E32 K variant affects LDL-C levels and could exacerbate the clinical phenotype of heterozygous FH carrying 3 types of *LDLR* mutations.[15] In Caucasian and Cameroun populations, 2 *PCSK9* mutations (R469 W and R496 W) have been reported to exacerbate the clinical phenotype of FH patients carrying an *LDLR* mutation.[16,17] In addition, a *PCSK9* L21_22insL variant has been reported to be associated with a reduction of LDL-C levels in Lebanese FH patients carrying an *LDLR* C681X mutation.[18] However, it has not been fully clarified whether *PCSK9* mutations/variants modify the clinical phenotype of FH.

In the present study, we examined the distribution of *PCSK9* variants in Japanese FH heterozygotes and investigated whether *PCSK9* variants or the combination of *LDLR* mutations and *PCSK9* variants affect the clinical phenotypes, including the prevalence of CAD.

Materials and methods

Subjects

We analyzed 269 Japanese patients with clinically diagnosed heterozygous FH who were visiting the lipid clinic of the National Cerebral and Cardiovascular Center (NCVC) hospital in Osaka, Japan. The diagnosis of FH was made following the Japanese guidelines: having 2 or more of the factors of LDL-C ≥180 mg/dL, tendon/skin xanthomas, and a familial history of FH or premature CAD within the second degree of kinship.[19] The protocol of this study was approved by the Ethics Review Committee of the National Cerebral and Cardiovascular Center (M17-56). Each patient gave written informed consent to participate in the study. All clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki.

Clinical and laboratory characteristics

Serum lipid and lipoprotein levels were measured at the time of initial diagnosis before any lipid-lowering treatments. Serum levels of total cholesterol (TC), triglyceride (TG), and high-density cholesterol (HDL-C) were measured using enzymatic methods (Sekisui Medical Co., Tokyo) and an automated analyzer (Hitachi Labospect 008, Hitachi-Hitec, Tokyo). LDL-C levels were calculated by the Friedewald formula except for TG levels >400 mg/dL. Achilles tendon thickness (ATT) was measured by x-ray as described.[20] The prevalence of CAD was evaluated in patients aged ≥30 years by identifying coronary arteries with ≥75% stenosis by coronary angiogram. The ATT values of the patients aged ≥30 years were also measured. Cigarette smoking information was obtained from the patient report at the first visit to the NCVC.

DNA analysis

Genomic DNA was extracted from whole blood of the patients, using an automated DNA extraction machine