

# A novel type of familial hypercholesterolemia: Double heterozygous mutations in LDL receptor and LDL receptor adaptor protein 1 gene

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## **Abstract**

**Background:** Autosomal recessive hypercholesterolemia (ARH) is an extremely rare inherited hypercholesterolemia, the cause of which is mutations in low-density lipoprotein (LDL) receptor adaptor protein 1 (LDLRAP1) gene.

**Methods:** A total of 146 heterozygous familial hypercholesterolemic (FH) patients with a mutation in LDLR gene were screened for genes encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) and LDLRAP1.

**Results:** Among the 146 subjects, we identified a 79-year-old Japanese female with double mutations in LDLR gene (c.2431A>T) and LDLRAP1 gene (c.606dup). Two other relatives with double mutations in those genes in her family were also identified. Although the proband exhibited massive Achilles tendon xanthoma and coronary and aortic valvular disease, serum LDL-C level of subjects with double mutations was similar with that of subjects with single LDLR mutation (284.0±43.5 versus 265.1±57.4mg/dl).

**Conclusion:** Additional mutation in LDLRAP1 may account for severer phenotype in terms of xanthoma and atherosclerotic cardiovascular disease in FH patients.

## **A Novel Type of Familial Hypercholesterolemia: Double Heterozygous Mutations in LDL Receptor and LDL Receptor Adaptor Protein 1 gene**

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## **1. Introduction**

Familial hypercholesterolemia (FH) is an inherited disease characterized by the triad of (1) hypercholesterolemia due to a high level of plasma LDL, (2) tendon xanthomas and (3) premature coronary artery disease [1]. Patients with homozygous FH have been defined as who have two mutant alleles of either of three following FH-associated genes: LDLR, apolipoprotein B (ApoB) gene and proprotein convertase subtilisin/kexin type 9 (PCSK9) [2]. Previously, we identified several homozygous FH patients who possessed double heterozygous mutations in LDLR gene and PCSK9 gene in relatively mild phenotypic patients compared with those with double mutations in LDLR gene [3]. In addition to autosomal dominant types of FH, recessive form of FH-associated gene was identified in 1992 [4]. The null mutations in the LDL receptor adaptor protein 1 (LDLRAP1) gene, which serves as an adaptor for LDLR endocytosis in the liver, causes autosomal recessive hypercholesterolemia (ARH) [5]. It is described that several heterozygous LDLRAP1 mutation carrier showed elevated LDL-C levels [6, 7]. However, there is no data on clinical significance of adding a mutation in LDLRAP1 gene onto single LDLR gene mutation.

## **2. Methods**

### *2.1. Study subjects*

This study was approved by the Ethics Committee of Graduate School of Medical Science, Kanazawa University, and all study subjects gave their written informed consent to participate. We examined consecutive unrelated 146 subjects with a single mutation in the LDLR gene (male=96, mean age=56.5±16.0, mean LDL-C=265.6±57.7mg/dl) since 2003 to 2008. All the participants were free from unstable or acute cardiovascular diseases. All the lipid-lowering therapy had been transiently suspended for one to three months to diagnose lipid disorders correctly. Although it has

been described the existence of the rebound effect after transient suspension of statin therapy [8], it is also reported that short-term suspension of statins is safe for at least patients with stable cardiovascular disease [9]. Complications related to this short-term suspension of lipid-lowering therapy have not been observed so far in our institute. The characteristics of the study subjects were listed in Table 1 and supplementary Table.

### *2.2 Biochemical analysis*

Serum concentrations of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were determined enzymatically. LDL-C concentrations were derived using the Friedewald formula. Apolipoprotein E (ApoE) phenotype was separated by isoelectric focusing and detected by Western blot with apoE polyclonal antibody (phenotyping apoE IEF system, JOKOH, Tokyo, Japan). Plasma cholesteryl ester transfer protein (CETP) levels were determined by a specific ELISA [10].

### *2.3 Genetic analysis*

Genomic DNA was isolated from peripheral blood white blood cells according to standard procedures and was used for PCR. Primers for the study were as used previously [3, 11]; PCR products were purified by Microcon (Millipore Corp., Bedford, MA) and used as templates for direct sequencing. DNA sequencing was carried out according to the manufacturer's instructions using a dye terminator method (ABI PRISM™ 310 Genetic Analyzer (PerkinElmer Biosystems, Waltham, MA). We screened the study subjects for all coding region of PCSK9 and LDLRAP1 genes as candidate genes that could affect their lipid profile and clinical phenotype. In addition, we analyzed the two common mutations of the CETP gene (c.1321+1G>A, previously described as Int14A and c.1376A>G, previously described as D442G) among Japanese population as previously described [12].

### **3. Results**

#### *3.1. Biochemical analysis*

Serum lipids and apolipoproteins in the proband and her pedigree are presented in Table 2.

#### *3.2. Sequence of LDLR gene*

Mutation in LDLR gene of the proband (c.2431A>T) was one of the most common mutations in Japan [13] (Supplementary Figure 1A).

#### *3.3. Sequence analysis of candidate genes for inherited hypercholesterolemia*

Although there was no genetic abnormality in her PCSK9 gene, we identified another heterozygous mutation in her LDLRAP1 gene (c.606dup, Supplementary Figure 1B).

#### *3.4. Clinical course of the proband*

At the age of 67, she was diagnosed as FH due to severe hypercholesterolemia with Achilles' tendon thickness (Supplementary Figure 2). Initial levels of TC, TG, and HDL-C concentrations were 367, 108, and 46 mg/dl, respectively under statin therapy (pravastatin 20mg daily). She underwent coronary artery bypass graft surgery at the age of 70 due to angina pectoris. The more intensive cholesterol lowering therapy using atorvastatin 20mg daily was introduced for secondary prevention of cardiovascular disease. She was referred to our hospital for further examination of her hypercholesterolemia and coronary artery disease at the age of 78. Although her coronary atherosclerosis including bypass grafts did not progress substantially during 8 years (Supplementary Figure 3), severe aortic valve stenosis developed causing her chest pain (Supplementary Figure 4). Although aortic valve replacement surgery was recommended, she refused due to potential complications derived from extreme high age.

#### *3.5. Family study*

Family study was performed as intensively as possible to find another family member with LDLR or LDLRAP1 mutation. We identified two other relatives with double mutations, and one obligate carrier who died suddenly probably due to cardiac event in his forties (Figure 2).

### 3.6. Genetic analysis for CETP gene

There was no carrier for both of common CETP gene mutation in this family.

## 4. Discussion

Patients with homozygous FH have two mutant alleles of either of three FH-associated genes (FH genes), namely LDLR, apolipoprotein B-100 and PCSK9 genes. In addition to those dominant form inherited gene mutation recessive form of null mutations in LDLRAP1 gene also causes FH (autosomal recessive hypercholesterolemia:ARH). There are few published data about the clinical characteristics of LDLRAP1 heterozygous mutation carriers because of rarity of this disorder. Previously, we have shown that c.606dup mutation carriers in LDLRAP1 gene had elevated LDL-C concentrations compared with non-carrier family members [14], suggesting that “autosomal recessive hypercholesterolemia “ is not necessarily a correct term.

In this paper, we report the first family which exhibit double mutations in LDLR and LDLRAP1 gene with severe xanthomas and coronary artery disease as well as the episode of ventricular fibrillation due to aortic valve stenosis. Besides the proband, we found two other relatives in her family with the same double mutations in LDLR and LDLRAP1 gene.

Some of the pedigrees, including double mutation carriers exhibit relatively high HDL-C level. Previously, we reported that the CETP gene mutations causing higher HDL-C levels are common in Japan [12]. However, there was no carrier of two common CETP gene mutations (c.1321+1G>A and c.1376A>G) among this family member. The plasma levels of CETP of this family member

were within normal limit, suggesting absence of CETP deficiency. It has been reported that the causes of high HDL-C level were quite heterogeneous [15]. Thus, we cannot exclude the possibility that unknown genetic factors may be involved in their high HDL-C levels. Another possibility of higher HDL-C is their excessive alcohol drinking. The pedigrees whose HDL-C levels were more than 90 mg/dl (II-1 and II-2) were both heavy drinkers (ethanol > 120g/day).

In conclusion, we report the first family with double mutation in LDLR and LDLRAP1 genes associated with autosomal dominant and recessive form of hypercholesterolemia. Although the proband exhibited massive Achilles tendon xanthoma and severe coronary and aortic valvular disease, serum LDL-C level of subjects with double mutations was similar with that of subjects with single LDLR mutation. We suggest that an additional mutation in LDLRAP1 may account for severer phenotype in terms of xanthoma and atherosclerotic cardiovascular disease in FH patients.

#### **Conflict of interest statement**

The authors have no conflict of interest.

#### **Acknowledgements**

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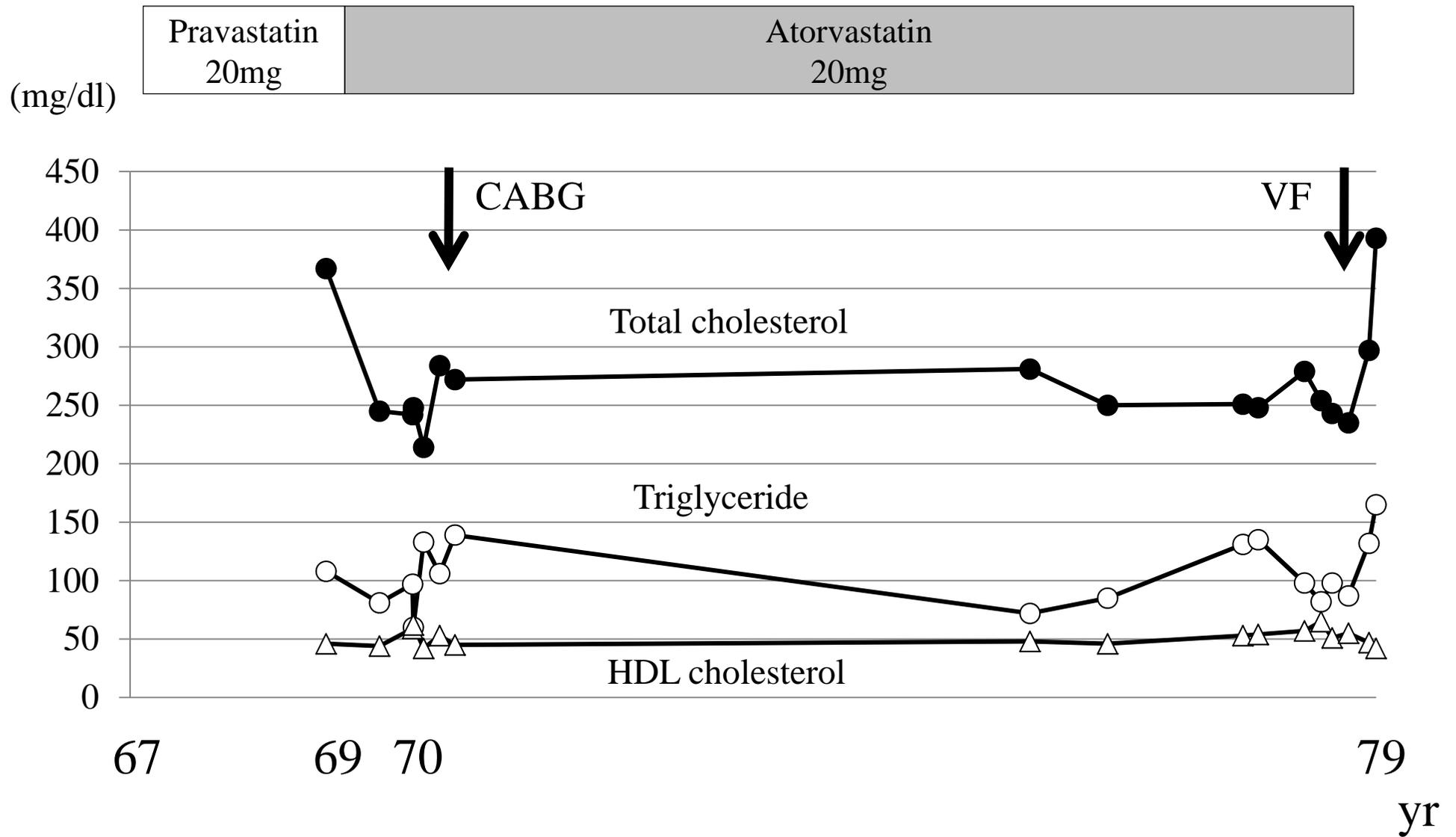
## Figure Legends

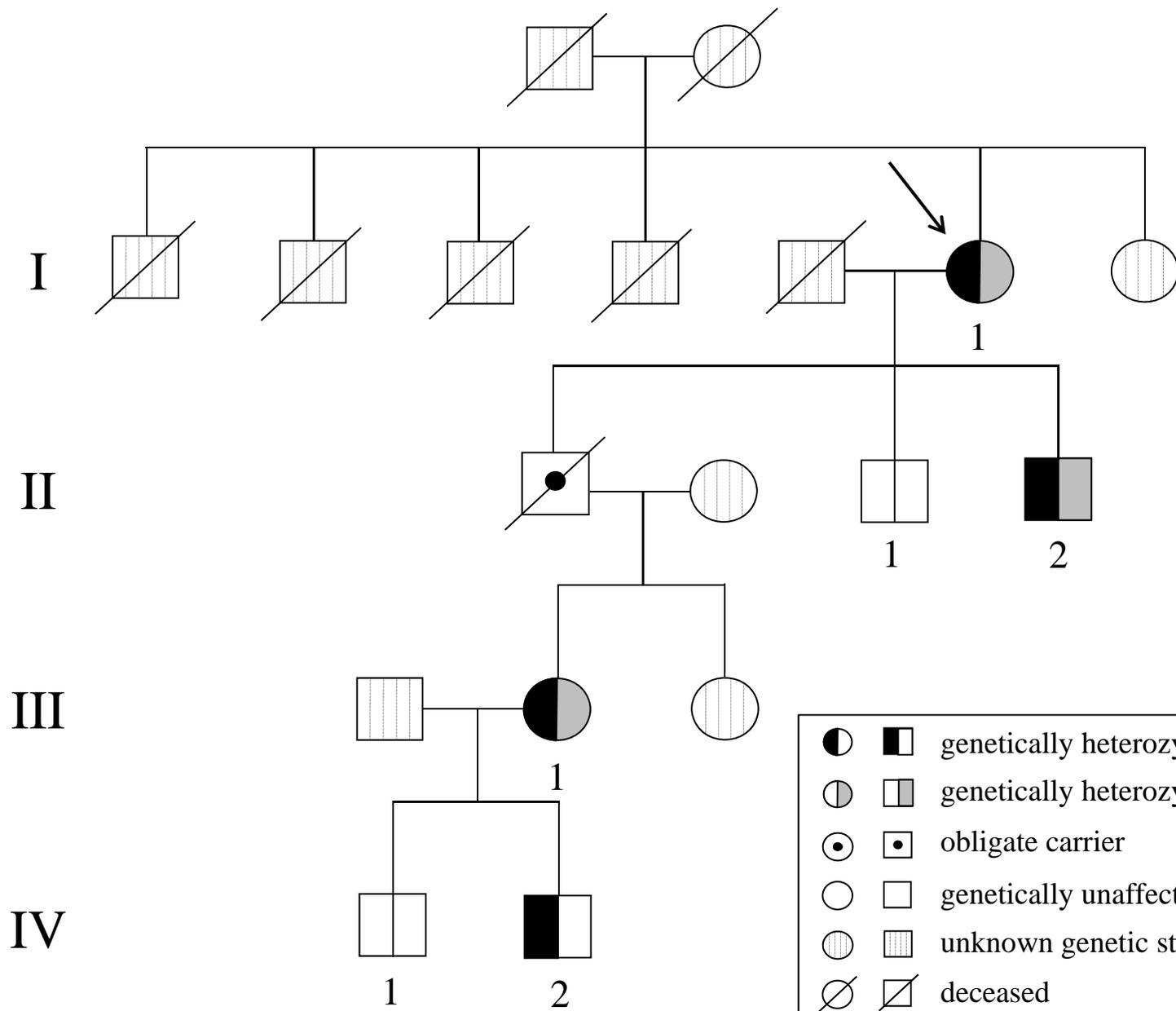
### Fig. 1. Clinical course of the proband

Plasma concentration of the total cholesterol (solid circle), triglyceride (open circle), and HDL-C (open triangle) in the proband, and the major clinical events were illustrated. CABG; coronary artery bypass grafting, VF; ventricular fibrillation.

### Fig. 2. Pedigree of the proband

Half-filled by black squares or circles indicate the heterozygous mutation carrier in LDLR (c.2431A>T). Half-filled by brown squares or circles indicate the heterozygous mutation carrier in LDLRAP1 (c.606dup). Square with a dot indicates the obligate carrier. Open squares or circles indicate unaffected subjects. Hatched squares or circles indicate the genetically unknown subjects.



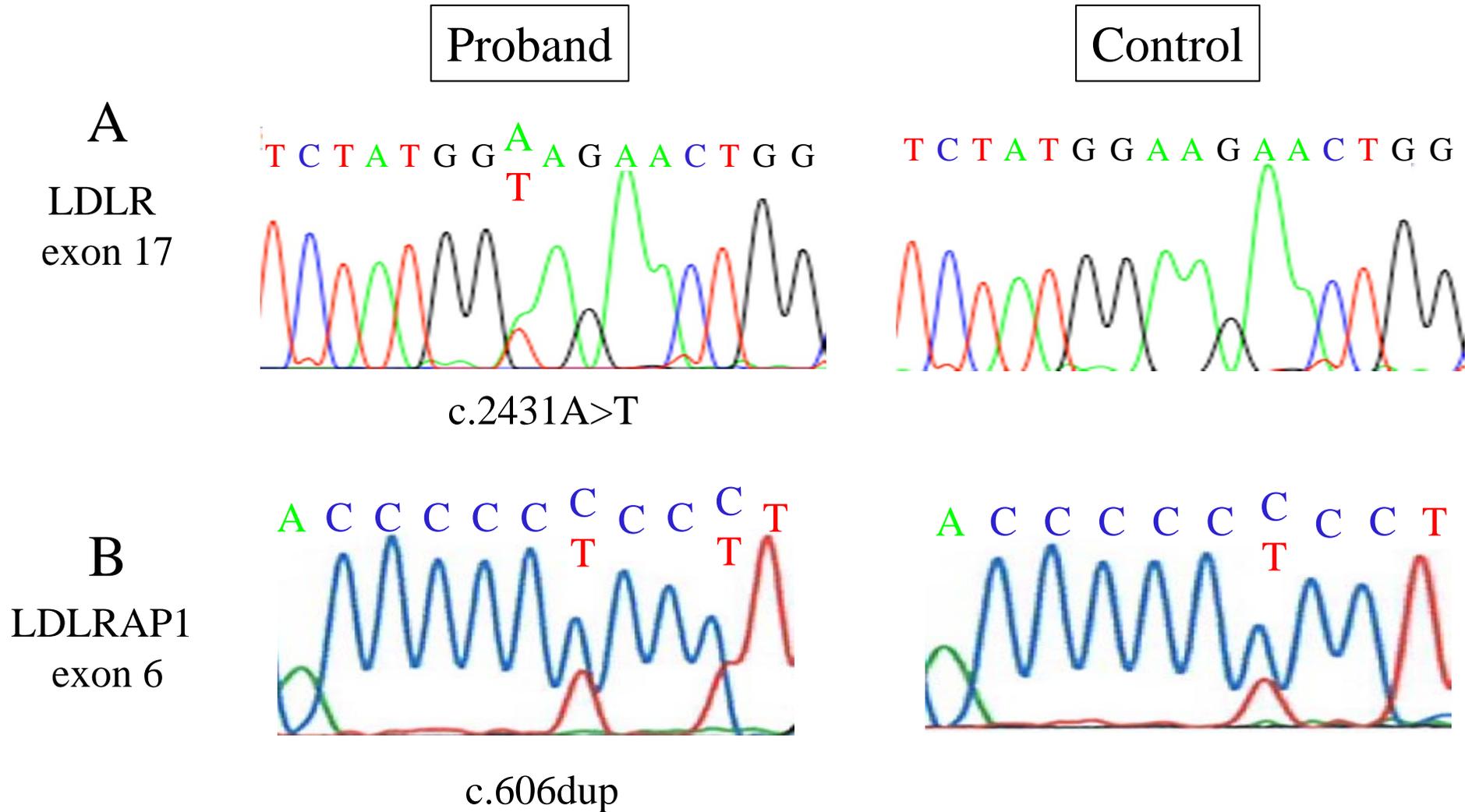


		genetically heterozygous carrier (LDLR:c.2431A>T)	
		genetically heterozygous carrier (LDLRAP1:c.606dup)	
		obligate carrier	
		genetically unaffected status	
		unknown genetic status	
		deceased	
		female	male

Location	Nucleotide change	Mutation type	Effect on protein (mutation class)	Numbers of patients	TC	TG	HDL-C	LDL-C
Intron 1-Intron 3	c.68-?_313+?del	Large deletion	Binding defective (3)	14	316±61	122±100	48±13	244±60
Exon 2	c.137G>A	Missense	Cys-Tyr	1	342	124	45	272
Exon 4	c.344G>A	Missense	Arg-His	2	300±1	300±1	300±1	300±1
Exon 4	c.611G>C	Missense	Cys-Ser	4	324±67	144±26	45±18	256±59
Exon 5	c.796G>A	Missense	Asp-Asn	3	319±38	87±45	50±12	252±38
Intron 7-Intron 14	c.941-?_2140+?del	Large deletion	Binding/recycling defective (3/5)	2	346±118	92±21	49±5	279±118
Exon 9	c.1297G>C	Missense	Asp-His	6	366±56	105±60	47±11	297±51
Exon 10	c.1567G>A	Missense	Val-Met (2A)	2	336±44	120±60	47±15	255±60
Exon 11	c.1689dupC	Insertion	Frameshift/stop	2	315±60	207±142	56±21	223±54
Exon 13	c.1871_1873delTCA	Deletion	602 Ile deletion	4	335±42	139±43	42±10	264±46
Exon 14	c.2054C>T	Missense	Pro-Leu (2B)	19	349±46	162±125	40±13	288±55
Exon 15-Exon 18	c.2141-?_2583+?del	Large deletion	Truncated protein	14	322±58	110±48	48±13	252±55
Intron 15	c.2312-3C>A	Splicing	Exon 16 skip	12	335±68	89±65	43±7	258±61
Exon 17	c.2431A>T	Nonsense	Lys-stop	61	334±55	126±60	48±21	257±63

Value are ± SD; TC; total cholesterol, TG; triglyceride, HDL-C; high-density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol

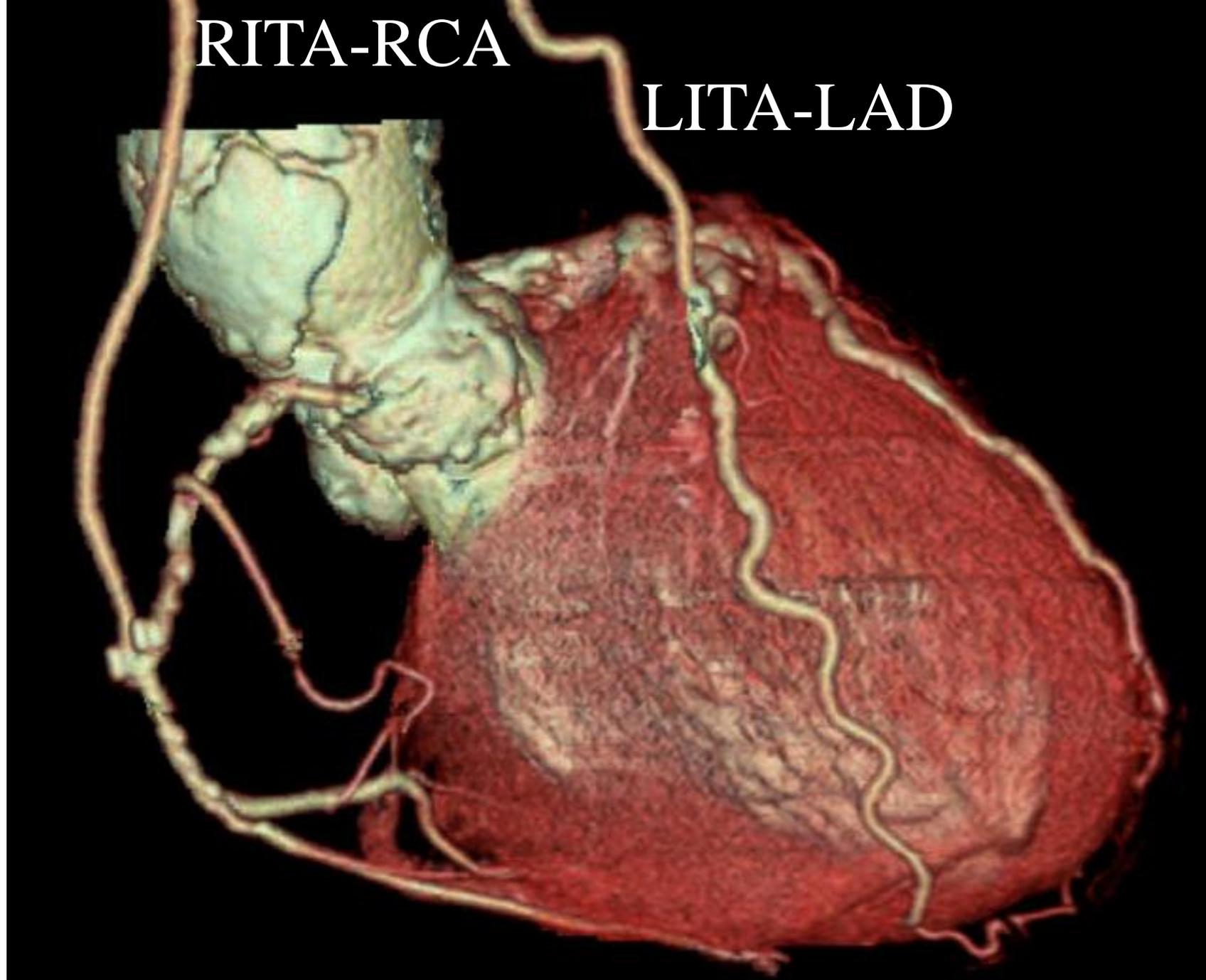
**Supplementary Table. Type of LDLR mutations of screened FH subjects. c.2431A>T mutation was the most**



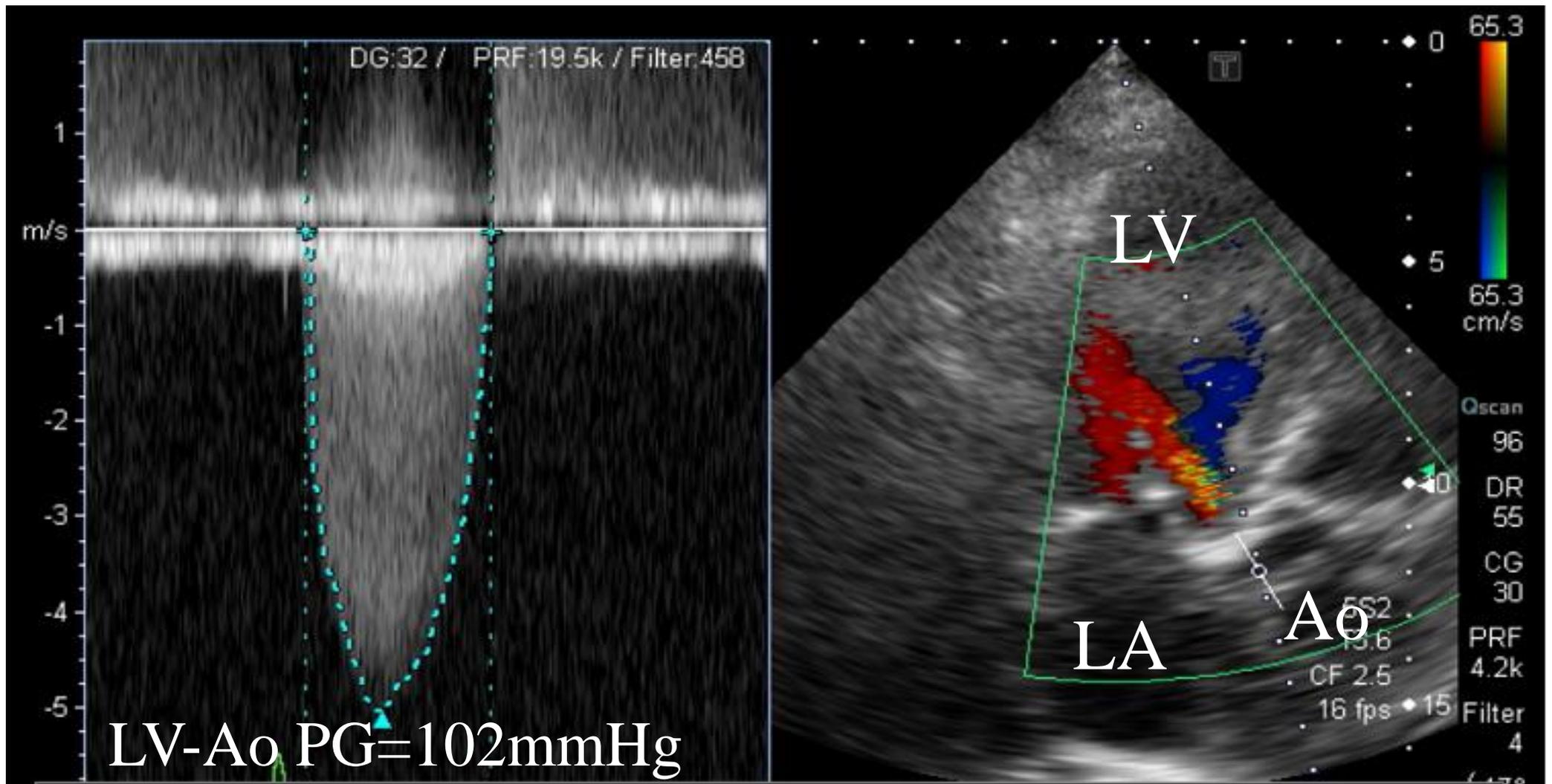
**Supplementary Figure 1. DNA sequence data of the proband (left panel) and a control subject (right panel) for the LDLR gene exon 17 (A) and LDLRAP1 gene exon 6 (B).** (A): Heterozygosity for a substitution of adenine to thymine was shown in the proband. (B): Heterozygosity for an extra cytosine insertion mutation in eight sequential cytosines between the nucleotide positions 599 and 606 (nucleotides are numbered from the first nucleotide that encodes the starting methyonine codon) was shown in the proband, with another single nucleotide polymorphism (c.604 C>T) in this lesion.



**Supplementary Figure 2. X-ray of proband's Achilles' tendon.** The Achilles' tendon thickness of the proband was as much as 24 mm with severe calcification



**Supplementary Figure 3. Coronary CT of the proband.** Bypass grafts (RITA-RCA, LITA-LAD) were patent. RITA; right internal thoracic artery, RCA; right coronary



**Supplementary Figure 4. Echocardiogram of the proband.** The peak velocity measured by continuous-wave Doppler through the aortic valve also increased from 5.05m/s, which corresponded to 102 mmHg of pressure gradient from the left ventricle to aorta. LV; left ventricle, LA; left atrium, Ao; aorta.

**Table 1**  
Characteristics of the screened FH subjects

Age (yr)	56.5±16.0
Sex (male/female)	96/50
BMI (kg/m <sup>2</sup> )	23.2±3.8
ATT (mm)	12.5±3.5
TC (mg/dl)	330.1±43.1
TG (mg/dl)	114.6±35.1
HDL-C (mg/dl)	42.3±8.7
LDL-C (mg/dl)	265.6±57.7
ApoA-I (mg/dl)	121.8±29.4
ApoB (mg/dl)	189.6±25.8

Values are mean±SD

**Table 2**

Clinical data of the pedigree

Subject (gender)	I-1 (female)	II-1 (male)	II-2 (male)	III-1 (female)	IV-1 (male)	IV-2 (male)
LDLR genotype	W/M1	W/W	W/M1	W/M1	W/W	W/M1
LDLRAP1 genotype	W/M2	W/W	W/M2	W/M2	W/W	W/W
Age (yr)	79	51	45	32	3	2
ATT (mm)	24	n.d.	n.d.	13	n.d.	n.d.
TC (mg/dl)	393	224	365	392	166	286
TG (mg/dl)	165	46	63	60	39	92
HDL-C (mg/dl)	42	97	96	61	59	62
LDL-C (mg/dl)	318	118	235	299	99	205
ApoA-I (mg/dl)	114	n.d.	n.d.	136	136	141
ApoB (mg/dl)	232	n.d.	n.d.	174	68	129
ApoE phenotype	3/3	3/3	3/3	3/3	3/3	3/3
CETP ( $\mu$ g/ml)	4.2	2.0	3.2	2.6	n.d.	n.d.

LDLR genotype:W=wild type, M1=c.2431A&gt;T, LDLRAP1 genotype:W=wild type, M2=c.606dup