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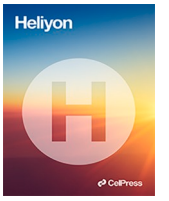

Case report

The first Japanese case with familial combined hypolipidemia without any complications caused by loss-of function variants in *ANGPTL3*: Case report

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ABSTRACT

Familial combined hypolipidemia, previously known as Familial hypobetalipoproteinemia 2 (FHBL2) is considered as an extremely rare recessive disease. Here, we present the case of familial combined hypolipidemia with homozygous loss-of function (LOF) variants in angiopoietin-like protein 3 (*ANGPTL3*) ((NM_014495.4) c.439_442del (p.Thr146_Asn147insTer)) using panel sequencing (46 yr male whose LDL cholesterol = 34 mg/dL). The serum level of ANGPTL3 was quite low (undetectable). Despite of extreme decreasing LDL cholesterol, this case did not have any complications as hypobetalipidemia (HBL), such as steatorrhea vomiting, hematological, neuromuscular, or ophthalmological symptoms. In addition, we did not find any systemic atherosclerosis in his carotid arteries and in coronary arteries. Based on the findings suggest that inhibition of ANGPTL3 effectively reduce LDL cholesterol without any apparent side effects, although it is still unclear if he will suffer any disadvantages because of this situation in the future.

1. Introduction

Hypobetalipoproteinemia (HBL) is an unique phenotype where their total cholesterol, LDL cholesterol, and apolipoprotein B (APOB) exhibit very low (<5th percentile of sex- and age-matched individuals in the population) [1]. Heritable primary causes of HBL are currently classified into 2 groups; Class 1: Familial HBL (FHBL) caused by lipoprotein assembly and secretion defects, and Class 2: FHBL caused by enhanced lipoprotein catabolism [2]. Class 1 includes abetalipoproteinemia (ABL), FHBL caused by LOF variant(s) in *APOB* (previously called as FHBL1), and chylomicron retention disease (CRD) due to loss-of function (LOF) variant(s) in secretion associated ras related GTPase 1B (*SAR1B*). Class 2 includes familial combined hypolipidemia caused by LOF variant(s) in angiopoietin-like 3 (*ANGPTL3*) (previously called as FHBL2), and FHBL caused of LOF variant(s) in proprotein convertase subtilisin/kexin type 9 (*PCSK9*) (previously called as FHBL3).

Notably, most of these molecules are now therapeutic targets for LDL-lowering based on these so-called "human-knockout findings [3–6]. Among them, familial combined hypolipidemia is an extremely rare recessive disorder where only few data are available regarding their genotypes and phenotypes [7–9]. In this sense, at least a part of Class-1 FHBL has been associated with fatty liver

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disease [10], while Class-2 FHBL caused by *PCSK9* variant(s) has not, although the situation of low LDL cholesterol is shared between them. It is interesting to note that these observations in extreme human-knockout are in line with the findings obtained in clinical trials using APOB inhibitor and PCSK9 inhibitor [4,5]. Under the circumstances, evinacumab, ANGPTL3 inhibitor has been developed to further reduce LDL cholesterol, especially of the patients with homozygous familial hypercholesterolemia (FH) [6]. In this report, we are presenting the first Japanese case with familial combined hypolipidemia caused by novel LOF homozygous variants in *ANGPTL3*, who had quite low LDL cholesterol level without any complications associated with HBL.

2. Methods

2.1. Study subjects

A 46-year-old Japanese male was referred to Kanazawa University Hospital because of his extremely low LDL cholesterol level (34 mg/dL). He did not exhibit any secondary causes of HBL, such as hyperthyroidism, bleeding, or malignancies. His parents had blood relationships. His LDL cholesterol level has been continued low since he was 22 years old at the first health check-up. His parents, his older sister, his younger brother and his children were also included in this study.

2.2. Biochemical analyses

Fasting blood samples were used. The serum concentrations of total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were determined enzymatically (Qualigent; Sekisui Medical, Tokyo, Japan). Those of L-aspartate: 2-oxoglutarate aminotransferase (AST), L-alanine: 2-oxoglutarate aminotransferase (ALT), γ-glutamyl transferase (γ-GTP) were determined enzymatically (Qualigent; FUJIFILM Wako Pure Chemical, Osaka, Japan). The apolipoprotein E (APOE) phenotype was determined by a commercial assay (JOKOH, Tokyo, Japan). Serum apolipoproteins, cholesteryl ester transfer protein, vitamin A and 25-OH vitamin D were determined using turbid metric immunoassay method, enzymatically method, HPLC, and a CLEIA (BML, Tokyo, Japan), respectively. Serum vitamin E was measured using a fluorescence method (LSI Medience Corp., Tokyo, Japan). Serum ANGPTL3 protein level was measured with a specific ELISA (IBL, Fujioka, Japan) [11].

2.3. Genetic analyses

Genetic analyses were performed using a panel sequencing based on next-generaion sequencer (iSeq: Illumina, USA). We sequenced the coding lesions of 21 genes known to be associated with Mendelian lipid disorders, including primary HBL (*ANGPTL3*, *APOB*, *MTTP*, and *PCSK9*). Details are described elsewhere [12].

Table 1
Characteristics of the family.

Subject (gender)	I.1 (male)	I.2 (female)	II.1 (female)	II.2 (male)	II.3 (male)	III.1 (female)	III.2 (male)
ANGPTL3 c.439_442del (p.Asn147Ter) status	M/W	M/W	M/W	M/M	M/W	M/W	M/W
Age (yr)	70	69	48	46	40	10	8
Total cholesterol (mg/dL) (142–219)	210	218	184	78	197	180	178
Triglyceride (mg/dL) (40–149)	125	96	80	32	114	60	55
HDL cholesterol (mg/dL) (40–90)	50	60	56	37	50	70	66
LDL cholesterol (mg/dL) (65–139)	134	138	112	34	124	96	100
Lp(a) (<30 mg/dL)	21.4	5.9	5.6	10.8	8.4	12.3	10.4
Apolipoprotein AI (mg/dL) (119–155)	136	141	133	98	129	134	144
Apolipoprotein AII (mg/dL) (25.9–35.7)	31.2	36.5	30.2	25.2	33.5	40.8	33.5
Apolipoprotein B (mg/dL) (73–109)	99	101	88	36	97	88	80
Apolipoprotein CII (mg/dL) (1.8–4.6)	2.8	2.4	3.1	2.2	3.8	2.4	2.9
Apolipoprotein CIII (mg/dL) (5.8–10.0)	6.8	5.9	6.7	2.3	6.7	5.9	6.1
Apolipoprotein E (mg/dL) (2.7–4.3)	3.8	3.4	2.9	2.4	3.1	2.8	3.1
Apolipoprotein E phenotype	E3/E3	E3/E3	E3/E3	E3/E3	E3/E3	E3/E3	E3/E3
CETP (μg/mL)	2.4	2.2	1.8	1.9	2.1	1.8	1.8
Vitamin A (IU/L) (27.2–102.7)	84.5	43.2	39.7	41.9	60.5	37.8	36
25-OH Vitamin D (ng/mL) (>30)	32.1	34.4	40.6	34.4	40.3	44.5	31.6
Vitamin E (IU/L) (0.75–1.41)	1.12	0.99	1.11	0.89	1.12	1.03	0.87
AST (IU/L) (13–30)	24	26	30	26	28	17	26
ALT (IU/L) (10–30)	30	17	24	24	23	12	14
γ-GTP (IU/L) (13–64)	16	13	10	33	22	16	13
ANGPTL3 (ng/mL) (346.8–584.5)[a]	123.9	168.8	185.4	undetectable	201.4	235.4	176.4

ANGPTL3: angiopoietin-like protein 3, Lp(a): lipoprotein (a), CETP: cholesteryl ester transfer protein, AST: aspartate transaminase, ALT: alanine transaminase, γ-GTP: γ-glutamyl transpeptidase.

[a] Interquartile range of Japanese general population [11].

2.4. Ethical considerations

The protocols of our genetic testing were approved by the Ethics Committee at Kanazawa University (study ID: 2016-021 [313]). All of the procedures in this study were in accordance with the ethical standards and the laws in Japan. All study subjects gave us written informed consents to participate in this study.

3. Case reports

3.1. Characteristics

The proband (II-2) was a Japanese male whose height was 173 cm and his body weight was 66 kg. He has no special habit of eating special foods and did not have any allergies to any specific foods. He did not exhibit any apparent metabolic diseases, including hypertension, diabetes, or hyperuricemia. Table 1 and Fig. 1 shows the biochemical profiles of the family and the family tree. They did not have any secondary hypobetalipoproteinemia causes, including hyperthyroidism, bleeding, or any malignancies. The proband (II-2) was born in a consanguineous marriage (his parents were cousin). He is currently working as a police officer. He had been pointed out extremely low LDL cholesterol level since he got the first medical check-up. He did not exhibit any symptoms associated with HBL, such as steatorrhea or vomiting, nor any other manifestations including hematological (acanthocytosis, anemia, bleeding tendency, etc.), neuromuscular (spinocerebellar ataxia, peripheral neuropathy, myopathy, etc.), and ophthalmological symptoms (e.g., retinitis pigmentosa). His serum fat-soluble vitamin levels, including vitamins A, 25-OH vitamin D and vitamin E, were within the normal range without any supplementations. His serum level of ANGPTL3 protein was undetectable. Serum lipoprotein (a) [Lp(a)] levels in his family members were within normal range.

There was no findings suggesting of fatty liver using ultrasound. In addition, there was no abnormalities identified using FibroScan (measuring steatosis and fibrosis). There was no plaque identified in his carotid arteries using carotid ultrasound nor in his coronary arteries using coronary computed tomography. We also assessed his families' lipid profiles, fat-soluble vitamins, and liver enzymes. We found no significant abnormalities among them, except for serum ANGPTL3 levels.

3.2. Genetic analyses

Panel sequencing identified homozygous variants in *ANGPTL3* (c.439_442del (p.Thr146_Asn147insTer)) in the proband (II-2). This variant has been shown to be extremely rare, the alle frequency = 0.0002697 (Genome Aggregation Database [gnomAD]) [13], and this variant is classified as pathogenic according to The American College of Medical Genetics and Genomics (ACMG) criteria [14]. In addition, there are 2 previous reports of this particular variant associated with familial combined hypolipidemia. Based on them, we determined this is a pathogenic variant of familial combined hypolipidemia. We identified s single variant in his parents, siblings, and children as well. We did not find any other variants in 21 genes associated with Mendelian lipid disorders, including *APOB*, *MTTP*, and

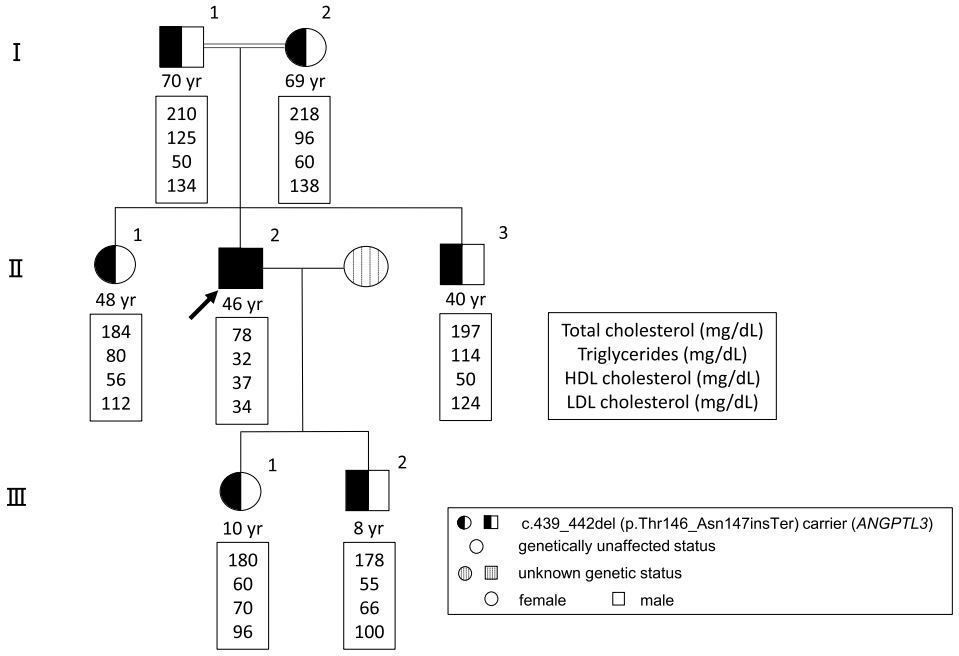

Fig. 1. Family tree. Black arrow shows the proband. The black color shows status of a c.439_442del (p.Thr146_Asn147insTer) (*ANGPTL3*).

PCSK9.

4. Discussion

We present the first Japanese case of familial combined hypolipidemia caused by LOF variants in *ANGPTL3*. Importantly, there are many similarities in phenotypes, including hypobetalipoproteinemia in a recessive manner without any other complications, such as hypertension and diabetes when compared with other cases previously reported.

LDL cholesterol is the most important causal factor or atherosclerosis [15], thereby, many types of medications targeting different pathways aiming to reduce LDL cholesterol have been developed. It is important to note that such targets have been discovered via discovery of monogenic hypercholesterolemia and monogenic hypocholesterolemia. Now, we have PCSK9 inhibitor, MTTP inhibitor, APOB inhibitor, and the ANGPTL3 inhibitor will be coming very soon. All of these 4 newer drugs are causal genes for HBL, and based on the findings of these "human knockout", we had estimated, and have experienced the effects as well as side effects of these drugs. Recently, D'Erasmo et al. demonstrated that the ANGPTL3 deficiency was not associated with risk of hepatic steatosis [16]. exhibit Accordingly, we are expecting to see LDL cholesterol reduction without any apparent side effects on liver as well as fat-soluble vitamins using ANGPTL3 inhibitor based on the findings in this study.

It is still unclear the mechanisms of this extremely low LDL cholesterol of the deficiency of ANGPTL3. However, it is obvious that this is completely independent of LDL receptor pathway, because ANGPTL3 inhibitor appears to reduce serum LDL cholesterol among patients with homozygous familial hypercholesterolemia (FH) regardless of their residual LDL receptor function [6]. Now, we have been operating a Nation-wide registry for primary dyslipidemias, including FH, ABL, and FHBL since 2015 in Japan [17–20]. We are expecting to see there are more individuals with ABL and FHBL who are currently considered as extremely rare disorders.

In conclusion, we present here a case of familial combined hypolipidemia caused by homozygous LOF variants in *ANGPTL3*. Despite of his HBL, fat-soluble vitamins concentrations was maintained without any manifestations associated with HBL.

Informed consent statement

Written informed consent was obtained from the patient for publication of this case report.

Ethics declarations

This study was reviewed and approved by Kanazawa University Ethics Committee, with the approval number: 2016-021 (313).

The patient (or their proxies/legal guardians) provided informed consent to participate in the study and the publication of their anonymized case details.

Sources of funding

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Data availability statement

Our IRB did not give us any approvals for data sharing in this case. However, data will be made available on reasonable request along with the approval from our IRB.

CRediT authorship contribution statement

Hayato Tada: Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nobuko Kojima:** Writing – original draft, Formal analysis, Data curation. **Masa-aki Kawashiri:** Writing – original draft, Supervision, Formal analysis, Data curation. **Masayuki Takamura:** Writing – original draft, Supervision, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Hayato Tada reports financial support was provided by Kanazawa University. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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