A case of ezetimibe-effective hypercholesterolemia with a novel heterozygous variant in ABCG5

Yujiro Nakano¹, Chikara Komiya¹, Hitomi Shimizu²,³, Hiroyuki Mishima³, Kumiko Shiba¹, Kazutaka Tsujimoto¹, Kenji Ikeda¹, Kenichi Kashimada⁴, Sumito Dateki², Koh-ichiro Yoshiura³, Yoshihiro Ogawa⁵ and Tetsuya Yamada¹

¹ Department of Molecular Endocrinology and Metabolism, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
² Department of Pediatrics, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki-shi, Nagasaki 852-8501, Japan
³ Department of Human Genetics, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki-shi, Nagasaki 852-8501, Japan
⁴ Department of Pediatrics and Developmental Biology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan
⁵ Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Fukuoka-shi, Fukuoka 812-8582, Japan

Abstract. Sitosterolemia is caused by homozygous or compound heterozygous gene mutations in either ATP-binding cassette subfamily G member 5 (ABCG5) or 8 (ABCG8). Since ABCG5 and ABCG8 play pivotal roles in the excretion of neutral sterols into feces and bile, patients with sitosterolemia present elevated levels of serum plant sterols and in some cases also hypercholesterolemia. A 48-year-old woman was referred to our hospital for hypercholesterolemia. She had been misdiagnosed with familial hypercholesterolemia at the age of 20 and her serum low-density lipoprotein cholesterol (LDL-C) levels had remained about 200–300 mg/dL at the former clinic. Although the treatment of hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors was ineffective, her serum LDL-C levels were normalized by ezetimibe, a cholesterol transporter inhibitor. We noticed that her serum sitosterol and campesterol levels were relatively high. Targeted analysis sequencing identified a novel heterozygous ABCG5 variant (c.203A>T; p.Ile68Asn) in the patient, whereas no mutations were found in low-density lipoprotein receptor (LDLR), proprotein convertase subtilisin/kexin type 9 (PCSK9), or Niemann-Pick C1-like intracellular cholesterol transporter 1 (NPC1L1). While sitosterolemia is a rare disease, a recent study has reported that the incidence of loss-of-function mutation in the ABCG5 or ABCG8 gene is higher than we thought at 1 in 220 individuals. The present case suggests that serum plant sterol levels should be examined and ezetimibe treatment should be considered in patients with hypercholesterolemia who are resistant to HMG-CoA reductase inhibitors.

Key words: Hypercholesterolemia, Sitosterolemia, ATP-binding cassette subfamily G member 5, ATP-binding cassette subfamily G member 8, Ezetimibe

SITOSTEROLEMIA is characterized by elevated levels of serum plant sterols including sitosterol [1, 2], and caused by homozygous or compound heterozygous gene mutations in either ATP-binding cassette subfamily G member 5 (ABCG5) or 8 (ABCG8) [2, 3]. Dietary plant sterols absorbed by Niemann-Pick C1-like intracellular cholesterol transporter 1 (NPC1L1) in the intestine are mostly excreted into feces and bile by heterodimeric transporters composed of ABCG5 and ABCG8 expressed in enterocytes and hepatocytes [1]. Functional loss of ABCG5 or ABCG8 leads to the accumulation of plant sterols in several tissues and results in symptoms such as xanthoma, hemolysis, and splenomegaly [3, 4]. Since ABCG5 and ABCG8 also have a role in excreting cholesterol, serum low-density lipoprotein cholesterol (LDL-C) levels can be increased in patients with sitosterolemia. Concomitant hypercholesterolemia, rather than excessive plant sterols themselves, is assumed to develop premature atherosclerosis [5].
While sitosterolemia is a rare disease, it has recently been reported that the incidence of heterozygous loss of function mutation in the ABCG5 or ABCG8 gene is higher than what we thought at 1 in 220 individuals [6]. Although the clinical significance of having heterozygous mutations in ABCG5 or ABCG8 remains unclear, it may affect cholesterol absorption and clearance. It has been demonstrated that ezetimibe, an inhibitor of NPC1L1, is highly effective at lowering the levels of serum LDL-C as well as plant sterols in patients with sitosterolemia whereas hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors are not [2-4]. Therefore, ezetimibe treatment may be a better option for lowering serum LDL-C levels in hypercholesterolemic patients with heterozygous mutation in ABCG5 or ABCG8.

Here we report a patient with a novel heterozygous ABCG5 variant. Her hypercholesterolemia was dramatically improved by ezetimibe treatment but not by HMG-CoA reductase inhibitors. A mild elevation of serum plant sterol levels led us to examine ABCG5 and ABCG8 genes, and a heterozygous variant in ABCG5 was detected by targeted analysis sequencing. No mutations were found in low-density lipoprotein receptor (LDLR), pro-protein convertase subtilisin/kevin type 9 (PCSK9), or NPC1L1. This case suggests the importance of measuring concentrations of serum plant sterols to distinguish hypercholesterolemic patients associated with impaired function of ABCG5 or ABCG8 from those with other primary hyperlipidemias such as familial hypercholesterolemia (FH).

**Methods**

**Measurement of serum lipids**

Serum levels of total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), and triglyceride were measured with enzymatic assays using LABOSPECT 008 (Hitachi High-Tech Corporation, Tokyo, Japan). Serum LDL-C concentrations were determined by a direct method with MetaboLead LDL-C kit (Hitachi Chemical Diagnostics System Co., Ltd., Tokyo, Japan). Serum lipoprotein fractionation was performed by electrophoresis on agarose gel. Serum concentrations of sitosterol, campesterol, and lathosterol were analyzed by gas chromatography subsequent to serum saponification, extraction, and silylation in a commercial laboratory (SRL, Inc., Tokyo, Japan) [7].

**Targeted analysis sequencing**

This study was approved by the Institutional Review Board at Nagasaki University Graduate School of Biomedical Sciences. We performed targeted analysis sequencing to identify germline pathogenic variants in the genes related to sitosterolemia or FH. Written informed consent was obtained from the patient and her father. Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany), in accordance with the manufacturer’s instructions. All of the coding exons and their flanking splice sites of ABCG5, ABCG8, LDLR, PCSK9, and NPC1L1 were sequenced by the targeted analysis sequencing, using Ion AmpliSeq™ technology (Thermo Fisher Scientific Inc., Waltham MA, USA) with originally designed primers. The PCR products were sequenced by MiSeq platforms (Illumina Inc., San Diego, CA, USA). Generated Fastq format files were aligned on the hg19/GRCh37 human reference genome sequence using the Novoalign software package (Novocraft Technologies, Kuala Lumpur, Malaysia). The Genome Analysis Toolkit (GATK HaplotypeCaller) was used for variant calling and consequently implemented in an in-house workflow management tool [8, 9]. Single nucleotide variations and insertions/deletions were annotated using the ANNOVAR software package [10]. The identified variants were confirmed via Sanger sequencing using a BigDye terminator and 3130xl genetic analyzer (Applied Biosystems, Carlsbad, CA, USA). The primer sequences used were as follows: 5'-TCTCCTCTTCACAA GCTTACC-3' (forward) and 5'-CCTCCTGCTCCTGGG GTTTC-3' (reverse).

**Bioinformatics analysis for predicting pathogenicity**

The influence of an amino acid substitution on protein function was predicted by PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (https://sift.bi.a-star.edu.sg/), and MutationTaster2 (http://www.mutationtaster.org/) [11-13]. The scores of PolyPhen-2 and MutationTaster2 show the probability of the results, and a substitution with the SIFT score of less than 0.05 is predicted to be damaging. The original and mutant ABCG5 structure images were obtained from the Protein Data Bank (https://www.rcsb.org/) and PyMOL software (http://www.pymol.org/).

**Case Presentation**

A 48-year-old woman was referred to our hospital for hypercholesterolemia. She had been misdiagnosed with heterozygous FH at the age of 20, and her serum LDL-C levels had remained at about 200–300 mg/dL despite medical treatments (precise pharmacotherapy unknown). She gave up taking medication around the age of 30, and then she started consuming foods that she believed would benefit her health, such as seed oil, cheese, and wine. She was asymptomatic and had no other remarkable medical history including obesity, diabetes, choles-
Hydrocholesterolemia with ABCG5 variant

The patient was 167 cm in height and 49.9 kg in weight, with a body mass index of 17.9 kg/m². Physical examination revealed no xanthomas, corneal rings, or splenomegaly. Blood chemistry showed high levels of serum total cholesterol (TC, 401 mg/dL) and LDL-C (289 mg/dL) (Table 1). Serum concentrations of high-density lipoprotein cholesterol (HDL-C, 107 mg/dL) were slightly high, and those of triglyceride (TG, 58 mg/dL) were in the normal range (Table 1). Although serum levels of apolipoprotein E (ApoE, 7.6 mg/dL) were elevated (Table 1), a broad β-band was not detected by serum lipoprotein fractions separated by electrophoresis (Fig. 1). Laboratory findings suggested that the patient did not have hypothyroidism or nephrotic syndrome, both of which are typical causes of secondary hypercholesterolemia (Table 1). Carotid ultrasonography revealed the presence of hyper-echoic plaques in bilateral bulbus.

First, we provided her with nutritional guidance including dietary cholesterol restriction and an increase of vegetable intake, but dietetic treatment demonstrated only slight reductions in serum TC and LDL-C levels (Fig. 2). Next, we started 1 mg of pitavastatin, an HMG-CoA reductase inhibitor, but no apparent improvement in the levels of serum TC and LDL-C were observed (Fig. 2). Finally, the treatment of pitavastatin was changed to that of ezetimibe (10 mg). The ezetimibe treatment showed dramatic reductions in the levels of serum TC and LDL-C.

The outstanding effectiveness of ezetimibe made us consider the possibility of sitosterolemia, and we measured the concentrations of serum plant sterols after the interruption of ezetimibe for 1 month. The levels of serum sitosterol (8.5 μg/mL) and campesterol

| Table 1  | Laboratory data of the patient on the first visit |
|----------|--------------------------------------------------|
| Parameter (unit) | Parameter (unit) | Parameter (unit) |
| WBC (/μL) | 5,900 | AST (U/L) | 19 | FPG (mg/dL) | 95 |
| Hb (g/dL) | 12.8 | ALT (U/L) | 15 | HbA1c (%) | 5.7 |
| Plt (10⁶/μL) | 21.8 | γ-GTP (U/L) | 18 | TSH (μU/mL) | 1.84 |
| TP (g/dL) | 6.8 | T-Bil (mg/dL) | 0.8 | FT4 (ng/dL) | 0.95 |
| Alb (g/dL) | 4.3 | CK (U/L) | 75 | FT3 (pg/mL) | 1.70 |
| BUN (mg/dL) | 14 | LDH (U/L) | 188 | ApoA-I (mg/dL) | 177 |
| Cre (mg/dL) | 0.74 | TC (mg/dL) | 401 | ApoA-II (mg/dL) | 30.1 |
| UA (mg/dL) | 3.6 | HDL-C (mg/dL) | 107 | ApoB (mg/dL) | 160 |
| Na (mEq/L) | 141 | LDL-C (mg/dL) | 289 | ApoC-II (mg/dL) | 6.3 |
| K (mEq/L) | 4.4 | TG (mg/dL) | 58 | ApoC-III (mg/dL) | 14.1 |
| Cl (mEq/L) | 105 | | | ApoE (mg/dL) | 7.6 |

WBC, white blood cell; Hb, hemoglobin; Plt, platelet; TP, total protein; Alb, albumin; BUN, blood urea nitrogen; Cre, creatinine; UA, uric acid; Na, sodium; K, potassium; Cl, chloride; CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyltransferase; T-Bil, total bilirubin; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; TSH, thyroid-stimulating hormone; fT4, free thyroxine; fT3, free triiodothyronine; ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II; ApoB, apolipoprotein B; ApoC-II, apolipoprotein C-II; ApoC-III, apolipoprotein C-III; ApoE, apolipoprotein E.

Fig. 1  Serum lipoprotein fraction of the patient

Serum lipoprotein fractionation was performed by electrophoresis on agarose gel.

CoA reductase inhibitor, but no apparent improvement in the levels of serum TC and LDL-C were observed (Fig. 2). Finally, the treatment of pitavastatin was changed to that of ezetimibe (10 mg). The ezetimibe treatment showed dramatic reductions in the levels of serum TC and LDL-C.

The outstanding effectiveness of ezetimibe made us consider the possibility of sitosterolemia, and we measured the concentrations of serum plant sterols after the interruption of ezetimibe for 1 month. The levels of serum sitosterol (8.5 μg/mL) and campesterol
(14.2 μg/mL) were relatively high, and were normalized after restarting ezetimibe (serum sitosterol and campes‐terol levels, 2.7 and 6.2 μg/mL, respectively) (Table 2). The level of serum lathosterol (1.7 μg/mL) was normal irrespective of the ezetimibe treatment (Table 2). The patient’s father showed no elevation in the levels of serum plant sterols (Table 2).

Targeted analysis sequencing and direct sequencing
revealed that the patient has a novel heterozygous \textit{ABCG5} variant (c.203A>T; p.Ile68Asn) in exon 2 (Fig. 3A). No mutations or pathogenic variants in \textit{ABCG8}, \textit{LDLR}, \textit{PCSK9}, or \textit{NPC1L1} were detected. The patient’s father also has the same variant in the \textit{ABCG5} gene. Ile68 is located in the nucleotide binding domain and is three-dimensionally located adjacent to a walker-A motif, which is known as the ATP-binding site (Fig. 3B and C) [14]. A molecular visualization tool demonstrated that the p.Ile68Asn variant protein has an abnormal hydrogen bond between an amino acid in the walker-A motif and displaced Asn (Fig. 3D). Tools for predicting the impact of an amino acid substitution suggest that this novel missense variant leads to a malfunction of ABCG5 (Table 3).

### Table 3

| Software       | Result             | Score  |
|----------------|--------------------|--------|
| PolyPhen2      | Probably damaging  | 0.992  |
| SIFT           | Damaging           | 0.000  |
| MutationTaster2| Disease causing    | 0.999  |

**Discussion**

Since patients with inherited hypercholesterolemia develop premature atherosclerotic cardiovascular diseases due to a lifelong elevation of serum LDL-C levels, it is important to distinguish them from those with lifestyle-related or other secondary hypercholesterolemia. Heterozygous FH is one of the most common inherited types of hypercholesterolemia, the prevalence of which in Japan is estimated to be as high as 1 in 200 individuals [15]. Although sitosterolemia is a rare disease, the incidence of heterozygous loss-of-function mutation in \textit{ABCG5} or \textit{ABCG8} has recently been reported to be as high as 1 in 220 [6]. Another recent report has demonstrated that 8% of clinically diagnosed FH patients have deleterious mutations in \textit{ABCG5} or \textit{ABCG8} without FH causative gene mutations [16]. Therefore, not a few patients with hypercholesterolemia might be affected by the functional loss of ABCG5 or ABCG8.

We have encountered a case of hypercholesterolemia with a heterozygous variant in the \textit{ABCG5} gene. This patient hardly responded to HMG-CoA reductase inhibitor treatment, but was remarkably improved by ezetimibe. While HMG-CoA reductase inhibitors reduce the levels
of serum LDL-C to some extent in patients with heterozygous FH [17], hypercholesterolemia accompanied by sitosterolemia is refractory to HMG-CoA reductase inhibitor treatment [18]. Although the precise mechanisms by which sitosterolemia leads to hypercholesterolemia have not been fully elucidated, inadequate cholesterol excretion to feces and bile and insufficient cholesterol uptake into hepatocytes due to downregulated hepatic LDLR expression are probably the cause of hypercholesterolemia. ABCG5 and ABCG8 are upregulated by liver X receptor (LXR), a transcription factor that responds to increased intracellular cholesterol levels, and plays a role in the excretion of excessive cholesterol [19]. Accumulated plant sterols in hepatocytes inactivate sterol regulatory element-binding protein 2 (SREBP-2), which results in the downregulation of hepatic LDLR expression and consequently in the decreased uptake of serum LDL-C [20, 21]. Inactivation of SREBP-2 also downregulates HMG-CoA reductase, which can explain why patients with sitosterolemia are unresponsive to HMG-CoA reductase inhibitor treatment. These findings suggest that patients possessing a heterozygous mutation in ABCG5 or ABCG8 might share common pathophysiological properties in cholesterol metabolism. In fact, the patient’s serum lathosterol/cholesterol ratio, which is a marker of endogenous cholesterol synthesis [22], was low. Although we did not attempt to increase the dose of pitavastatin, it seems unlikely that high-dose HMG-CoA reductase inhibitors could have reduced her serum LDL-C levels because cholesterol synthesis had already been suppressed.

In contrast to the patient, her father showed a normal concentration of serum plant sterols and a slight elevation in the level of serum LDL-C, although they have the same variant in ABCG5. It has been recognized that serum levels of sitosterol and cholesterol are quite variable in patients with sitosterolemia even if they possess the identical mutations in the ABCG5 or ABCG8 gene [3, 23-25]. While the patient had consumed an excessive amount of seed oil, her father had intentionally cut fat-rich ingredients including seed oil and nuts from his diet to avoid caloric overload. We speculate that this difference in dietary habit of consuming plant sterols might have caused the phenotypic discrepancy between them. Although dietary fibers and plant sterols are well known to attenuate cholesterol absorption in the intestine, they can exacerbate hypercholesterolemia if patients possess a loss-of-function mutation in ABCG5 or ABCG8. As a reason for dietary treatment failing to improve the patient’s serum LDL-C levels, an increase of vegetable intake might deteriorate the benefit of dietary cholesterol restriction.

Since the nucleotide change detected in this case is a novel missense variant, we performed bioinformatics analyses to predict the pathogenicity of the p.Ile68Asn variant in ABCG5. Although we did not perform in vitro studies on the function of the mutant protein, it may be assumed that the amino acid substitution adversely affects protein function, as all three of the prediction software programs estimated. An abnormal hydrogen bond possibly forming at the ATP-binding site in the mutant protein could alter the protein structure and function because intramolecular hydrogen bonds are generally recognized as essential for maintaining proper protein conformation [26, 27]. A previous study indicated that there was substantial heterogeneity in the serum levels of sitosterol (3.96 [3.08–5.04] μg/mL) and LDL-C (199 [188–255] mg/dL) in 37 individuals with heterozygous mutations in ABCG5 or ABCG9 [16]. Further studies are needed to elucidate the association between each mutation and phenotype in those with heterozygous mutations in these genes.

One of the limitations of this study is that we did not perform whole-genome sequencing. It thus cannot be completely ruled out that genetic mutations or pathogenic variants other than ABCG5 have a causative effect on the difference of lipid profiles between the patient and her father. However, the dramatic effectiveness of ezetimibe cannot be explained by other causal genes for dyslipidemia such as apolipoprotein B (APOB), apolipoprotein E (APOE), and low-density lipoprotein receptor adaptor protein 1 (LDLRAP1). Although the patient might also have heterozygous cholesteryl ester transfer protein (CETP) deficiency because of the mild elevation in serum HDL-C concentration, this appears to bear little relation to the high level of serum LDL-C and ezetimibe efficacy.

In conclusion, this case suggests that heterozygous mutations in ABCG5 or ABCG8 affect the response to cholesterol-lowering agents in patients with hypercholesterolemia and that measuring the concentrations of serum plant sterols can be useful to predict the possibility of harboring mutations in these genes. Ezetimibe may be a better therapeutic option to lower serum LDL-C in hypercholesterolemic patients with ABCG5 or ABCG8 mutation.

**Disclosure**

The authors declare no conflict of interest.
References

1. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, et al. (2000) Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* 290: 1771–1775.

2. Tada H, Nohara A, Inazu A, Sakuma N, Mabuchi H, et al. (2018) Sitosterolemia, hypercholesterolemia, and coronary artery disease. *J Atheroscler Thromb* 25: 783–789.

3. Park JH, Chung IH, Kim DH, Choi MH, Garg A, et al. (2014) Sitosterolemia presenting with severe hypercholesterolemia and intertriginous xanthomas in a breastfed infant: case report and brief review. *J Clin Endocrinol Metab* 99: 1512–1518.

4. Tada H, Kawashiri M, Tanaka M, Matsunami K, Imamura A, et al. (2015) Infantile cases of sitosterolaemia with novel mutations in the ABCG5 gene: extreme hypercholesterolaemia is exacerbated by breastfeeding. *JIMD Rep* 21: 115–122.

5. Genser B, Silbernagel G, De Backer G, Bruckert E, Carmena R, et al. (2012) Plant sterols and cardiovascular disease: a systematic review and meta-analysis. *Eur Heart J* 33: 444–451.

6. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, et al. (2016) Exome aggregation consortium: analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536: 285–291.

7. Kidambi S, Patel SB (2008) Sitosterolemia: pathophysiology, clinical presentation and laboratory diagnosis. *J Clin Pathol* 61: 588–594.

8. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, et al. (2010) The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 20: 1297–1303.

9. Mishima H, Sasaki K, Tanaka M, Tatebe O, Yoshiura K (2011) Agile parallel bioinformatics workflow management using Pwrrake. *BMC Res Notes* 4: 331.

10. Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* 38: e164.

11. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, et al. (2010) A method and server for predicting damaging missense mutations. *Nat Methods* 7: 248–249.

12. Sim NL, Kumar P, Hu J, Hemikoff S, Schneider G, et al. (2012) SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res* 40: W452–W457.

13. Schwarz JM, Cooper DN, Schuelke M, Seelow D (2014) MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 11: 361–362.

14. Lee JY, Kinch LN, Borek DM, Wang J, Wang J, et al. (2016) Crystal structure of the human sterol transporter ABCG5/ABCG8. *Nature* 533: 561–564.

15. Harada-Shiba M, Arai H, Ishigaki Y, Ishibashi S, Okamura M, et al. (2018) Guidelines for diagnosis and treatment of familial hypercholesterolemia 2017. *J Atheroscler Thromb* 25: 751–770.

16. Tada H, Osaka H, Nomura A, Yashiro S, Nohara A, et al. (2019) Rare and deleterious mutation in ABCG5/ABCG8 genes contribute to mimicking and worsening of familial hypercholesterolemia phenotype. *Circ J* 83: 1917–1924.

17. Versmissen J, Oosterveer DM, Yazdanpanah M, Defesche JC, Basart DCG, et al. (2008) Efficacy of statins in familial hypercholesterolaemia: a long term cohort study. *BMJ* 337: a2423.

18. Cobb MM, Salen G, Tint GS, Greenspan J, Nguyen LB (1996) Sitosterolemia: opposing effects of cholestyramine and lovastatin on plasma sterol levels in a homozygous girl and her heterozygous father. *Metabolism* 45: 673–679.

19. Back SS, Kim J, Choi D, Lee ES, Choi SY, et al. (2013) Cooperative transcriptional activation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 genes by nuclear receptors including Liver-X-Receptor. *BMB Rep* 46: 322–327.

20. Radhakrishnan A, Sun L, Kwon HJ, Brown MS, Goldstein JL (2004) Direct binding of cholesterol to the purified membrane region of SCAP: mechanism for a sterol-sensing domain. *Mol Cell* 15: 259–268.

21. Brown AJ, Sun L, Feraimisco JD, Brown MS, Goldstein JL (2002) Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. *Mol Cell* 10: 237–245.

22. Kempen HJ, Glatz IF, Gevers Leuven JA, van der Voort HA, Katan MB (1988) Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J Lipid Res* 29: 1149–1155.

23. Wang J, Joy T, Mymin D, Frohlich J, Hegele RA (2004) Phenotypic heterogeneity of sitosterolemia. *J Lipid Res* 45: 2361–2367.

24. Mannucci L, Guardamagna O, Bertucci P, Pisciotta L, Liberatoscioli L, et al. (2007) Beta-sitosterolemia: a new nonsense mutation in the ABCG5 gene. *Eur J Clin Invest* 37: 997–1000.

25. Veit L, Allegri Machado G, Bürer C, Speer O, Häberle J (2019) Sitosterolemia-10 years observation in two sisters. *JIMD Rep* 48: 4–10.

26. Dill KA (1990) Dominant forces in protein folding. *Biochemistry* 29: 7133–7155.

27. Tomlinson JH, Craven CI, Williamson MP, Pandya MJ (2010) Dimerization of protein G B1 domain at low pH: a conformational switch caused by loss of a single hydrogen bond. *Proteins* 78: 1652–1661.