Genome sequencing identifies uniparental isodisomy of chromosome 2p and a homozygous *ABCG5* variant, enabling effective treatment of pediatric hypercholesterolemia after 13 years of therapeutic resistance: A case report

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Abstract. Sitosterolemia is a rare inherited disorder caused by mutations in *ABCG5* or *ABCG8* genes, resulting in abnormal accumulation of phytosterols. Clinically, it often resembles familial hypercholesterolemia (FH), which is much more common. The present study described the first reported case of sitosterolemia caused by paternal segmental uniparental isodisomy of chromosome 2p, leading to a homozygous pathogenic variant in *ABCG5*. The patient developed xanthomas at age two and was initially misdiagnosed with FH. Despite statin therapy and a cholesterol-restricted diet, the LDL cholesterol of the patient remained elevated, and the patient experienced intermittent knee periarthritis. At age 15, genome sequencing revealed sitosterolemia. After introducing dietary phytosterol restriction and ezetimibe, the lipid levels of the patient normalized within 4 months, with marked clinical improvement. The present case highlights the importance of genetic testing in patients with treatment-resistant hyperlipidemia and represents the first reported instance of sitosterolemia caused by uniparental disomy.

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Abbreviations: FH, familial hypercholesterolemia; UPD, uniparental disomy; UPiD, uniparental isodisomy

Key words: sitosterolemia, UPD, phytosterols, hypercholesterolemia, *ABCG5*

Introduction

Sitosterolemia is a rare metabolic disease that results in the buildup of phytosterols in the body. Phytosterols are dietary plant sterols found in high-fat plant-derived foods such as nuts and oils. While healthy individuals absorb ~5% of these sterols, patients with sitosterolemia absorb 15-60%, resulting in phytosterol accumulation in the blood and arteries (1). This lipid buildup can lead to heart attack, stroke, and death, making its early diagnosis essential. Sitosterolemia is estimated to affect 1 in every 200,000 individuals globally and is often detected as early as childhood (2). However, this rate may be underestimated, as sitosterolemia is often misdiagnosed as other more common hyperlipidemia disorders, such as familial hypercholesterolemia (FH), which affects an estimated 1 in every 250 individuals globally, due to their similar signs of hypercholesterolemia as well as the lack of plant sterol-specific testing (2,3).

Sitosterolemia has been associated with variants in either *ABCG5* or *ABCG8* on chromosome 2, which code for sterol efflux transporters in the intestines (1). Sitosterolemia is an autosomal recessive disease and biallelic missense, nonsense, frameshift, and splice site variants have all been previously cited as causes of sitosterolemia (4). Similar to other autosomal recessive disorders, this mode of inheritance requires a variant to be inherited from each parent.

In the present study, the first reported case of sitosterolemia caused by uniparental disomy (UPD) is identified. Specifically, a homozygous variant in *ABCG5* was found as a result of paternal segmental uniparental isodisomy (UPiD) of the p arm of chromosome 2. The molecular diagnosis enabled effective treatment after 13 years of therapeutic resistance.

Case report

A 2-year-old female patient from non-consanguineous parents presented with xanthomas on both achilles tendons, knees and

toe knuckles (Fig. 1A-C) at King Chulalongkorn Memorial Hospital (Bangkok, Thailand). The patient had high total cholesterol (542 mg/dl; normal range, <200 mg/dl) and high LDL cholesterol (419 mg/dl; normal range, <130 mg/dl) (5). The father of the patient had high cholesterol levels (230 mg/dl) in his 30s, and the mother of the patient developed hypercholesterolemia (220 mg/dl) in her 40s. The patient was initially clinically diagnosed with FH. The patient was advised to follow the Cardiovascular Health Integrated Lifestyle Diet (CHILD) 2 diet including consumption of skim milk, cholesterol restriction <200 mg/day, avoidance of saturated fat and trans-fat, shifting to monounsaturated fat and plant sterols, in addition to regular physical activity (5). The patient was encouraged to participate in school athletics and reported running as much as 4-5 km on multiple days per week at the age of 7. Despite these lifestyle changes and strict dietary control (a more plant-based, pescatarian diet) the cholesterol levels of the patient did not improve over time, as observed in Table I. Around the age of 10, the patient was prescribed simvastatin. The compliance of the patient with the CHILD 2 diet worsened during adolescence, and the medication was changed to atorvastatin with modest improvement. Around the age of 13, the patient experienced the first episode of acute arthritis in both knee joints (Fig. 1D and E). The investigations showed acute inflammation, but none indicated other autoimmune diseases. Therefore, it was likely 'periarthritis' resulted from hypercholesterolemia. The arthritis recurred and subsided periodically once or twice a year, requiring school breaks and short-term NSAIDs. Atorvastatin was then titrated upward together with stricter cholesterol restriction. With the lack of improvement and this 'periarthritis' manifestation, genetic testing was requested and revealed a sitosterolemia diagnosis at the age of 15. Thus, in addition to the current treatment, the patient was instructed to avoid phytosterols such as canola oil, olive oil, nuts, whole grains, legumes, and avocados. The patient was also prescribed ezetimibe which can lower cholesterol and phytosterol absorption. Notably, 4 months later, on the first visit of the patient to the hospital following diagnosis, the total and LDL cholesterol levels of the patient returned to normal levels (Table I).

Genetic testing. After written informed consent was obtained from the patient and both parents of the patient, peripheral blood from the patient and the parents was collected and short-read genome sequencing was conducted on the extracted genomic DNA. The present study was approved (approval no. 264/62) by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (Bangkok, Thailand). Genome sequencing was performed on the DNBSEQ-T7 platform (BGI), achieving an average coverage depth of 30x. Variant calling was conducted using GATK 4.0 against the human genome reference GRCh38 (6). Variants were annotated with Variant Effect Predictor version 110, dbNSFP version 4.4a and our in-house Thai genome databases (7-9). The in-house Thai databases included the Thai Exome Database (ThEx) and Thai Long Read Genome Database (Th-LR), both developed by the Center of Excellence for Medical Genomics, Chulalongkorn University (Bangkok, Thailand). genmod (version 3.1; https://github.com/Clinical-Genomics/genmod), a variant-inheritance annotation tool developed by Måns Magnusson, available at GitHub, Inc. was used for Trio analysis. All SNVs and indels were filtered by the following filtering criteria: i) Located in genes related to the phenotype of the patient; ii) located in exons or flanking introns of the phenotype-associated genes; iii) not synonymous; iv) total read depth ≥10x; v) present at >1% in the Genome Aggregation Database (gnomAD) v4.1 and/or our in-house Thai population database; and vi) predicted to be damaging (≥0.644) by REVEL score (for missense variants) or predicted to be splice-altering (≥0.2) by SpliceAI (for splice variants) (10-12). UPD detection was performed with TrioMix v.0.0.1 (13).

Short-read genome sequencing identified a homozygous splice variant c.904+1G>A in the *ABCG5* gene (NM_022436.3). The father of the patient is heterozygous for this variant. This variant is present in gnomAD in 8 alleles of 1,613,770 with no homozygotes and is absent from our in-house Thai population database. SpliceAI predicts this variant to strongly disrupt the exon 7 donor splice site with a score of 0.99. This variant has been reported to occur *in trans* with other *ABCG5* variants in two other unrelated individuals affected with sitosterolemia (14). As a result, the variant was classified as pathogenic in accordance with ACMG/AMP guidelines (PVS1 + PM3_Strong + PM2_Supporting) (15). Examination of the BAM file showed paternal UPiD of the p arm of chromosome 2 in the patient [seq(GRCh38) 2p25.3p11.2 (1-85,128,508)x2upat]. The TrioMix tool was employed to visualize relevant single nucleotide polymorphisms across the genome of the patient and to infer paternal UPiD of the p arm of chromosome 2 as observed in Fig. 2.

Discussion

UPD is rare and occurs in only ~0.2% of the population (16). There are numerous forms of UPD, but all result in the inheritance of two alleles of a gene from one parent. Typically, whole chromosomes from the same parent, or complete disomy, are observed in these cases. However, rare occurrences of mitotic recombination can lead to segmental UPD in which only a part of the chromosome is identically inherited (17). The patient in the present case report exhibited uniparental paternal segmental isodisomy in the p arm of chromosome 2. Therefore, it is possible that a double-stranded DNA break near the centromere occurred, leading to mitotic recombination as an attempted repair, resulting in a daughter cell with segmental UPiD of the p arm of chromosome 2.

The father of the patient is a carrier of the *ABCG5* c.904+1G>A variant that has previously been reported to cause sitosterolemia with compound heterozygosity (14,18). Although the mother did not have sitosterolemia and the father is an asymptomatic carrier, the 2-year-old female patient inherited two copies of the father's mutated *ABCG5* gene through segmental UPiD, resulting in a homozygous recessive genotype and manifestation of sitosterolemia. Although both parents reported at-risk levels of high cholesterol, they were likely as a result of lifestyle factors as their onset was late with only moderately high lipid levels. They also did not present xanthomas and no genetic causes were found, supporting the sitosterolemia diagnosis resulting from UPiD in the patient. To date, this is the first reported case of sitosterolemia caused by UPD of any kind.

Table I. Lipid profiles of the patient before and after diagnosis of sitosterolemia.

Lipid profile	Reference range	First diagnosis (2 years old)	After dietary counseling (Cardio-vascular Health Integrated Lifestyle Diet 2)	After simvastatin treatment (starting at 10 years old)	After atorvastatin titration from 10-80 mg/day 12 years old)	During the diagnostic visit for sitosterolemia, (starting at before initiating new treatment (15 years old)	After ezetimibe treatment and avoidance of phytosterols (15 years old, 4 months)
Total cholesterol (mg/dl)	<200	542	187-457	217-384	155-244	242	117
LDL (mg/dl)	<130	419	138-384	168-345	101-195	193	59
HDL (mg/dl)	>40	52	26-45	30-44	39-47	46	48
Triglycerides (mg/dl)	<100-130	72	56-127	71-106	57-103	57	59

Reference ranges adapted from Ref (5).

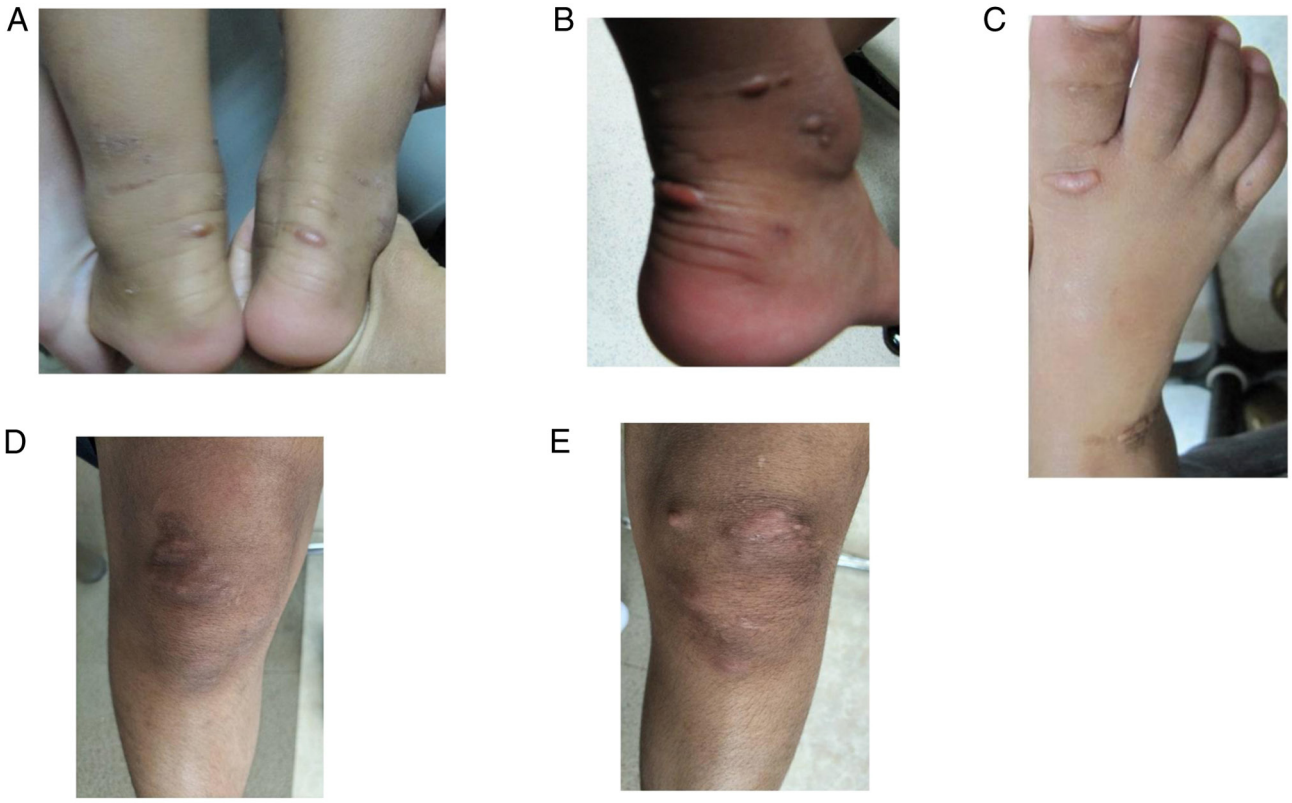

Figure 1. Images of the clinical manifestations of the patient. (A) Tendinous xanthomas on both Achilles tendons (2 years old). (B) Tendinous xanthomas on right Achilles tendon and ankle (2 years old). (C) Tendinous xanthoma on right toe knuckle (2 years old). (D) Tendinous xanthomas and inflammation of right knee (periarthritis) (13 years old). (E) Tendinous xanthomas and inflammation of left knee (periarthritis) (13 years old).

With cases of UPD, gene imprinting may also cause phenotypic abnormalities as some genes are only expressed when inherited maternally or paternally. However, there are currently no known paternally imprinted genes on chromosome 2 and no other phenotypic abnormalities were reported for this patient (19). Furthermore, patients with UPiD can be prone to homozygosity when the parent could be a carrier for autosomal recessive disorders. However, in this case, no other

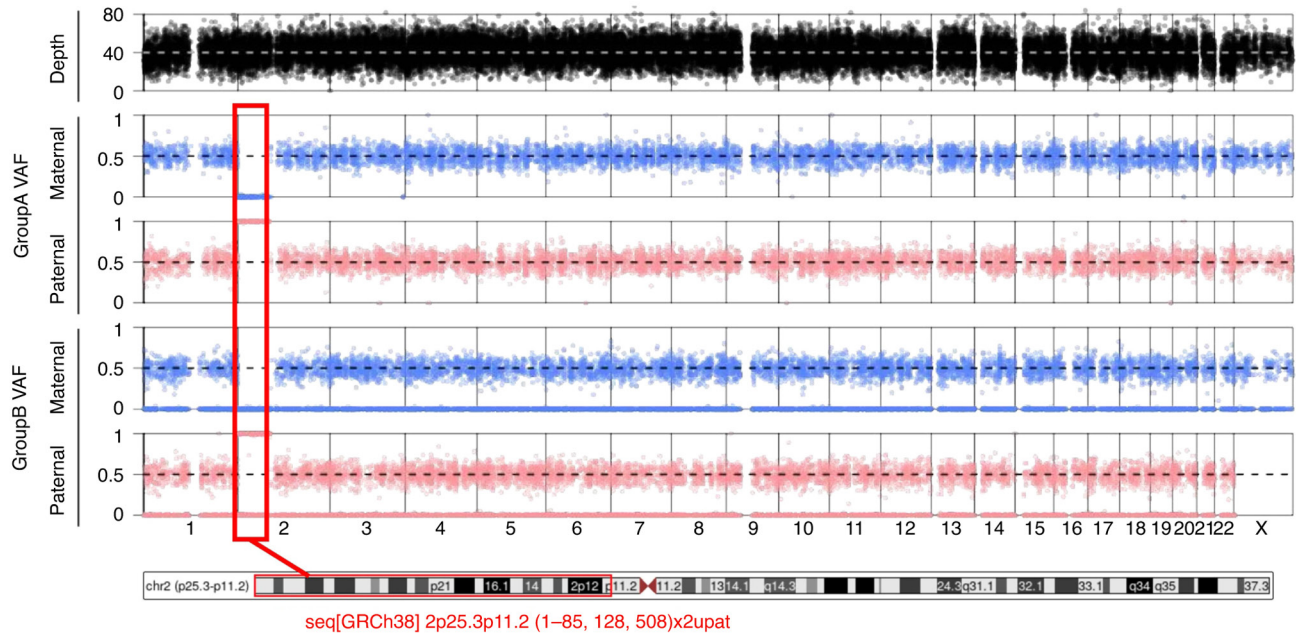

Figure 2. TrioMix tool displays the VAF for SNPs on each chromosome, separated by maternal and paternal contributions. In Group A SNPs (where only SNPs that are homozygous in either parent are considered), the maternal allele frequency plot for SNPs on the short arm of chromosome 2 revealed a value of ~0, while the paternal plot registered at ~1. This indicates that the patient inherited parental homozygous SNPs on the p arm of chromosome 2 solely from the father. In Group B SNPs (where only SNPs that are heterozygous in either parent are considered), the VAF of SNPs on the p arm of chromosome 2 exhibits a maternal allele frequency of 0, coupled with a paternal plot showing allele frequencies predominantly at 0 and 1, with an absence of heterozygous SNPs (0.5) along the p arm. This pattern suggests paternal uniparental isodisomy of most of the p arm of chromosome 2, wherein the patient inherited two identical copies of this chromosomal segment from the father, with no contribution from the mother. VAF, variant allele frequencies; SNPs, single nucleotide polymorphisms.

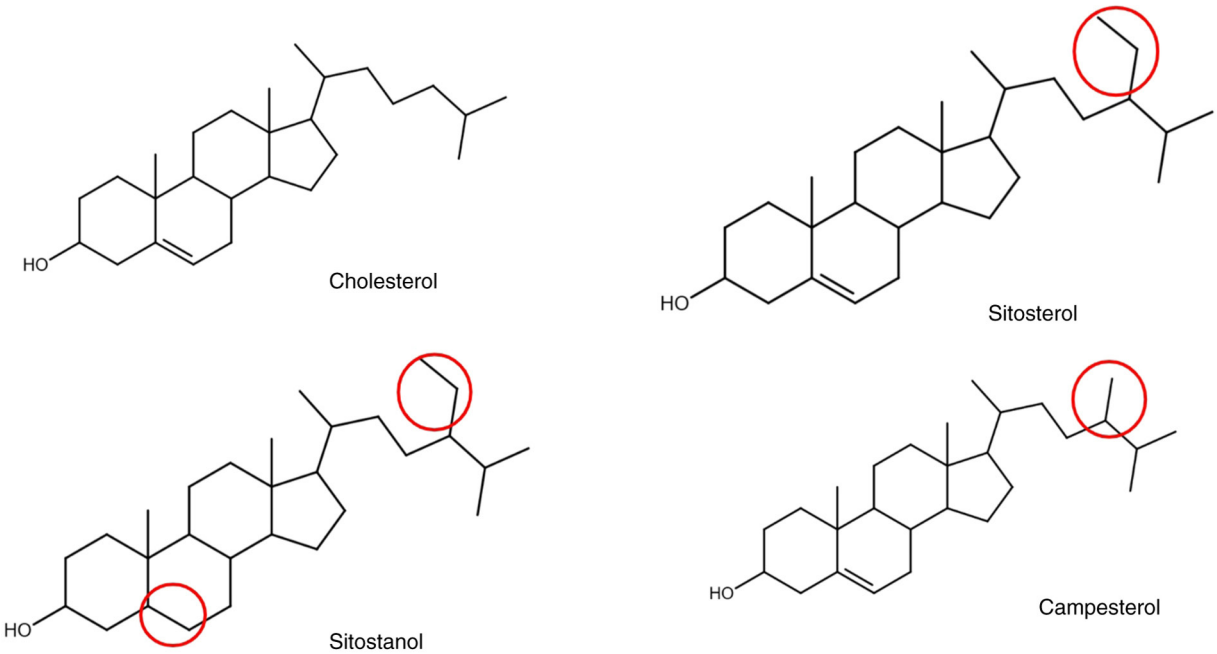

Figure 3. Chemical structures of cholesterol and three common phytosterols. The structural differences between each plant sterol and cholesterol are circled in red.

homozygous pathogenic variants in genes on the short arm of chromosome 2 were present.

This genetic testing was essential to diagnosis. As is common for patients with sitosterolemia, including this patient, numerous individuals are initially misdiagnosed with FH due to the lack of plant sterol-specific testing and early genetic testing. More specifically, total cholesterol tests cannot differentiate between cholesterol and phytosterols due to their similar structures, resulting in high total cholesterol levels in patients with sitosterolemia (Fig. 3). LDL levels may also be high particularly in children such as this patient due to immature intestinal cells and breast milk consumption (1). Suspected patients with FH are typically

advised to shift from saturated animal fat sources to monounsaturated fats, such as phytosterols, to help control their cholesterol levels. As a result, the patient generally avoided animal fat, which is high in saturated fat and cholesterol and instead consumed plant-based sources of fat such as canola and olive oil. The patient also reported eating numerous fruits, vegetables, avocado, nuts, whole grains, and legumes rather than animal protein-based and processed snacks. However, these plant-based foods typically contain phytosterols, which patients with sitosterolemia are unable to excrete properly. As a result, this consumption likely resulted in the deterioration of the condition of the patient at the time.

However, after receiving genetic testing and the sitosterolemia diagnosis, the diet and management plan of the patient were better curated to the condition. Dietary removal of phytosterols along with the addition of ezetimibe, which inhibits the absorption of cholesterol and phytosterols from the small intestine, significantly reduced the total and LDL cholesterol to their lowest levels since infancy in only 4 months of this new treatment plan. Had the patient known of this diagnosis sooner, the cholesterol levels and symptoms experienced by the patient, likely could have been improved earlier.

This is not the only case where genetic testing has proven useful for patients with treatment-resistant hyperlipidemia. A previous case of a 3-year-old female patient with xanthomas and high total and LDL cholesterol was initially misdiagnosed with FH and struggled to show improvement over time (20). After the patient received genetic testing, a correct diagnosis of sitosterolemia due to *ABCG5* variants was made. After starting ezetimibe and removing phytosterols from the diet, the lipid levels and xanthomas of the patient significantly improved. Similarly, two 7- and 9-year-old patients with high cholesterol and xanthomas were also initially misdiagnosed with FH (21). However, on an FH low-cholesterol diet, lipid levels did improve significantly in one patient, and xanthomas were still present in the other. However, after receiving genetic testing, variants in *ABCG5* were identified, leading to a correct sitosterolemia diagnosis. Following subsequent phytosterol removal from the diet, significant xanthoma regression and reduced lipid levels were observed. In all three patients, genetic diagnosis resulted in a change of treatment and improvements to health (20,21).

Thus, genetic testing is an important aspect of diagnosis, especially for those with treatment-resistant hyperlipidemia and rarer manifestations such as periarthritis, as it can provide important information on the conditions of patients that can significantly alter their treatment plan and ultimately lead to an improved quality of life for a number of these patients.

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Availability of data and materials

The data generated in the present study may be found in the NCBI Sequence Read Archive under the BioProject accession no. PRJNA1281833. The in-house Thai databases, Thai Exome Database (ThEx) and Thai Long Read Genome Database (Th-LR), both developed by the Center of Excellence for Medical Genomics, Chulalongkorn University (Bangkok, Thailand) may be requested from the corresponding author.

Authors' contributions

HK was involved with the case analysis and was the primary author of the manuscript. CC edited the manuscript and was involved with analysis and variant identification. SK analyzed the bioinformatics data for the creation of Fig. 2. KS analyzed and identified the variant responsible for the disease. SC was the pediatrician nutrition specialist involved with the treatment of the patient, and edited the manuscript. RT was the clinical geneticist involved with the treatment of the patient. VS designed and supervised the study, analyzed patient data, and obtained funding. VS and SK confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. 264/62) by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (Bangkok, Thailand). Written informed consent from the patient and both parents of the patient was obtained.

Patient consent for publication

Written informed consent was obtained from the patient and both parents of the patient for the publication of any patient data and associated images.

Competing interests

The authors declare that they have no competing interests.

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