CASE REPORT

Complex Phenotype of Hypercholesterolemia in a Family with Both *ABCG8* and *APOB* Mutations

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Abstract

Familial hypercholesterolemia is a common genetic hypercholesterolemia caused by mutations in *LDLR*, *APOB* and *PCSK9* that leads to premature atherosclerosis. Other rare disorders like sitosterolemia can present the same phenotype but have distinct therapeutic interventions. We present a case of severe hypercholesterolemia in a 5-year-old child found to have both familial hypercholesterolemia and sitosterolemia. The proband was diagnosed initially as familial hypercholesterolemia, but the lack of pathogenic variants with Sanger approach questioned this hypothesis. High levels of sitosterol established the diagnosis of sitosterolemia, genetically confirmed by an *ABCG8* homozygous variant c.1974C>G/p.(Tyr658*). Next-generation sequencing re-sequence for familial hypercholesterolemia genes revealed an *APOB* heterozygous functional variant (c.11477C>T/p.(Thr3826Met), in a region previously unstudied. The mother presented with the same genotype but a milder phenotype. Control of low-density lipoprotein cholesterol levels was only accomplished with dietary and therapeutic intervention for both sitosterolemia and familial hypercholesterolemia. The correct diagnosis of dyslipidemia is important to establish proper dietary and pharmacological intervention for atherosclerosis prevention.

Keywords: Child, Preschool; Hyperlipoproteinemia Type II/diagnosis; Hyperlipoproteinemia Type II/diet therapy; Hyperlipoproteinemia Type II/drug therapy; Hyperlipoproteinemia Type II/genetics; Hypercholesterolemia; Intestinal Absorption/genetics; Mutation; Risk Factors

Introduction

Familial hypercholesterolemia (OMIM 143890) is the most common of all genetic hypercholesterolemias, with defects in *LDLR*, *APOB* and *PCSK9* genes accounting for the majority of cases.[1] However, there are other rare disorders presenting with the same phenotype,[1] as sitosterolemia (OMIM 210250). In this disease, patients can present with high levels of low-density lipoprotein cholesterol (LDL-C) and plant sterol accumulation in tendons and arteries, also contributing to increased cardiovascular risk.[2] The defect in this case is in *ABCG5* / *ABCG8* genes, that encode two transporter proteins expressed in enterocytes and hepatocytes that participates in the excretion of absorbed dietary sterols, preventing their accumulation in blood and tissues.[3] Clinically it can present with tuberous xanthomas, accelerated atherosclerosis, and occasionally macrothrombocytopenia.[4] Correct diagnosis is important to guide proper dietary and/or pharmacological interventions for the prevention of atherosclerosis.

The aim of this study is to describe a case of severe hypercholesterolemia in a child and mother found to have both sitosterolemia and familial hypercholesterolemia.

Case Report

The proband was a 5-year-old girl referred for severe hypercholesterolemia and xanthomas. Clinical, biochemical, and genetic investigation of the proband and all available family members was performed. She had normal growth and psychomotor development and normal physical examination, except for yellowish plaques on knees and ankles. Biopsy of the dermal plaques confirmed tuberous xanthomas.

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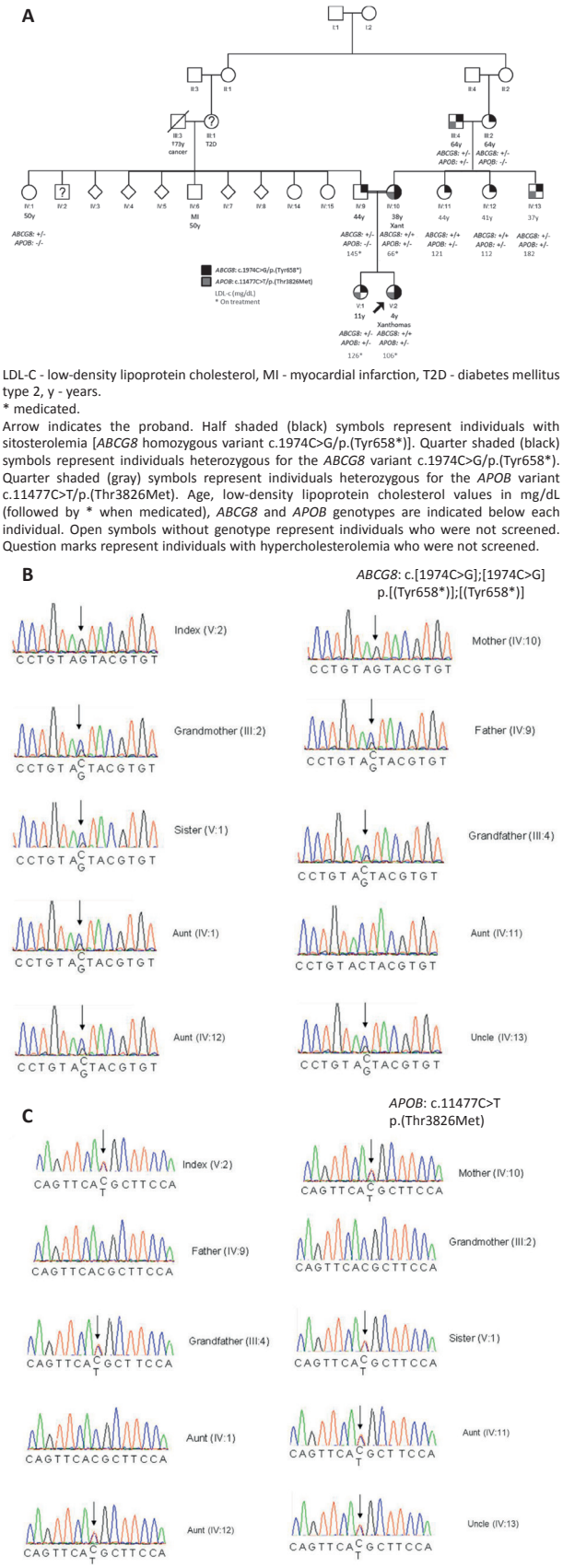
Initial lipid profile showed LDL-C 391 mg/dL (reference values < 130 mg/dL), high-density lipoprotein cholesterol (HDL-C) 34 mg/dL (reference values 36-73 mg/dL), triglycerides 89 mg/dL (reference values < 150 mg/dL), apolipoprotein B 208 mg/dL (reference values 51-126 mg/dL), apolipoprotein A1 99 mg/dl (reference values 86-179 mg/dL), apolipoprotein B / apolipoprotein A1 ratio 2.6 (reference values < 0.6) and lipoprotein (a) 78 mg/dL (reference values < 30 mg/dL). Apart from intermittent macrothrombocytopenia and slight elevation of aspartate aminotransferase and alanine aminotransferase, laboratory evaluations were considered unremarkable.

Cardiovascular exam was normal except for ambulatory monitoring of blood pressure which showed systolic pre-hypertension and nocturnal systolic grade 2 hypertension and a non-dipping pattern. Carotid intima-media thickness was on percentile 50-75.[5]

The proband (V:2 in Fig. 1A) is the second child of consanguineous parents, both with hypercholesterolemia. The mother (IV:10 in Fig. 1A) was first diagnosed with hypercholesterolemia at 28 years and had xanthomas as a child. She tried many statins with diverse side effects and no significant improvement on the lipid profile. She was also under methotrexate for psoriatic arthritis that had to suspend for thrombocytopenia. Lipid profile only improved after she suspended methotrexate and later when ezetimibe was added to a statin (Fig. 2B). The father (IV:9 in Fig. 1A) was also treated with a statin. Three of the proband grandparents (III.1, III.2 and III.4 in Fig. 1A), a paternal aunt and an uncle (IV:1 and IV:2 in Fig. 1A) were also referred to have hypercholesterolemia, although we could not determine the lipid profile. Another paternal uncle had a myocardial infarction when he was 50 years old. From the maternal side only one maternal uncle (IV:13) was reported to have hypercholesterolemia (Fig. 1A).

Due to severe hypercholesterolemia in the proband and parents, a presumptive diagnosis of familial hypercholesterolemia was given according to Simon Broome criteria.[6] However, the diagnosis of familial hypercholesterolemia was questioned when the first molecular results using the Sanger protocol found no mutations in the *LDLR*, *APOB* (two fragments of exons 26 and 29) and *PCSK9* genes. The Sanger protocol included polymerase chain reaction amplification followed by Sanger sequencing of[7]:

- Fragments of exon 26 (c.10423_10765) and exon 29 (c.12987_13321) where the most commons *APOB* mutations have been found;
- Promoter region, coding, and splicing regions (including ±50bp of intron regions flanking the exons) of the *LDLR* and *PCSK9* genes[7].



LDL-C - low-density lipoprotein cholesterol, MI - myocardial infarction, T2D - diabetes mellitus type 2, y - years.
* medicated.
Arrow indicates the proband. Half shaded (black) symbols represent individuals with sitosterolemia [*ABCG8* homozygous variant c.1974C>G/p.(Tyr658*)]. Quarter shaded (black) symbols represent individuals heterozygous for the *ABCG8* variant c.1974C>G/p.(Tyr658*). Quarter shaded (gray) symbols represent individuals heterozygous for the *APOB* variant c.11477C>T/p.(Thr3826Met). Age, low-density lipoprotein cholesterol values in mg/dL (followed by * when medicated), *ABCG8* and *APOB* genotypes are indicated below each individual. Open symbols without genotype represent individuals who were not screened. Question marks represent individuals with hypercholesterolemia who were not screened.

Figure 1. Results of the genetic diagnosis: A. Pedigree of the family. B. Sequencing electropherograms with the molecular results for the *ABCG8* variant. C. Sequencing electropherograms with the molecular result for the *APOB* variant.

It also includes the study of *LDLR* large rearrangements by multiplex ligation-dependent probe amplification technique.

At this point, genetic investigation progressed to next-generation sequencing methodology that included the study of the three genes causing familial hypercholesterolemia (*LDLR*, *APOB* and *PCSK9*) and three other genes considered alternative molecular etiologies (*LDLRAP1*, *LIPA*, *APOE*). For all genes the promotor region, exons, and introns up to ±15bp were analyzed as previously described.[8] Briefly, a gene panel was sequenced including *LDLR*, *APOB*, *PCSK9*, *LDLRAP1, APOE* and *LIPA* genes. SureSeq™ (Oxford Gene Technology, OGT, Oxford, United Kingdom) next-

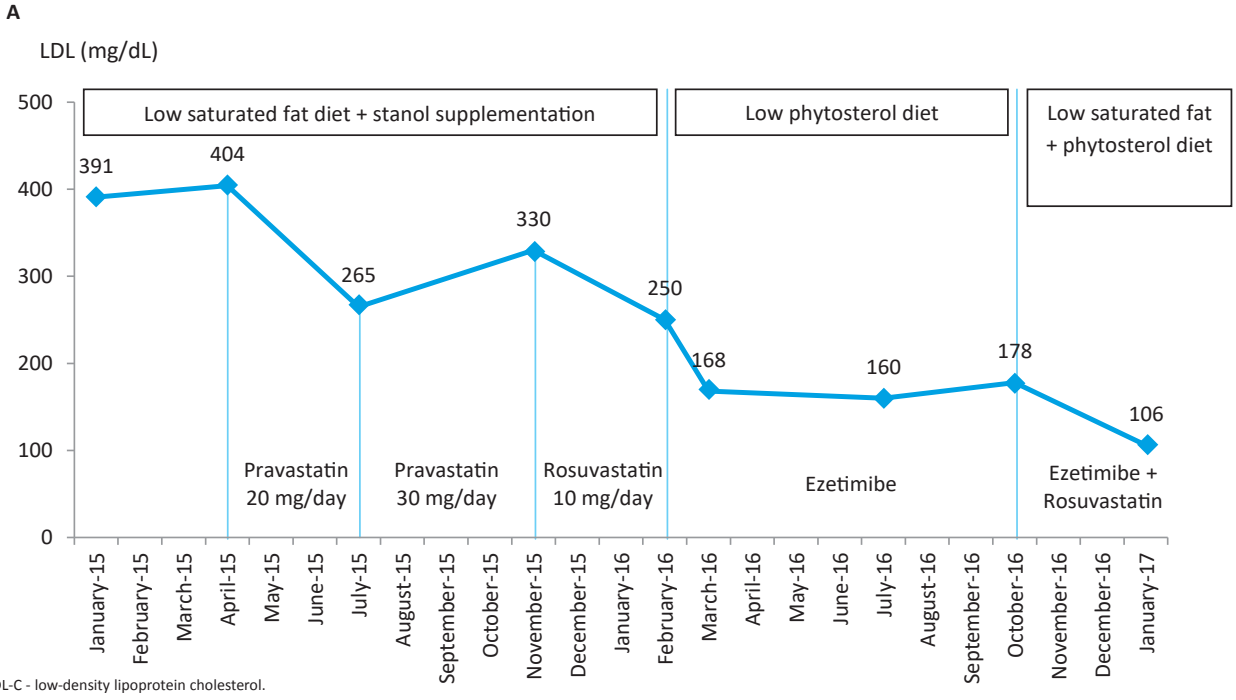


Figure 2. Low-density lipoprotein cholesterol evolution of the proband (A) and her mother (B).

generation sequencing library preparation was prepared following manufacturer protocol. A 4nM pool consisting of 24 individual DNA libraries was loaded into a v2 300 cycle Miseq cartridge and run on an Illumina Miseq (Illumina, Little Chesterford, Essex, United Kingdom). Of the targeted regions, 97% were covered at ≥ Q30. The FASTQ files were analysed using OGT SureSeq Interpret software (Oxford Gene Technology, Oxford, United Kingdom).

The next-generation sequencing study uncovered a heterozygous variant in exon 26 of the *APOB* gene (c.11477C>T/p.(Thr3826Met) (rs61744153), not detected by the methodology previously used. This variant has been proven to affect the binding of apolipoprotein B / *LDLR* by functional studies as reported elsewhere.[8]

At the same time, on clinical ground, other etiologies for hypercholesterolemia were being investigated. Elevation of transaminases and macrothrombocytopenia prompted the investigation of cholesteryl ester storage disease and sitosterolemia.

Sterol analysis[9] in the index patient revealed an increased cholestanol (43.6 µmol/L, controls 3.52-23.8 µmol/L) and β-sitosterol (470 µmol/L, not detected in controls). The mother, at that time under ezetimibe and a statin, showed normal cholestanol and high β-sitosterol (224 µmol/L, not detected in controls). Sterol analysis was normal in the father and sister.

After the sterol chromatography, sitosterolemia was confirmed by the finding of a homozygous known nonsense mutation (c.1974C>G/p.(Tyr658*)) (rs137852989) in the *ABCG8* gene (c.[1974C>G]; [1974C>G]/p.[(Tyr658*)]; [(Tyr658*)]). All exons of *ABCG8* gene were analyzed by polymerase chain reaction and Sanger sequencing.

Family studies revealed that the proband mother (IV:10) had the same mutations in the *ABCG8* gene (in homozygosity) and in the *APOB* gene (in heterozygosity) (Figs. 1B and 1C). Both maternal grandparents, as expected, have the *ABCG8* nonsense variant in heterozygosity and all carriers of the *APOB* variant have a hypercholesterolemic phenotype, showing this way co-segregation of the hypercholesterolemia with this genotype (c.11477C>T/p.(Thr3826Met) (Fig. 1A).

When a familial hypercholesterolemia diagnosis was assumed, the proband was put on a low saturated fat diet with vegetable stanol supplementation. After six months, she was started on pravastatin (up to 30 mg/day) and then changed to rosuvastatin 10 mg/day, resulting only then in a moderate reduction of LDL-C levels (Fig. 2A).

After the diagnosis of sitosterolemia the child changed to a low phytosterol diet, stopped rosuvastatin and initiated ezetimibe 10 mg/day. Five months later the tuberosum xanthomas had attenuated, phytosterols and LDL-C diminished (Fig. 2A).

When the *APOB* variant was found, the child was put on a diet restricted in both saturated fat (meat and dairy sources) and phytosterol (plant source). Rosuvastatin 10 mg/day was also added to ezetimibe as the combination of diet intervention with ezetimibe was not enough to control LDL-C levels (Fig. 2A). After five years of follow-up, the child is growing and developing normally, xanthomas have disappeared, phytosterols diminished from 470 mmol/L to 242 mmol/L, and vitamin A supplementation was needed.

Discussion

Here we present a case of severe hypercholesterolemia with both a homozygous null variant in the *ABCG8* gene and a heterozygous variant in the *APOB* gene, causing sitosterolemia and familial hypercholesterolemia, respectively. The mother was found to have the same genotype, although now she presents a milder phenotype. It has been reported that the phenotype of sitosterolemia subjects improves with age[10] explaining the difference between the proband and the mother.

Until recently, the routine genetic diagnosis of familial hypercholesterolemia comprised the study of the entire *LDLR* gene and only two *APOB* fragments located in the *LDLR* binding region, and in some cases the study of *PCSK9* gene.[11] So, when the routine familial hypercholesterolemia diagnosis test was found to be negative, a next generation sequencing protocol was performed which included the complete study of six genes (all exons, promotor, and splice regions (+/-50 bp) of the three familial hypercholesterolemia causing genes, plus three genes responsible for alternative molecular causes of the familial hypercholesterolemia phenotype: autosomal recessive hypercholesterolemia (*LDLRAP1*), lysosomal acid lipase deficiency (*LIPA*) and dysbetalipoproteinemia (*APOE*). Only one variant was found in *APOB,* outside the described binding region to the *LDLR*. This variant has been found before in familial hypercholesterolemia cohorts worldwide and was showed to affect apolipoprotein B / *LDLR* binding by functional analysis.[8] However, following the American College Medical Genetics and Genomics[12] this variant is classified as variant of unknown significance, since there is not enough evidence to classify it as pathogenic or benign. Nevertheless, since the functional study showed that the apolipoprotein B / *LDLR* binding was affected, the finding of this *APOB* variant established a possible diagnosis of familial hypercholesterolemia in the

proband. More than 50% of *LDLR* variants found in familial hypercholesterolemia patients worldwide are classified as variant of unknown significance[13] and are described as familial hypercholesterolemia causing variants.

Sitosterolemia was also investigated since it is a known alternative molecular etiology for the familial hypercholesterolemia phenotype and the child had intermittent macrothrombocytopenia. The sterol chromatography confirmed the presence of large concentrations of β-sitosterol and cholestanol. A homozygous pathogenic stop codon variant was found in the last exon, exon 13 of *ABCG8* gene, confirming the diagnosis.

Determining the complex genotype was essential to establishing the correct patient management. Control of LDL-C levels was only achieved with a combined dietetic (restriction in both saturated fat and phytosterol) and pharmacological (statin and ezetimibe) approach for both sitosterolemia and familial hypercholesterolemia, which can be considered a therapeutic proof that the patient has indeed sitosterolemia and familial hypercholesterolemia.

This study highlights the importance of the study of all familial hypercholesterolemia genes and other alternative molecular etiologies with a more complete next-generation sequencing panel to fully understand the patient phenotype.

Our lab has now decided to change the genetic diagnosis of familial hypercholesterolemia to a next generation sequencing panel of eight genes (*LDLR, APOB, PCSK9, LDLRAP1, APOE, LIPA, ABCG5* and *ABCG8*). So, whenever possible this novel eight-gene next generation sequencing panel should be implemented for the molecular study of familial hypercholesterolemia as suggested by international familial hypercholesterolemia experts.[1] This will improve the knowledge of the etiology of the familial hypercholesterolemia phenotype and will allow better patient identification and management.

The clinical phenotype presented by patients with sitosterolemia, and familial hypercholesterolemia have a considerable overlap, but each entity has specific treatment approaches. Target sequencing panels allow for the study of familial hypercholesterolemia genes and other alternative molecular etiologies in one run and increase the correct identification of the disorder and the understanding of the underlying pathophysiology. This study provides strong evidence that next generation sequencing panels for familial hypercholesterolemia diagnosis should include the sitosterolemia genes. The correct identification of the origin of the dyslipidemia is important to establish the correct diagnosis and to establish the different dietary and pharmacological interventions for the prevention of atherosclerosis.

WHAT THIS CASE REPORT ADDS

- The genetic diagnosis of hypercholesterolemia is important for the correct management of the disease.
- Next-generation sequencing panels for familial hypercholesterolemia diagnosis should be frequently updated as the number of causal genes increases.
- To our knowledge this is the first reported case of diagnosis and management of both sitosterolemia and familial hypercholesterolemia.

Conflicts of Interest

The authors declare that there were no conflicts of interest in conducting this work.

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Consent for publication was obtained.

Confidentiality of data

The authors declare that they have followed the protocols of their work centre on the publication of patient data.

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References

1. Sturm AC, Knowles JW, Gidding SS, Ahmad ZS, Ahmed CD, Ballantyne CM, et al. Clinical genetic testing for familial hypercholesterolemia: JACC Scientific Expert Panel. J Am Coll Cardiol 2018;72:662-80. doi: 10.1016/j.jacc.2018.05.044.
2. Kidambi S, Patel SB. Sitosterolaemia: Pathophysiology, clinical presentation and laboratory diagnosis. J Clin Pathol 2008;61:588-94. doi: 10.1136/jcp.2007.049775.
3. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000;290:1771-5. doi: 10.1126/science.290.5497.1771.
4. Myrie SB, Steiner RD, Mymin D. Sitosterolemia. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al, editors. GeneReviews [Internet]. Seattle: University of Washington; 2013-2020.
5. Doyon A, Kracht D, Bayazit AK, Deveci M, Duzova A, Krmar RT, et al. Carotid artery intima-media thickness and distensibility in children and adolescents: Reference values and role of body dimensions. Hypertension. 2013;62:550-6. doi: 10.1161/HYPERTENSIONAHA.113.01297.
6. Scientific steering committee on behalf of the Simon

Broome register group. Risk of fatal coronary heart disease in familial hypercholesterolaemia. BMJ 1991;303:893-6. doi: 10.1136/bmj.303.6807.893.
7. Medeiros AM, Alves AC, Bourbon M. Mutational analysis of a cohort with clinical diagnosis of familial hypercholesterolemia: Considerations for genetic diagnosis improvement. Genet Med 2016;18:316-24. doi: 10.1038/gim.2015.71.
8. Alves AC, Benito-Vicente A, Medeiros AM, Reeves K, Martin C, Bourbon M. Further evidence of novel APOB mutations as a cause of familial hypercholesterolaemia. Atherosclerosis 2018;277:448-56. doi: 10.1016/j.atherosclerosis.2018.06.819.
9. Amaral C, Gallardo E, Rodrigues R, Pinto Leite R, Quelhas D, Tomaz C, et al. Quantitative analysis of five sterols in amniotic fluid by GC-MS: Application to the diagnosis of cholesterol biosynthesis defects. J Chromatogr B Analyt Technol Biomed Life Sci 2010;878:2130-6. doi: 10.1016/j.jchromb.2010.06.010.
10. Mymin D, Salen G, Triggs-Raine B, Waggoner DJ, Dembinski T, Hatch GM. The natural history of phytosterolemia: Observations on its homeostasis. Atherosclerosis 2018;269:122-8. doi: 10.1016/j.atherosclerosis.2017.12.024.
11. Motazacker MM, Pirruccello J, Huijgen R, Do R, Gabriel S, Peter J, et al. Advances in genetics show the need for extending screening strategies for autosomal dominant hypercholesterolaemia. Eur Heart J 2012;33:1360-6. doi: 10.1093/eurheartj/ehs010.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24. doi: 10.1038/gim.2015.30.
13. Chora JR, Medeiros AM, Alves AC, Bourbon M. Analysis of publicly available LDLR, APOB, and PCSK9 variants associated with familial hypercholesterolemia: Application of ACMG guidelines and implications for familial hypercholesterolemia diagnosis. Genet Med 2018;20:591-8. doi: 10.1038/gim.2017.151.

Fenótipo Complexo de Hipercolesterolemia numa Família com Mutações *ABCG8* e *APOB*

Resumo

A hipercolesterolemia familiar é uma hipercolesterolemia genética comum causada por mutações nos genes *LDLR*, *APOB* e *PCSK9*, que leva a aterosclerose prematura. Outros alterações raras, como a sitosterolemia, podem apresentar o mesmo fenótipo, mas a intervenção terapêutica é distinta. Apresentamos um caso clínico de hipercolesterolemia grave numa criança de 5 anos com hipercolesterolemia familiar e sitosterolemia. O probando foi diagnosticado inicialmente como hipercolesterolemia familiar, mas a ausência de variantes patogénicas com a abordagem de Sanger pôs em causa essa hipótese. Níveis elevados de sitosterol permitiram estabelecer o diagnóstico de sitosterolemia, confirmado geneticamente por uma variante homozigótica *ABCG8*, c.1974C> G / p. (Tyr658 *). A nova sequência por sequenciamento de nova geração para genes de hipercolesterolemia familiar revelou uma variante funcional heterozigótica *APOB* (c.11477C> T / p. (Thr3826Met), numa região não estudada anteriormente. A mãe apresentou o mesmo genótipo, mas um fenótipo mais ligeiro. O controlo dos níveis de colesterol das lipoproteínas de baixa densidade só foi conseguido com intervenção dietética e terapêutica, tanto para sitosterolemia como para hipercolesterolemia familiar. O diagnóstico correto da dislipidemia é importante para estabelecer uma intervenção dietética e farmacológica adequada para a prevenção da aterosclerose.

Palavras-Chave: Absorção Intestinal/genética; Fatores de Risco; Hiperlipoproteinemia Tipo II/diagnóstico; Hiperlipoproteinemia Tipo II/dietoterapia; Hiperlipoproteinemia Tipo II/genética; Hiperlipoproteinemia Tipo II/tratamento farmacológico; Hipercolesterolemia; Mutação; Pré-Escolar