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Case Report

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Importance of the detection of genetic diseases associated with cardiovascular risk in pediatric age

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Abstract

Familial Hypercholesterolemia (FH) is a disease with a homozygous or heterozygous autosomal dominant inheritance pattern. It is characterized by high plasma concentrations of Low-Density Lipoprotein (LDL) cholesterol. It can lead to the formation of atherosclerotic plaque in the coronary arteries and proximal aorta at a young age, increasing the risk of premature cardiovascular events such as angina and acute myocardial infarction. The diagnosis can be made clinical or genetic. We present the case of an asymptomatic adolescent with relevant first and second degree family history of consanguinity of cardiovascular disease, difficult-to-manage hyperlipidemia and premature deaths. Given the family history and the importance of ruling out a hereditary genetic disease associated with cardiovascular risk, in order to carry out anticipatory and preventive actions, a lipid profile was requested with subsequent hyperlipidemia at the expense of LDL. After this, a molecular panel of genes associated with FH was demonstrated using the NGS+CNVs methodology, which discovered the ABCG5, ABCG8, APOB, LDLR, LDLRAP1, PCSK9 genes, obtaining pathogenic variants in the gene that encodes LDL Receptors (LDLR) (OMIM*606945) and in the LDL Receptor Adapter Protein 1 (LDLRAP1) gene (OMIM*605747). Both associated with HF, identified early in an asymptomatic adolescent patient. In this way, it is possible to carry out an adequate intervention that is predictive, preventive, personalized and participatory. In order to establish a targeted treatment, carry out adequate follow-up and genetic advice, including the risk of heritability, active search for relatives who are carriers of the disease, and thus affect morbidity and mortality.

Keywords: Familial hypercholesterolemia; LDL-cholesterol; Cardiovascular disease; Atherosclerosis; Congenital genetic diseases; Genetic tests; Early diagnosis; Precision medicine; Disease prevention; Specific treatment.

Introduction

Familial Hypercholesterolemia (FH) is a genetic condition with high prevalence in the general population around the world. It is characterized by elevations in plasma cholesterol concentrations, particularly Low-Density Lipoprotein (LDL) cholesterol levels [1]. People affected by HF are at increased risk of early and progressive development of atherosclerotic cardiovascular disease, in children and adolescents, which can be suspected by classic clinical signs, such as tendon xanthomas [2].

The inheritance pattern of FH is autosomal dominant, which can be Homozygous (HoFH) or Heterozygous (HeFH). The most frequent cause are variants in the gene that encodes the LDL receptors (LDLR), less frequently it occurs due to variants in the apolipoprotein B100 (ApoB 100) gene, in the subtilisin/kexin 9 proprotein convertase gene (PCSK9) or in the LDL Receptor Adapter Protein 1 (LDLRAP1) gene [3].

HeFH has a prevalence of 1 per 311-313 people, which translates into an estimated 6.8-8.5 million children and adolescents in the world [4]. Although, there are no official statistics reported nationally; however, with global statistics, it can be estimated that the prevalence of HeFH in Colombia is between 96,000-240,000 [4-6].

In Colombia, HoFH is part of the recognized orphan diseases (Resolution 5265 of 2018, 2018). HeFH is not recognized. The Colombian Society of Cardiology and Cardiovascular Surgery convened specialists from multiple fields to prepare a review document on FH. Due to the fact that search and early diagnosis actions are not undertaken at national level, and there are also no consolidated statistics on the population burden, prevalence and incidence of this pathology, the proposal was made to carry out a Colombian FH registry that would allow the detailed knowledge of the frequencies and distributions of FH forms, a document that is still developing [6].

Given the clinical relevance of this pathology, which is considered a public health problem, FH screening and early detection programs have been created. However, diagnosis at an early age remains a challenge because on many occasions, in the pediatric age, there will be no classic clinical manifestations or the family history will not be adequately documented [7]. The three main characteristics that determine FH are elevated plasma LDL levels, the presence of tendon xanthomas, and premature onset of atherosclerotic cardiovascular disease [2].

The diagnosis of HF can be made with two aspects: clinical and genetic. Regarding the clinical component, different diagnostic criteria have been described in multiple guidelines and consensuses worldwide. The phenotypic diagnosis can be made taking into account 3 instructions. The first stipulates a plasma concentration of LDL >190 mg/dL in 2 different blood samples and after a period of 3 months of adequate nutrition. The second, serum LDL levels >160 mg/dL and concomitantly having a 1st degree relative of consanguinity with high values of this or a history of premature coronary events. The third is LDL blood values >130 mg/dL and having one of the parents diagnosed with FH. Each one is highly suggestive of HF [8,9]. Most pediatric patients do not show clinical manifestations, however, among them, tendon xanthomas are classic findings, their presence orients more towards HoFH instead of HeFH [10]. There are other causes that can lead to HF, such as hypothyroidism, nephrotic syndrome, congenital analbuminemia, obesity or anorexia, obstructive liver disease, among others [11].

The genetic diagnosis component is performed by identifying a pathogenic variant, performed with genetic tests such as Next Generation Sequencing (NGS), which is the gold standard for diagnosing FH [2]. Patients with FH can be classified as monogenic or polygenic, which occurs by the association of common genetic variations; as well as in polygenic form plus hypertriglyceridemia, which derives from variants also of the genes associated with triglycerides and also of those associated with LDL. It has been described that the FH that is more likely to cause coronary events more prematurely is monogenic [12].

The management of FH depends on its inheritance pattern, HeFH or HoFH. In the first, lifestyle changes should be made from the diagnosis, then if the infants are older than 8 years old and the LDL is >154 mg/dL, it is necessary to start statins. Also, if the infant is older than 10 years old and LDL is >135 mg/dL, ezetimibe should be added to drug therapy. Whereas, in HoFH, lifestyle changes should be made and both statins and ezetimibe should be started together [2]. It is described that if serum LDL values are >135 mg/dL, new and unconventional therapies should be started in adults. If they exceed 308 mg/dL, it is suggested to start lipoprotein apheresis, and if the latter is not available, the last measure is liver transplantation, in children this therapeutic option has not been described [13].

Currently, there are several novel drugs as treatment options for adults with FH; however, few have been approved for the pediatric population with this disease. In one hand, it was known that the long-lasting inhibition of PCSK9 synthesis by inclisiran, a small interfering RNA, can lead to a 47.9% decrease in serum LDL, compared to adults with HeFH who they received a placebo [14]. Its use for the treatment of primary dyslipidemia in adults was approved by the European Medicines Agency (EMA) in 2020, while it is still under evaluation for adult HoFH patients. Another drug, evolocumab, received US Food and Drug Administration (FDA) approval for HoFH patients >12 years of age, and has also shown potential as a treatment for HeFH children with high serum LDL concentrations [15]. Also, studies are being carried out to test the use of alirocumab, a proprotein convertase subtilisin-kexin (PCSK9) inhibitor, in children and adolescents (8-17 years) with HeFH and HoFH, studies that have had promising results [3,16]. In 2020, the FDA-approved bempedoic acid, which lowers blood LDL levels through inhibition of ATP-citrate synthase, was declared only for the treatment of adult patients with HeFH [17]. Finally, a randomized controlled trial in pediatric patients with HoFH was carried out to evaluate the use of mipomersen as adjuvant therapy, with successful results regarding the efficacy of parameters in long-term treatment [18]; however, there are no similar trials conducted in children and adolescents with HeFH.

Case presentation

A 12-year-old male patient, asymptomatic, who consults due to a paternal family history with a clinical and paraclinical diagnosis of FH in treatment with statin and biological therapy, without prior genetic study and relatives of 2nd and 3rd degree of consanguinity with a history of dyslipidemia and early death from heart disease.

On physical examination, the individual had no palpable xanthelasmas, cutaneous or tendon xanthomas. Vital signs were normal for his age, weight, and gender. His body mass index was between 0 to +1 standard deviations. He did not present with hepatomegaly or corneal alterations. Had a serum lipid profile with total cholesterol levels of 372 mg/dL (normal value <200 mg/dL), HDL cholesterol of 38 mg/dL (normal value >60 mg/dL), LDL cholesterol of 304 mg/dL (normal value <130 mg/dL) and triglycerides of 159 mg/dL (normal value <150 mg/dL).

Given the relevant family history associated with cardiovascular risk of genetic origin without family molecular studies and given the importance of an accurate and specific diagnosis, in order to establish targeted and personalized treatment, follow-up, prognosis and genetic counseling including heritability risk, a molecular panel of genes associated with familial hypercholesterolemia was requested. NGS methodology (New Generation Sequencing) + CNVs (Copy Number Variants) included ABCG5, ABCG8, APOB, LDLR, LDLRAP1, and PCSK9 genes. Pathogenic variants with heterozygous autosomal dominant inheritance pattern were reported in the LDLR (Online Mendelian Inheritance in Man (OMIM) *606945) and LDLRAP1 (OMIM *605747) genes, which are respectively associated with familial hypercholesterolemia 1 (MIM #143890) of autosomal dominant inheritance; and familial hypercholesterolemia 4 (MIM #603813) of autosomal recessive inheritance.

The molecular panel of genes associated with familial hypercholesterolemia using NGS (Next Generation Sequencing) + CNVs (Copy Number Variants) methodology, reported a heterozygous pathogenic variant in the LDLR gene, consisting of the change of a cytosine for a thymine in position 1246 of the cDNA, in exon 9/18 of the gene (c.1246C>T), which at the protein level generates the missense change of a arginine by a tryptophan at codon 416 (p.Arg216Trp), a highly conserved amino acid residue located in the functional domain "LDL-receptor class B1". It also reported a probably pathogenic heterozygous variant in the LDLRAP1 gene, consisting of a deletion at position 604 of the cDNA corresponding to exon 6/9 (c.604del), which at the protein level produces the frameshift change of the reading frame leading to a premature stop signal at codon 204 (p.Ser202ProfsTer2) in a 309 amino acid protein. Pathogenic variants in the LDLR gene (OMIM *606945) are associated with familial hypercholesterolemia 1 (MIM #143890) of autosomal dominant inheritance. As well as, pathogenic variants in the LDLRAP1 gene (OMIM *605747) are associated with familial hypercholesterolemia 4 (MIM #603813) of autosomal recessive inheritance, which confers heritability risk. It was documented that the patient is a carrier of the heterozygous pathogenic variant c-1236C>T; p. Arg416Trp in the LDLR gene; being the dyslipidemia phenotype concordant and listed in association with hypercholesterolemia. This variant is classified as pathogenic given the apparent alteration in the recycling of the defective receptor, which also impacts the response of patients to the pharmacological use of statins. Likewise, he is a carrier of the probably pathogenic heterozygous variant c.604del; p.Ser202ProfsTer2 in the LDLRAP1 gene. This variant probably produces an mRNA transcript degraded by the NMD (nonsense mediated decay) system or a non-functional protein.

LDLR and LDLRAP1 genes

In 1970, Brown and Goldstein identified abnormalities of the LDLR in 85-90% of patients, which is considered the main determinant of this disorder, finding little or no accumulation of LDL particles in plasma [19]. The LDLR gene is located on chromosome 19 and to date more than 1,700 variants have been described [20].

Different variants in alleles of the gene can affect the activity of the LDLR, which makes the severity of the disease variable, since depending on the variant it is related to little or no activity of the receptor. On some occasions, LDLR activity can be normal but with elevated serum LDL levels as well, due to variants in LDLRAP1, whose mechanism has not been fully deciphered [2].

According to the degree of residual activity of LDLR, its variants have been conventionally classified as null allele variant if the activity is less than 2%, or defective allele if the activity is between 2 and 25% [21]. Homozygous patients with null allele variants usually have very high plasma LDL values and a poor prognosis. In patients with HeFH, the clinical expression will depend on the type of variant and the degree of receptor activity dependent on the healthy allele [22].

The cell has control over cholesterol levels thanks to two main mechanisms: the expression of HMG CoA reductase (it can increase or decrease cholesterol synthesis), and the uptake of extracellular cholesterol. The process of cholesterol uptake requires the expression of LDLR and the activity of LDLRAP1, which intervenes in the entry of the receptor into the cell, once it has taken up cholesterol [23]. LDLRAP1 is located on the short arm of chromosome 1. Its homozygous variants produce a condition similar to HoFH, known as autosomal recessive hypercholesterolemia, and it represents less than 1% of cases [24].

The LDLR, in association with the LDLRAP1 protein, takes cholesterol and facilitates endocytosis of the cell. LDLR is a protein that is expressed on the outer surface of the cell and acts as a receptor for ApoB, which is on the outer surface of LDL phospholipids. LDLR also acts as a receptor for ApoE, which is on the surface of chylomicron remnants and Intermediate Density Lipoproteins (IDL). When the receptor and LDL bind, an invagination of the membrane occurs, which then closes and fuses, forming a clathrin-coated intracellular vesicle, which carries LDL into the cell to its intracellular destination [25].

This process occurs in all cells with a nucleus, but mainly in the liver, which removes more than 70% of LDL from the circulation. Once the vesicle has entered the cell medium, the change in pH produces a conformational change in the receptor, which releases the LDL particle and the receptor returns to the cell surface, where the neutral pH returns it to its original conformation. and disposes it to receive another LDL particle [25].

LDLR synthesis depends on the level of intracellular free cholesterol. When there is excess free cholesterol, receptor gene transcription is inhibited. A second mechanism for cholesterol regulation has been described, which occurs through the destruction of LDLR before its release to the cell surface. From inside the cell PCSK9 comes out and binds to LDLR. When the complex of PCSK-9 and LDLR + LDL enters the cell, LDL is released, and PCSK9 induces receptor degradation [26]. When there is a gain in the function of the PCSK9 gene, the number of receptors for LDL on the cell surface is decreased. This can cause that, since there is no entry of LDL, its plasmatic levels increase; and also, the lack of cholesterol inside the cell, interpreted as a deficit by the nucleus, translates into an increase in the expression of HMGCoA reductase, which leads to an increase in cholesterol synthesis and, therefore, in cholesterol plasma concentrations [23].

The primary defect in the quantity of LDLR or in the quality of

their activity translates into deficits in cholesterol uptake. The poor conformation of the LDLR leads to the difficulty or impossibility to take the LDL, with the consequent elevation of the lipid levels in the blood [6]. On the other hand, because LDLRAP1 allows easy binding of the receptor to ApoB, the variant of this gene decreases the function and hinders the formation of the LDLR-ApoB-LDL complex, which leads to elevated serum LDL levels [26].

Discussion

FH is a disease characterized by elevated plasma cholesterol concentrations, particularly LDL cholesterol levels. It can lead to the formation of atherosclerotic plaque in the coronary arteries and proximal aorta at an early age, increasing the risk of premature cardiovascular events such as angina and acute myocardial infarction.

The diagnosis of FH can be made with two aspects: clinical and genetic. This patient complies with both aspects. Regarding the clinical component, he presented serum LDL levels >160 mg/dl and complied with a family history of premature coronary diseases and a diagnosis of hypercholesterolemia in a first-degree relative. The molecular diagnosis of familial hypercholesterolemia involves the identification of heterozygous pathogenic variants in the APOB, LDLR or PCSK9 genes, and as an alternative possibility, biallelic pathogenic variants in the LDLRAP1 gene can be identified. The process of cholesterol uptake requires the expression of LDLR and the activity of LDLRAP1, which intervenes in the entry of the receptor into the cell, once it has taken up cholesterol [23].

FH patients can be classified as monogenic or polygenic, which is caused by the association of common genetic variations. It has been described that the FH that is more likely to cause coronary events more prematurely is monogenic [12]. In this patient, a pathogenic heterozygous variant in the LDLR gene and a probably pathogenic heterozygous variant in the LDLRAP1 gene were found. The first of them is associated with familial hypercholesterolemia with autosomal dominant inheritance and the second with autosomal recessive inheritance, which confers heritability risk. Interactions in these genes lead to greater severity and early adverse cardiovascular outcomes.

The hereditary pattern of FH is autosomal dominant, it can be Homozygous (HoFH), which is caused by a deleterious double variant in the FH gene; or Heterozygous type (HeFH), which is caused by the variant of only one allele. The most frequent cause is variants in the gene that encodes the receptors for LDLR and, less frequently, it occurs due to variants in the LDLRAP1 gene [3]. HeFH has a prevalence of 1 per 311-313 people, which translates into an estimated 6.8-8.5 million children and adolescents in the world [4]. Although there are no official statistics reported nationally, it can be estimated that the prevalence of HeFH in Colombia is between 96,000-240,000 [4-6].

In Colombia, there is no protocol or guide that dictates pediatric population screening for this disease. On the other hand, at a global level, universal screening at birth is not recommended, despite the fact that it is feasible in the context of neonatal screening for some metabolic diseases; however, government support is required for this. In Italy, selective screening is recommended between 2 and 10 years of age, since at this age serum lipid values are constant, being values similar to those found in early adulthood [9]. In recent years, some authors in developed first world countries recommend performing a cascade screening for children who have 1st and 2nd degree relatives of consanguinity with the disease [7,20].

Currently, there are several novel drugs as treatment options for adults with FH; however, few have been approved for the pediatric population with this disease. Among the drugs that stand out for pediatric use, evolocumab is described, which received approval from the FDA for patients with HoFH > 12 years of age, and has also demonstrated its potential as a treatment for HeFH children with high serum LDL concentrations [15]. Also, studies are being carried out to test the use of alirocumab, a proprotein convertase subtilisin-kexin (PCSK9) inhibitor, in children and adolescents (8-17 years) with HeFH and HoFH, studies that have had promising results [3,16]. Due to first and second degree family history of consanguinity with death at an early age and a history of dyslipidemia, it is necessary to carry out targeted pharmacological and non-pharmacological management with this patient, in addition to strict follow-up to prevent premature acute coronary events and outcomes. adverse.

Conclusion

FH is a genetic condition with a high prevalence in the general population around the world. It is characterized by elevated plasma cholesterol concentrations, particularly LDL cholesterol levels, which leads to a higher risk of early and progressive development of atherosclerotic cardiovascular disease. The most frequent cause is variants in the gene that encodes LDLR and less frequently occurs due to variants in the LDLRAP1 gene.

Given the clinical relevance of this pathology, which is considered a public health problem, FH screening and early detection programs have been created. However, diagnosis at an early age remains a challenge because on many occasions, in the pediatric age, there will be no classic clinical manifestations or the family history will not be adequately documented [7]. The patient in the reported case was asymptomatic and had no abnormal findings on physical examination.

In Colombia, HeFH is not part of the orphan diseases (Resolution 5265 of 2018, 2018). There is also no publication aimed at the diagnosis and treatment of this disease in any of its inheritance patterns. Nationally, there are no statistics on the prevalence of the disease, much less in pediatrics. A proposal was made to create a Colombian FH registry that would allow detailed knowledge of the frequencies and distributions of the forms of FH, a document that is still being published is gestating [6]; indeed a necessary study to know the population burden of this pathology, characterize patients with this disease, carry out an early identification through the affected families and thus be able to carry out an adequate follow-up to manage patients and thus avoid early outcomes that increase morbidity-mortality due to cardiovascular diseases in this population. It is necessary to increase screening, to raise awareness among the health personnel to consider this pathology as a differential diagnosis for good genetic counseling, even if patients are asymptomatic, since they may be at risk of developing early cardiovascular disease and thus avoid them.

It is also essential to strengthen public health strategies for the promotion and prevention of health in the entire population. An early identification of this disease is a priority through a complete clinical history, physical examination, knowing family genetic risks and the importance of screening the population, approaching anticipatory, preventive, predictive, participatory and preventive medicine.

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