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HDL deficiency due to Tangier disease. A novel mutation of ABCA1 cholesterol tranporter gene

Meritxell Torrabías Rodas[1], Ana Bárbara García García[2], Anna Rodríguez Ferre[3], Lluís Rodríguez Fernández[4]

[1]Sección de Endocrinología infantil, Servicio de Pediatría. Hospital General de Vic. Vic, Barcelona (España)
[2]CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM). Unidad de Genotipado y diagnóstico genético. Fundación Investigación Clínico de Val. Hospital Clínico de Valencia . Valencia (España)
[3]Medicina familiar y comunitaria. Centro de Atención Primaria Calldetenes. Calldetenes, Barcelona (España)
[4]Hematología . Hospital General de Vic. Vic, Barcelona (España)

Abstract

Context: Tangier disease (TD) is a rare autosomal recessive genetic disorder caused by a mutation in the ATP binding cassette transporter (ABCA1) that is characterized by reduced levels of plasma high density lipoproteins (HDL) resulting in tissue acumulation of cholesterol. Clinical features include large and orange tonsils, hepatosplenomegaly, hemolytic anemia, neuropathy, corneal opacity and increased risk for coronary heart disease.

AIM: We describe the clinical features and the genetic study of a familial HDL deficiency caused by Tangier disease.

Methods: The ABCA1 gen was sequenced.

Results: A 12 years-old boy with growth hormone deficiency and her 15 years old sister with stomatocytosis, present both high density lipoprotein (HDL) deficiency. Their parents present low values of HDL. We report a novel homozygous mutation in ABCA1 transporter (c.2193delG) in the index case and her sister and in the parents in heterozygous form.

Conclusion: This study reveals a novel ABCA1 gene mutation that has not been previously described.

Correspondencia:
Meritxell Torrabías Rodas
Sección de Endocrinología infantil, Servicio de Pediatría, Hospital General de Vic, Francesc Pal "El vigatà", 1, 08500, Vic, Barcelona, España, Tel: 937027722
E-mail: mtorrabias@chv.cat
E-mail: txelltr@gmail.com

Key Words: ATP-binding cassette transporter (ABCA1), Apolipoprotein, Tangier Disease, High-density lipoprotein deficiency, Hypoalphalipoproteinemia, Analphalipoproteinemia

Resumen

Antecedentes: La Enfermedad de Tangier (ET) es una enfermedad rara, autosómica recesiva, que está causada por una mutación genética en el transportador de colesterol ABCA1, y que provoca una disminución del colesterol de alta densidad (HDL) en plasma y un acúmulo lipídico patológico en otros tejidos que puede manifestarse con amígdalas grandes y anaranjadas, hepatoesplenomegalia, anemia hemolítica, neuropatía, opacidad corneal y aumento del riesgo cardiovascular.

Objetivos: Describir las manifestaciones clínicas y el estudio genético de un déficit familiar de HDL causado por enfermedad de Tangier.

Métodos: Secuenciación del gen ABCA1.

Resultados: Paciente de 12 años con déficit de hormona de crecimiento, y su hermana con estomatocitosis, que presentan un déficit de HDL, cuyos padres presentan niveles bajos de HDL. Se reporta una nueva mutación en el gen ABCA1 (c.2193delG) en el caso índice y en su hermana en homocigosis, y en sus padres en heterocigosis.

Conclusiones: Este estudio revela una nueva mutación genética en ABCA1 no descrita previamente.

Palabras clave: *ATP-binding cassette transporter (ABCA1), Apolipoproteína, Enfermedad de Tangier, Déficit de lipoproteínas de baja densidad, Hipoalfalipoproteinemia, Analfalipoproteinemia*

Introduction

Hypocholesterolemia due to abnormal low levels of high-density lipoprotein (HDL) (under 10 mg/dl) is an uncommon disease and its etiology can be primary or secondary, including hypertriglyceridemia, liver failure, monoclonal gammopathy and iatrogenic. The first is caused by three well known genetic disorders, which are the apolipoprotein A-I (ApoA-I) deficiency, Tangier Disease (TD) and lecithin cholesterol acyltransferase (LCAT) deficiency [1].

TD is an autosomal recessive genetic disorder caused by a mutation in the adenosine triphosphate (ATP) binding cassette transporter (ABCA1), which function consists on regulating the secretion of free cholesterol and cellular phospholipids to an extracellular acceptor (Apo A-I) in order to form HDL. TD is characterized by abnormal low values for HDL with a decrease of low density lipoprotein (LDL) to the half, Apo A-I levels below 10 mg/dL and normal or increased triglycerides levels. The clinical features of TD are hepatosplenomegaly, large yellow-orange tonsils or history of tonsillectomy, hemolytic anemia, neuropathy, mild corneal opacity and an increased prevalence of coronary heart disease (CHD) or stroke [2, 3].

We present a family with HDL deficiency caused by TD with a novel gene mutation.

Case Report

The index case was a 12 year old Moroccan boy visited at the pediatric endocrinology consultation for short stature with no other relevant medical history. The family consisted of parents with heights of 173 cm (-0.67 SD) for the father, 150 cm (-2.34 SD) for the mother and 4 siblings with normal growth. Consanguinity was ruled out. Physical examination noticed short stature of 136.7 cm (-2.35 SD) with a normal weight of 31 kg (-1.62 SD) and body mass index (BMI) of 16.66 (-1.0 SD). The cardiorespiratory and gastrointestinal functions were normal and there was no goiter. Pubertal development was: Tanner pubertal stage of genitalia 2 (G2) and pubic hair 2 (P2) with a testicular volume of 12 mL.

A blood analysis was performed showing normal values for blood cell count, renal function, transaminases, proteins, thyroid function, endomysial antibodies and immunoglobulin A (IgA).

However, abnormal low values within the lipid profile were found including a total cholesterol level of 46 mg/dL (normal: 121-216), HDL levels below 1 mg/dl (normal: 35-59) and LDL levels of 33 mg/dl (normal: 50-140). Triglyceride levels were found normal at 81 mg/dl (normal: 35-148). Several hormone levels were also tested showing the following results: Insulin-like Growth Factor 1 (IGF-1) 146 ng/mL (normal: 160-332 for a Tanner II), Insulin-like Growth Factor Binding Protein 3 (IGFBP3) 3.84 µg/mL (normal: 1.53-5.11), Testosterone 165 ng/dl (normal: 262-1593), Sex Hormone Binding Globulin (SHBG) 112 nM (normal: 13-71) and Free Androgen Index 12% (normal: 35-140). A wrist radiography showed a slight bone maturation delay with an age of 11 years and a half. The diagnostic approach was of short stature and hypocholesterolemia. The study of short stature revealed a growth hormone (GH) deficiency (propranolol-exercise and L-Dopa tests with GH peak levels of 3.9 and 2.6 ng/mL, respectively). Consequently, treatment with GH was started with good response. This was maintained for 3 years. The nuclear magnetic resonance (NMR) of the pituitary-hypothalamic area was normal.

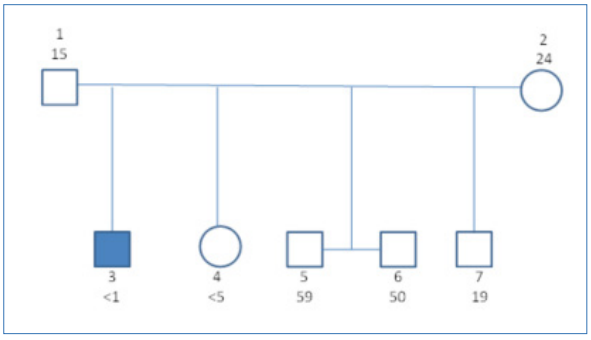

Figure 1. HDL study of all family members (numbered from 1 to 7 in order of age) with the value of HDL (mg/dL) included below. Black figure corresponds to the index case. First row indicates the case number and second row the serum HDL concentrations (see Table 1).

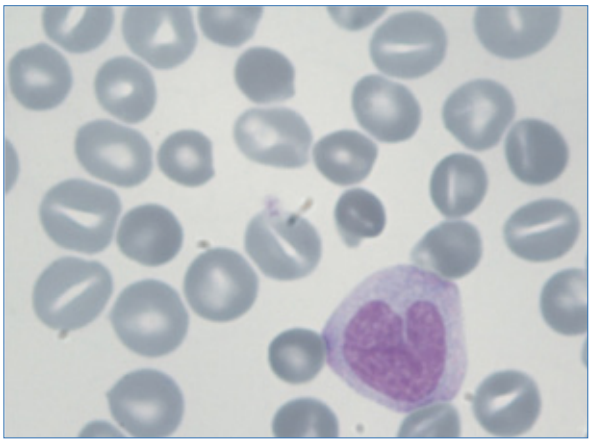

Figure 2. Peripheral blood smear test of the sister where stomatocytes can be observed.

Hypocholesterolemia was confirmed by new tests showing total cholesterol levels of 42 and 30 mg/dL (121-216) and the main apolipoprotein levels showed low values highlighting Apo A-I levels below10 mg/dL (115-190) and an apolipoprotein B (Apo B) of 58 mg/dL (70-160).

The rest of the family was also considered for further study. The 15 year-old sister presented hemolytic anemia and splenomegaly due to stomatocytosis, as well as an HDL deficiency (<5 mg/dL). The father, the mother and a 10 year-old brother presented low HDL values (15, 24 and 19 mg/dL, respectively), whereas the two 14 year-old twin brothers had normal lipid profiles (Table 1 and Figure 1). The Apo A-I levels were very low in the sister (<11 mg/dL), slightly low in the father and the 10 years old brother (both with 92 mg/dL) and normal in the rest of the family. From the standpoint of hematology, the index case and his sister presented hemolysis indicative parameters and the latter was accompanied by anemia and stomatocytosis (Figure 2). The study of erythrocyte morphology in peripheral blood revealed the presence of some stomatocytes (<5%) in the mother and anisocytosis in the index case and the 10 year-old brother (Table 1).

An exploration of tendon reflex and sensitivity levels was carried out to the whole family which did not show any deficiency. The electromyogram tests carried out to the index patient, his sister and the parents were also normal. An ophthalmological examination was also performed to the index case showing no alterations.

Bearing in mind those findings, a genetic severe deficiency of HDL was suspected, the causes of which are currently described in Apo A-I deficiency, Tangier Disease (ABCA1) and LCAT deficiency. The biochemical parameters pointed towards a TD and a study of ABCA1 gene in the index patient was performed (Table 2).

The complete ABCA1 coding regions, splicing boundaries and part of the promoter region were analysed. This genetic analysis revealed a novel mutation in exon 16 of the ABCA1 transporter, c.2193delG in homozygosis, which has not been previously described. The mutation consisted of a base deletion which would alter the reading frame of amino acid 731 and an early stop codon at position 735 leading to a shorter protein that could not trigger its function properly and therefore originating the disease.

This mutation was analysed in other family members observing the same mutation in homozygosis in the sister and in heterozygosis in both parents. These genetic studies were not conducted to the rest of the family members.

Table 1. Lipid profile, apoprotein levels, hematological parameters and age of the family members at the time of determination.

Case	Family member	Age (years)	Mutation	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Apo A-I (mg/dl)	Apo B (mg/dl)	Peripheral blood smear
1	Father	52	hZ	159	44	**15***	**82***	**92***	**63***	Normal
2	Mother	45	hZ	135	75	**24***	98	128	81	Stomatocytosis<5%
3	Index case	12	HZ	**46***	81	**<1****	**33***	**<10****	**58***	Anysocitosis
4	Sister	15	HZ	**20***	92	**<5****	**-**	**<11****	**37***	Stomatocytosis 40%
5	Twin Brother	14	-	139	33	59	66	152	**38***	Normal
6	Twin Brother	14	-	123	31	50	67	138	**40***	Normal
7	Brother	10	**-**	**80***	39	**19***	**-**	**92***	**41***	Anisocytosis

TC (Total cholesterol); TG (triglycerides); hZ (Heterozygote); HZ (Homozygous).

Table 2. Characteristics of severe deficiencies in HDL and Apo A-I.

Disease	ApoA-I (mg/dl)	LDL (mg/dL)	Triglycerides (mg/dL)	Clinical features	Early cardiovascular disease risk
Apo A-I gene mutation	<1	Normal	Normal	Xanthomas Corneal opacity + Steatorrhea	Yes +++
ABCA 1 gene mutation (TD)	<10	Decrease of 50%	Normal-slightly increased	Yellow-orange tonsils Neuropathy Hepatosplenomegaly Corneal opacity	Yes +
LCAT deficiency	<40	Decrease of 50%	Normal-slighty increased	Corneal opacity +++ Anemia Splenomegaly Renal insufficiency	?

Discussion

Tangier Disease was first identified in 1961 in two brothers aged 5 and 6 in the Tangier island (United States of America (USA) whose tonsils had been removed because of their yellow-orange colour due to the accumulation of cholesterol in the macrophages. Moreover, abnormal low values for HDL and splenomegaly were also found as well as decreased levels of HDL in their parents.

Later on, TD has been reported worldwide. Since its discovery it has been diagnosed in around 100 patients. Its clinical features are due to the accumulation of cholesterol in various tissues producing yellow-orange enlarged tonsils (pathognomonic) or a history of tonsillectomy. In addition, a low cholesterol content in the cell membrane of red cells is present which causes stomatocytosis [4] and hemolytic anemia. Other features are hepatosplenomegaly, gastrointestinal disorders, transient or permanent peripheral neuropathy in 50% of cases (some authors recommend including lipid profile in the study of the demyelinating neuropathy) [5], mild corneal opacity and an increased risk of developing premature CHD (around 30% of Tangier disease cases) [6]. Kocen *et al.* [7] discussed that whilst the characteristic pharyngeal appearance had been the main feature for children, for adolescents there was a tendency to present relapsing peripheral neuropathy and adults presented hyperesplenism or precocious coronary artery disease. It has been suggested that homozygotes are at increased risk of CHD in the early 50s or 60s but not earlier because LDL levels decrease approximately to the half by then.

The index case this study focused on presented yellow-orange tonsils and hemolytic anemia and his sister presented hemolytic anemia with hepatosplenomegaly. There has been no reported case of ABCA1 mutation and GH deficiency so far.

In recent years, a relationship between the genetic variants in ABCA1, neurodegenerative disease and Alzheimer disease has been described [8], as well as with recurrent brain hemorrhage caused by amyloidosis and participation in the proliferation and cellular immunity [9].

The lipid disorders are typical in TD, including very low levels of HDL and Apo A-I (on both < 10 mg/dL), moderate to low LDL and Apo B levels and normal or elevated triglycerides. These disorders are caused by a defect in the ABCA1 which disrupts the outflow of cholesterol and consequently causes its accumulation in tissues and the formation of a few small HDL particles, leading to an increase in the renal clearance. These findings have only been described in homozygous. Also, a study of heterozygotes has revealed a 50% of cholesterol output reduction, showing only HDL and Apo A-I low levels, without clinical features. All previously mentioned traits were observed in the index case and his sister (who were homozygotes) and in both parents and a brother (who were all heterozygotes). The heterozygotes members of this family, although asymptomatic, had abnormal erythrocyte forms, which suggested that a decrease in HDL levels could also affect the physical properties of the cell membrane.

A differential diagnosis was made with the other two entities that produce severe hereditary HDL deficiency. Firstly, when there is a genetic deficiency of Apo A-I a lack of synthesis of Apo A-I (< 1 mg/dL) is present due to a mutation in the gene that regulates its production. This results in a severe deficiency of

HDL. However, this does not affect the LDL production. The primary symptom is the occurrence of premature cardiovascular disease. They may also show xanthomas and moderate corneal opacity. Secondly, LCAT deficiency must be also taken into consideration. This enzyme is responsible for the transformation of free cholesterol into its esterified form. Its deficiency produces a decrease in the synthesis of both LDL and HDL but with an Apo A-I not as low as seen in TD (< 40 mg/dL). The main clinical feature in this case is corneal opacity which has given name to the disease as "Fish eyes disease" (Table 2).

The index case focus of this study presented low levels of Apo A-I (< 10 mg/dL) and approximately half of average levels for LDL which directed the researchers towards a diagnosis of TD.

The first ABCA1 gene mutations in patients with TD homozygous was firstly described in 1999. Currently, the Human Genome Mutation Database describes more than 100 mutations responsible for Tangier disease or HDL deficiency, being more than 45 responsible for Tangier. ABCA1 gene involves 50 exons and codes for a 2261 aminoacid protein. Mutations are mostly missense (although splicing, small and large insertions or deletions have also been described) and are distributed along the entire gene [10]. In our case study, the researchers identified a novel mutation of ABCA1 transporter in c.2193delG in the index case and in his homozygous sister. It corresponds with subjects with low levels of HDL (<10 mg/dL) and symptomatic with features of yellow-orange tonsils and hemolytic anemia with hepatosplenomegaly, respectively. The mutation was also seen in both parents in heterozygous form; i.e. showing only lower levels of HDL without the clinical features described for ABCA1 mutations.

To date there is no specific treatment for TD. Old and recently designed drugs which increase HDL levels have been proven to be ineffective for treating Tangier patients. An effective therapeutic strategy should aim to selectively increase the mature HDL concentration levels in order to restore cholesterol efflux. Additionally, recently designed drugs such as Cholesterol Ester Transfer Protein (CETP) inhibitors and reconstituted forms of HDL might also be considered for treating these cases until the development of gene therapy takes place [11].

Conclusion

This case report reveals a novel mutation in ABCA1 gene identified in two siblings with TD in homozygous form which affected mainly the tonsil and the hematology system. The index case presented a GH deficiency wich was probably unrelated to the ABCA1 gene mutation. It also identifies this mutation in their parents in heterozygous form. The therapeutic strategy considered as treatment in this case has identified the need for development of gene therapy in the future in order to effectively treat this type of patients.

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