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Diagnostic and therapeutic particularities in a case of familial hypercholesterolemia secundary to a new mutation in the APOB gene

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ABSTRACT

Throughout the hereby article I will detail the case of a five-years old patient diagnosed with a new form of familial hypercholesterolemia. This way we wish to highlight the importance of swift diagnosis of all forms of familial dyslipidaemia. A particularity of this pathology is the long amount of time when no symptoms are present, thus creating the false impression that it is a benign, and very well tolerated condition.
Currently the incidence of familial dyslipidaemia is continuously increasing, posing a real public health issue, both due to the complications it displays, and the degree of disability it produces to the patients. That is why it is extremely important that the lipidic profile be included in the package of regular laboratory tests of the paediatric practice.

Keywords: dyslipidaemia, hypercholesterolemia, precocious atherosclerosis

INTRODUCTION

The metabolism of lipids is formed of catabolism and anabolism, found on a permanent balance. Ingested lipids are undergoing a degradation process until they reach the stage of fatty acids, which are then transported to the mitochondria. There they are undergoing a degradation named beta-oxidation, which has as final result acetyl-CoA [1].

Also, lipids may be used for the synthesis of other elements such as cellular membranes or signalling molecules [1].

Lipids are of many types such as cholesterol and triglycerides, but they are also found in the structure of some complex molecules such as lipoproteins.

Elevated serum cholesterol is called hypercholesterolemia and it may be the result of certain genetic anomalies (primary hypercholesterolemia), or secondary to some other diseases such as obesity [2].

This elevation of the cholesterol level has as result the formation of atheroma plaques in the blood vessels, which will lead to ischaemic effects in the organs operated by them, and the elevation of arterial blood pressure [3].

Precocious diagnosis and correct and efficient treatment of this pathology are essential to decreasing morbidity and mortality related to it. This can be done through special diets, medication, or a mixed, diet-medication therapy [4].

METHOD

Throughout the hereby article I will present the case of a family (father and son) diagnosed with a new form of familial hypercholesterolemia. The diagnosis was suspected after a lipidic profile of the child was performed, which highlighted an important hypertriglyceridemia, while a hypercholesterolemia was found on the father. What is interesting

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is the fact that even though the two have the same genetic mutation, the manifestation is not the same for the paediatric and the adult age.

The diagnosis was genetically confirmed by the NGS technique.

MEDICAL CASE

Presenting concerns

We present you the case of a male patient aged 5, and that of his father, aged 46.

The child was born on time, without any complications, without significant pathological history. Following a routine examination a hypertriglyceridemia is discovered (400 mg/dl), with no hypercholesterolemia. Repeated determinations of the lipidic profile confirmed its presence.

No treatment is initiated, and the patient doesn't follow any diet.

The persistence of the anomalies of the lipidic profile determines the parents to also require an examination for a metabolic congenital disease.

The father was not known to have dyslipidemia or any cardiac pathology.

Clinical findings

The clinical examination of the child at the time of the consultation doesn't reveal significant pathological elements. The patient didn't have any xanthomas, nor xanthelasmas, at the time of the examination. No hepatomegaly is detected, and no associated cardiac pathology either. The weight at the time of the diagnosis was 18 kg, the height 110 cm, and IMC of 14.9 kg/m2.

The parents do not describe any intolerance to effort, and neither is there any transit disorder present.

In the case of the father, IMC is of 21.9 kg/m2.

Diagnostic findings and assessment:

A new full lipidic profile is performed for the child (Table 1), which indicated the following anomalies:

TABLE 1. Biochemical results for the pediatric patient

Parameter	Result (mg/dl)	Normal values (mg/dl)
Total cholesterol	170	<200
HDL cholesterol	82	>60
LDL- cholesterol	78.23	<100
VLDL- cholesterol	63.6	<50
Triglycerides	318	<150
Total lipids	790.5	400-800
Apolipoprotein B	0.64 g/l	0.55-1.40
Apolipoprotein A1	1.81 g/l	1.10-2.05

Samples are also collected from the father and an LDL value of 196 mg/dl is found, with total lipids of 874 mg/dl. The full lipidic profile of the father is detailed in Table 3.

TABLE 2. Biochemical results for the adult patient

Parameter	Result (mg/dl)	Normal values (mg/dl)
Total cholesterol	292	<200
Total lipids	874	400-800
Triglycerides	127	<150
LDL- cholesterol	196	<100
HDL cholesterol	80	>60

The suspicion of a primary dyslipidaemia is raised, affecting the metabolism of the triglycerides. It is recommended that the investigations be completed by a genetic test, namely the NGS technical sequencing of a panel of genes involved in the metabolism of lipids.

The APOB gene is known as being involved in the determination of an autosomal dominant form of familial hypercholesterolemia. However, the c.3194A>C mutation is unknown, as it was never found in the human pathology before.

Given all of these, I have decided to perform the same test on the parents as well, the results being presented below.

Thus it can be observed that the father also has a dyslipidaemia with hypercholesterolemia. Therefore, we may conclude that this new mutation is, in fact, a pathogen one, its presence leading to a form of familial hypercholesterolemia. It is, however, interesting to observe that in the case of the patients with this mutation, hypertriglyceridemia apparently disappears at an adult age.

Nevertheless, the particularity of the case consists of the need to reduce the triglyceride intake from the diet, as well, which is not usual with the cases of familial hypercholesterolemia.

Therapeutic focus and assessment

The medical nutritional intervention on the child aimed for the reduction of the range of triglycerides, by limiting the quantity and the type of fat in the daily diet, therefore on September 9th, 2021, their value was of 318 mg/dl, and on September 29th, 2021, the value was of 242 mg/dl.

The fats were restricted from the diet, and the intake of lipids with medium chain of triglycerides was supplemented. The diet was substantially modified, but the compliance was very good, therefore the triglycerides profile improved in a short amount of time. Animal fats, vegetable oils, fried foods, processed meat, intensely processed foods, refined sweets were eliminated from the diet. The quantity of food was calculated in such a way that the fats intake does not exceed 10% of the overall intake of calories, the difference up to 25-30% being supplemented with MCT, there were provided 45-65% of carbohydrates, at least 25g of dietary fibers, minimum 0.9 g/kg of proteins, 1.5 litres of liquids, an optimal intake of minerals and vitamins.

Balancing the diet was done with the help of a special product with a low content of fat – 16% LCT,

Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
APOB	c.3194A>G (p.Gln1065Arg)	heterozygous	Uncertain Significance

FIGURE 1. Genetic results for the pediatric patient

Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
APOB	c.3194A>G (p.Gln1065Arg)	heterozygous	Uncertain Significance

FIGURE 2. Genetic results for the index's father

FIGURE 3. Genetic results for the index's mother

and high MCT – 84%, fully nutritional, of MCT oil and MCT emulsion for caloric balance. After 20 days of diet, the lipidic profile is analysed again, the results being presented in Table 2.

TABLE 3. Results for the index case after diet

Parameter	Result (mg/dl)	Normal values (mg/dl)
Total cholesterol	148	<200
HDL- cholesterol	61	>60
LDL- cholesterol	77.18	<100
Triglycerides	242	<150

In the case of the father, the diet sought to diminish the cholesterol intake to 200 mg/day, and the intake of saturated fat to less than 10% of the total intake of calories.

The diet was calorically and nutritiously balanced, as well as in terms of the intake of dietary fibres (minimum 38 grams/day), fibres deficient upon the nutritional assessment of the dietary journals. These ensure not only a normal intestinal transit, but also an optimal immunity. In addition, it also determines the decrease of the cholesterol range (soluble fibres).

The proteins intake was balanced, of the 52 grams/day (0.8 g/kg), half were proteins from animals, and half were vegetal proteins.

45-65% of carbohydrates were provided, most of them complex, 2.5 – 3 litres of liquids, and an optimum intake of minerals and vitamins.

Follow-up and monitoring

Both the child and the father followed a strict diet. The level of cholesterol was kept within normal range, somatic development not being influenced by the diet. The strict compliance with the diet was sought, the ranges of vitamins and minerals are monitored to prevent deficiencies, anthropometric parameters are monitored as well. A cardiac echography was performed, indicating an ejection fraction of 66.2%, a 1st degree tricuspid regurgitation, as well as a 1st degree pulmonary regurgitation.

After a few months the lipidic profile of the child is performed again, indicating a total cholesterol of 162 mg/dl, an HDL of 72 mg/dl, and total lipids of 741 mg/dl. However, despite the strict diet, the range of the triglycerides remains high (286 mg/dl). Consequently a change in the lifestyle is decided (giving up nocturnal meals, taking on swimming courses).

DISCUSSIONS

Familial hypercholesterolemia is currently the most frequent metabolism congenital disease. Its main particularity is the lack of clinical symptoms during childhood.

The most important complication of this condition is early atherosclerosis. It occurs by deposits of cholesterol crystals and by mobilizing the blood cells (granulocytes, monocytes) which release proinflammatory cytokines, and these will trigger an inflammatory response. These plaques can tear off, which leads to a thrombotic process, resulting, in the end in stopping the blood flow through such a vessel, thus causing an ischaemic process [5].

These ischaemic processes represent the main modality of debut with this disease, the most frequent being AVC and myocardial infarction. A beneficial role in the prevention of the atherosclerosis process seems to be played by the intake of a high dose of ascorbic acid [6].

Another effect of hypercholesterolemia is endothelial disfunction of cerebral arteriolas. This dis-

function occurs through an oxidative stress, generated by the hyperproduction of superoxide. What is interesting is the face that this effect is also displayed when atherosclerosis is not present [7].

The diagnosis of familial hypercholesterolemia (FH) was performed over a period of years, using a series of clinical criteria. Throughout the time, there were a various forms of these criteria, the most used being the one known as "The Dutch Diagnostic Criteria" [8]

TABLE 4. The Dutch Criteria

Criteria		*Score*
Family History	First-degree relative with premature coronary and/or vascular disease (men ≤55 years, women ≤60 years), OR First-degree relative with known LDL-cholesterol ≥95th percentile for age and sex	1
	First-degree relative with tendon xanthomata and/or arcus cornealis, OR Children aged ≤18 years with known LDL-cholesterol ≥95th percentile for age and sex	2
Clinical History	Patient with premature coronary artery disease (age as above)	2
	Patient with premature cerebral or peripheral vascular disease (age as above)	1
Physical Examination	Tendon Xanthomas	6
	Arcus cornealis at age ≤45 years	4
	LDL-C ≥8.5 (330)	8
	LDL-C 6.5-8.4 (250-329)	5
	LDL-C 5.0-6.4 (190-249)	3
	LDL-C 4.0-4.9 (155-189)	1
	DNA Analysis – functional mutation LDLR, APOB and PCSK9	8

Interpreting these criteria is performed as follows. If the result is superior to 8, than the diagnosis is certain. A result between 6 and 8 indicates a probable diagnosis and a result between 3 and 5 indicates a potential diagnostic. However if the result is less than 3, than the diagnostic is improbable.

Another diagnosis scale is the one called ''The Simon-Broome Diagnostic Criteria'' [8] detailed below.

The Simon-Broome Diagnostic Criteria:

1) A diagnosis of explicit FH is made if the cholesterol is higher than 7.5 mmol/L or LDL-cholesterol is above 4.9 mmol/L in adult AND tendon xanthomas in patient or a 1st degree relative (parent, sibling, child), or in a 2nd degree relative (grandparent, uncle, aunt)
A diagnosis can also be explicit if the total cholesterol is higher than 6.7 mmol/L or LDL-cholesterol above 4.0 mmol/L in a child under 16 years of age AND tendon xanthomas in patient or a 1st degree relative (parent, sibling, child), or in a 2nd degree relative (grandparent, uncle, aunt)

Also if a DNA based evidence of a functional LDLR, PCSK9 and APOB mutation is found the diagnosis is also certain.

2) A diagnosis of FH is probable if the total cholesterol is higher than 7.5 mmol/L or LDL-cholesterol above 4.9 mmol/L in adult AND family history of myocardial infarction (MI) before 50 years of age in a 2nd degree relative or below age 60 in a 1st degree relative

Also a probable diagnosis is made if total cholesterol higher than 6.7 mmol/L or LDL-cholesterol above 4.0 mmol/L in a child under 16 years of age AND family history of myocardial infarction (MI) before 50 years of age in a 2nd degree relative or below age 60 in a 1st degree relative

Family history of raised total cholesterol - higher than 7.5 mmol/L in adult 1st or 2nd degree relative or higher than 6.7 mmol/L in a child or sibling aged under 16 years can also be used to establish a probable diagnosis.

Nowadays these criteria are out of date, the diagnosis being set by NGS sequencing of genes involved in the metabolism of cholesterol. The main reason is the fact that these criteria cannot differentiate between real and fake familial hypercholesterolemia.

Familial hypercholesterolemia is a disease with genetic sublayer. The genes involved in its onset are LDLR, APOB, and PCSK9. Its incidence is an increased one, in Europe being calculated to one case every 500 births. Its spreading is dominant autosomal [9].

However, there is the possibility of the occurrence of hypercholesterolemia with recessive autosomal spreading. The genes responsible for the onset of this pathological entity are LDLRAP1, ABCG5, and ABCG8. The mutation occurring in these genes trigger either homozygous hypercholesterolemia (LDLRAP1), or a form of sitosterolaemia (ABCG5, ABCG8), and the spreading is recessive autosomal [10]. Given that these diseases do not follow the dominant autosomal spreading, they cannot be considered to be familial hypercholesterolemia, being known as homozygous hypercholesterolemia or fake familial hypercholesterolemia. However, the clinical picture is identic to that of FH, that is why the Dutch and Broom criteria are no longer deemed sufficient to determine the diagnosis, as they are used for reference only.

The treatment of hypercholesterolemia is the same for the homozygous types and for the familial forms (heterozygous). Diet is its central part. Its objective is decreasing the level of LDL cholesterol. This can also be obtained by increasing the number of cellular receptors for LDL, and by decreasing the hepatic synthesis of cholesterol. Also, the daily intake of fat must not exceed 10% of the intake of calories [11].

The foods very useful to generate this effect are nuts and soy-based products [12].

An important element of diet is represented by unsaturated fatty acids. The most important, due to the lipid-lowering effect, are linoleic acid, and Omega 3 fatty acids. Although the mechanism by which linoleic acid has a lipid-lowering effect is not very well explained, as far as Omega 3 fatty acids goes it is known that they have the ability to decrease the hepatic synthesis of cholesterol. They also decrease the level of triglycerides, VLDL, and E and B apolipoproteins [13].

Drug treatment is recommended starting from 8 years old. The most used pharmaceutical group is that of statins. They decrease the range of LDL-cholesterol down to 50% in the case of the heterozygous form, and down to 25% in the case of the homozygous one. This decrease may be increased up to 70% if ezetimibe is associated to statins [14].

Lomitapide was recently introduced in the treatment course of familial hypercholesterolemia. It inhibits the protein responsible for the microsomal transport of triglycerides, and it is used as treatment to help the diet [15].

Mipomersen is another new generation medicine which may be used to decrease the range of LDL-cholesterol [16]. Following a study conducted on 142 patients, the efficiency of this drug was assessed on patients suffering from familial hypercholesterolemia. After 104 weeks during which 200 mg/week were administered as subcutaneous injections, a decrease by 28% of the LDL range, by 31% and 12% of APOB and triglycerides range, but also an increase by 10% of HDL was noticed [17].

A molecule called Evolocumab can also be used for the treatment of adults suffering from familial hypercholesterolemia. It is a monoclonal antibody which fixes on a protein called PCSK9 (Proprotein Convertase Subtilisin/Kexin type 9) which it blocks, preventing it from destroying cellular receptors for LDL-cholesterol. Thus, these receptors which remained untouched unite with the LDL molecule and rush its elimination from the blood. A clinical study published in 2020 assessed its efficiency in paediatric practice as well. It included 104 patients aged minimum 10, who received 420 mg doses of Evolocumab as monthly subcutaneous injections. After 24 weeks a decrease by 44.5% of the LDL-cholesterol level was noticed, proving thus the efficiency and the safety of this drug for children as well [18].

Another new generation drug which may be used to treat familial hypercholesterolemia is Alirocumab. This is also a monoclonal antibody which fixes on the PCSK9 protein, just like Evolocumab, as it has a similar mechanism. Following a study conducted on patients suffering from familial hypercholesterolemia, after 24 weeks, a decrease by 45.7% of the LDL range was noticed, comparative to 6.6% in the placebo lot [19].

CONCLUSIONS

Familial hypercholesterolemia is a condition with important incidence amongst the paediatric population. Due to its long term effects, it represents a true public health issue.

Unfortunately, at this time there are no therapeutic solutions for paediatric patients to allow giving up the diet. That is why it is quite difficult to ensure therapeutic compliance on a long term.

Thus, this disease remains an important medical issue, aside from the complications it can trigger in time. Therefore it is very important that performing a lipidic profile on a child becomes a regular practice of paediatric physicians, so intervention on decreasing the LDL-cholesterol range be done swiftly.

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