Atherosclerosis 262 (2017) 71–77

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Timely diagnosis of sitosterolemia by next generation sequencing in two children with severe hypercholesterolemia

Paola Sabrina Buonuomo [a], Lorenzo Iughetti [b], Livia Pisciotta [c], Claudio Rabacchi [d], Francesco Papadia [e], Patrizia Bruzzi [b], Albina Tummolo [e], Andrea Bartuli [a], Claudio Cortese [f], Stefano Bertolini [c, **], Sebastiano Calandra [g, *]

[a] Rare Diseases and Medical Genetics, Bambino Gesù Children Hospital, Rome, Italy
[b] Department of Medical and Surgical Sciences of the Mothers, Children and Adults, Paediatric Unit, University of Modena & Reggio Emilia, Modena, Italy
[c] Department of Internal Medicine, University of Genova, Genova, Italy
[d] Department of Life Sciences, University of Modena & Reggio Emilia, Modena, Italy
[e] University Pediatric Hospital Giovanni XXIII, O.U. Metabolic and Genetic Diseases, Bari, Italy
[f] Department of Experimental Medicine and Surgery, University of Rome "Tor Vergata", Rome, Italy
[g] Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy

ARTICLE INFO

Article history:
Received 20 March 2017
Received in revised form
14 April 2017
Accepted 3 May 2017
Available online 4 May 2017

Keywords:
Hypercholesterolemia
ABCG5 gene
ABCG8 gene
Plasma phytosterols
Next generation sequencing

ABSTRACT

Background and aims: Severe hypercholesterolemia associated or not with xanthomas in a child may suggest the diagnosis of homozygous autosomal dominant hypercholesterolemia (ADH), autosomal recessive hypercholesterolemia (ARH) or sitosterolemia, depending on the transmission of hypercholesterolemia in the patient's family. Sitosterolemia is a recessive disorder characterized by high plasma levels of cholesterol and plant sterols due to mutations in the *ABCG5* or the *ABCG8* gene, leading to a loss of function of the ATP-binding cassette (ABC) heterodimer transporter G5-G8.

Methods: We aimed to perform the molecular characterization of two children with severe primary hypercholesterolemia.

Results: Case #1 was a 2 year-old girl with high LDL-cholesterol (690 mg/dl) and tuberous and interdigitous xanthomas. Case #2 was a 7 year-old boy with elevated LDL-C (432 mg/dl) but no xanthomas. In both cases, at least one parent had elevated LDL-cholesterol levels. For the molecular diagnosis, we applied targeted next generation sequencing (NGS), which unexpectedly revealed that both patients were compound heterozygous for nonsense mutations: Case #1 in *ABCG5* gene [p.(Gln251*)/p.(Arg446*)] and Case #2 in *ABCG8* gene [p.(Ser107*)/p.(Trp361*)]. Both children had extremely high serum sitosterol and campesterol levels, thus confirming the diagnosis of sisterolemia. A low-fat/low-sterol diet was promptly adopted with and without the addition of ezetimibe for Case #1 and Case #2, respectively. In both patients, serum total and LDL-cholesterol decreased dramatically in two months and progressively normalized.

Conclusions: Targeted NGS allows the rapid diagnosis of sitosterolemia in children with severe hypercholesterolemia, even though their family history does not unequivocally suggest a recessive transmission of hypercholesterolemia. A timely diagnosis is crucial to avoid delays in treatment.

1. Introduction

The presence of xanthomas and severe hypercholesterolemia (total cholesterol ≥500 mg/dl) in an infant or in a young child promptly suggests the suspicion that the patient might have homozygous familial hypercholesterolemia (autosomal dominant hypercholesterolemia, ADH). If the child's parents have hypercholesterolemia, the diagnosis of homozygous ADH is highly probable

* Corresponding author. Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Via Campi 287, I-41125 Modena, Italy.
** Corresponding author. Department of Internal Medicine, University of Genova, Viale Benedetto XV, no.6, I-16132 Genova, Italy.
E-mail addresses: stefbert@unige.it, stefbert42@gmail.com (S. Bertolini), sebcal@unimore.it (S. Calandra).

http://dx.doi.org/10.1016/j.atherosclerosis.2017.05.002
0021-9150/

[1]. This disorder is due in ~95% of cases to homozygous/compound heterozygous pathogenic variants in the *LDLR* gene [2–4]. If the child's parents are normocholesterolemic, suggesting a recessive transmission of hypercholesterolemia, other disorders should be considered such as autosomal recessive hypercholesterolemia (ARH) [5–7], sitosterolemia [8,9] or childhood/adult-onset lysosomal acid lipase deficiency (LALD) [10–13]. ARH is due to homozygous/compound heterozygous pathogenic variants in the *LDLRAP1* gene encoding the LDLR adaptor protein 1, which is required for the internalization of LDLR/LDL complex into the hepatocytes [5,14]. Sitosterolemia is caused by homozygous or compound heterozygous pathogenic variants in one of the two genes (*ABCG5* or *ABCG8*) located on chromosome 2p21; these encode the proteins ABCG5 (stereolin-1) and ABCG8 (sterolin-2), two ATP-binding cassette transporters which act, in heterodimer form, to pump phytosterols and cholesterol from enterocytes, in the proximal small intestine, into the gut lumen and from hepatocytes into the bile ducts [9,15]. LALD is due to pathogenic variants in the *LIPA* gene encoding the enzyme lysosomal acid lipase, which hydrolyses cholesteryl esters and triglycerides of plasma apoB containing lipoproteins internalized into the lysosomes [11].

The differential diagnosis among these four rare disorders is crucial as hypercholesterolemia in sitosterolemia is highly responsive to a diet low in saturated fat and cholesterol [9,16,17], while this diet has a negligible effect on plasma cholesterol of ADH, ARH or LALD patients. On the other hand, hypercholesterolemia in LALD can be greatly reduced by the recombinant LAL enzyme replacement therapy [18]. Therefore, identifying sitosterolemia as a separate entity from ADH, ARH and LALD is crucial as the delay between the onset of symptoms and the correct diagnosis may be extremely long and patients initially misdiagnosed may receive inappropriate treatment [19].

In the present study, we describe two hypercholesterolemic children with and without cutaneous xanthomas originally suspected to have homozygous ADH, in whom the parallel sequencing of hypercholesterolemia causing genes unexpectedly revealed the presence of nonsense mutations in the *ABCG5* and *ABCG8* genes, respectively, leading to a timely diagnosis of sitosterolemia.

2. Patients and methods

2.1. Case #1

A 2 year-old girl was referred to the Rare Diseases Unit of a hospital for the presence of cutaneous xanthomatosis associated with high serum cholesterol levels (total cholesterol 770 mg/dL, LDL cholesterol 690 mg/dL) (Table 1). The child was the only daughter of a 44 year-old Italian male and a 33 year-old Chinese female. Her development had been within the normal range; at 28 months of age the auxological parameters were the following: height 85 cm, weight 10 kg, BMI 13.8 kg/m^2 (BMI 3rd-5th percentile). There was no family history of sudden death, premature atherosclerosis or severe hypercholesterolemia; the proband's father had hypercholesterolemia, while the mother had a borderline cholesterol value for ethnicity (Table 1).

The physical examination revealed yellow plaques on the fold of the buttock, the posterior region of the right thigh, left elbow, knee, heel and intergluteal line, which had been noticed starting from 16 months of age (Fig. 1; A-C). Liver and spleen had a normal size; there were no signs of hemolysis, arthralgia and arthritis, or a history of bleeding. Routine biochemical and hematological tests were within the normal range, including liver function tests, erythrocyte count (4.40 $\times$ 10^6/μL), hemoglobin (13.0 g/dL), hematocrit (38.5%), MCH (29.4 pg/cell), MCHC (33.7 g/dL), MCV (87.4 fL), platelet count (208 $\times$ 10^3/μL) and platelet volume (9.8 fL). Two-dimensional echocardiographic examination did not reveal structural abnormalities and showed normal flow patterns. Abdominal ultrasound examination did not reveal hepatosplenomegaly or liver steatosis.

The child had been exclusively breast-fed for the first 6 months of life and then slowly weaned off breast milk over the following 10 months. From the age of 16 months, she had been fed with solid foods based on traditional Chinese diet, rich in soya, green vegetables and fish. The composition of a standard meal is given in Supplemental material. Taking into account this diet, it can be assumed that the daily intake of cholesterol and phytosterols was about 180–200 mg/day and 140–180 mg/day, respectively (about 19 mg/kg and 16 mg/kg of body weight, respectively).

2.2. Case #2

A 7 year-old Italian boy was referred to the outpatient Paediatric Unit for the incidental finding of severe hypercholesterolemia (total cholesterol 598 mg/dL, HDL cholesterol 46 mg/dL, triglycerides 118 mg/dL) during a routine laboratory check, for a putative development delay (height 115.9 cm, weight 19.4 kg, BMI 14.4 kg/m^2, a value below the 10th percentile of the distribution for Italian boys of the same age) [20]. Physical examination did not reveal xanthomas, xanthelasma, arcus cornealis or hepatosplenomegaly. The abnormal plasma lipoprotein profile at admission is shown in Table 1. Liver enzymes, thyroid hormones, creatinine levels and urine analysis were in the normal range. Blood cell parameters, including erythrocyte count (4.46 $\times$ 10^6/μL), hemoglobin (11.6 g/dL), platelets count (315 $\times$ 10^3/μL) and platelet volume (9.2 fL) were in the normal range. ECG and echocardiography did not reveal abnormalities.

The proband's parents were apparently unrelated. The proband's 46 year-old father had a family history of coronary artery disease and presented with a moderate hypercholesterolemia, while the proband's 42 year-old mother showed a normal lipoprotein profile (Table 1).

The study protocol was approved by the Institutional Ethics Committees and informed consent was obtained from the probands' parents.

Table 1
Plasma lipoprotein profile detected in the two patients (Case#1 and Case#2) and in their parents.

	Case 1 (female)			Case 2 (male)		
Age (years)	Patient (2 y)	Father (44 y)	Mother (33 y)	Patient (7 y)	Father (46 y)	Mother (42 y)
TC[a]	770	278	199	524	242	194
LDL-C[a]	690	198	112	432	160	103
HDL-C[a]	40	55	65	52	63	74
Triglycerides	202	73	72	55	91	71
ApoA-I	110	124	148	114	157	175
ApoB	394	159	90	253	108	85

[a] The values include cholesterol and plant sterols; lipid and apolipoprotein plasma concentrations are reported in mg/dl.