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Variable phenotypic expression of chylomicron retention disease in a kindred carrying a mutation of the Sara2 gene

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Abstract

Chylomicron retention disease is a recessive inherited disorder characterized by fat malabsorption and steatorrhea and is associated with failure to thrive in infancy. We describe a kindred carrying a mutation of Sara2 gene causing a chylomicron retention phenotype. The proband was a 5-month-old baby, born of consanguineous, apparently healthy parents from Morocco, with failure to thrive. There was a large quantity of fats in feces and malabsorption of fat-soluble vitamins. Intestinal biopsies showed a diffused enterocyte vacuolization with large cytosolic lipid droplets. Chylomicron retention disease or Anderson disease was hypothesized, and the Sara2 gene was analyzed by direct sequencing. Analysis of the Sara2 gene in the proband identified a 2-nucleotide homozygous deletion in exon 3 leading to a premature stop codon (c.75-76 del TG-L28fsX34). The father was heterozygous for the same mutation, whereas the proband's mother was homozygous, suggesting a variable phenotypic expression of the molecular defect. More studies are needed to understand the reasons of the phenotypic variability of the same molecular defect in the same family.

1. Introduction

Chylomicron retention disease (CMRD; Online Mendelian Inheritance in Man [OMIM] 246700) is a recessive inherited disorder characterized by fat malabsorption and steatorrhea and is associated with failure to thrive in infancy [1,2]. It is also characterized by deficiency in fat-soluble vitamins, low blood cholesterol, and a selective absence of chylomicrons in blood. Affected individuals accumulate chylomicron-like particles in the enterocytes, with intestinal histology showing a diffuse enterocyte vacuolization with large cytosolic lipid droplets.

Chylomicron retention disease shares common clinical features with Anderson disease (OMIM 607689) [3,4] and the neuromuscular disorder Marinesco-Sjogren syndrome (OMIM 607692) [5].

The molecular basis of these 3 different severe disorders of fat malabsorption resides in mutations of *SARA2* gene that encodes Sar1b protein. This protein is involved in the vesicular COPII-dependent transport of proteins from the endoplasmic reticulum (ER) to the Golgi apparatus [6-8].

Abetalipoproteinemia (OMIM 200100) and familial homozygous hypobetalipoproteinemia (OMIM 107730), 2 other inherited lipoprotein-deficiency diseases associated with fat malabsorption, could also be considered as differential diagnoses.

Here we report the clinical and genetic analysis of a Moroccan family, carrier of a mutation in the Sara2 gene, characterized by a variable phenotypic expression in 2 subjects homozygous for the same mutation.

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2. Case report

A 5-month-old female infant was referred to the Pediatric Gastroenterology Unit because of failure to thrive, diarrhea, and abdominal swelling. She was born in Italy of Moroccan consanguineous, apparently healthy parents (first cousins) after a 32-week + 5-day pregnancy. At birth, she weighed 2060 g (75th centile) and had an Apgar score of 9/9. She was never breast-fed. She weighed 4080 g (<third centile) at 5 months.

Four paternal uncles and 3 paternal aunts were in good health, as was 1 maternal uncle and 6 maternal aunts. In the paternal line, there were 9 first cousins (4 male [M]/5 female [F]), one of them (a male) with poor growth. In the maternal line, there were 8 first cousins (4 M/4 F), 2 (1 M/1 F) with poor growth.

Laboratory tests showed mild neutropenia (3.870/μL), altered liver function (aspartate aminotransferase, 95 U/L; alanine aminotransferase, 179 U/L; γ-glutamyl transferase, 152 U/L) with normal bilirubin values, and an altered lipid profile (total cholesterol [TC], 82 mg/dL; high-density lipoprotein cholesterol, 30 mg/dL; triglyceride [TG], 89 mg/dL; apolipoprotein [apo] A-1, 79 mg/dL; apo B, 47 mg/dL).

A 72-hour fecal fat collection revealed an abnormally increased fat loss, and plasma vitamin E concentration was found to be reduced to as low as 1 μg/mL (reference range values = 5-16 μg/mL).

A preliminary diagnosis of exocrine pancreatic insufficiency was hypothesized. Further investigation ruled out cystic fibrosis (sweat test and genetic test results were negative) and Shwachman-Diamond syndrome (white blood count, fecal elastase, and serum immunoreactive trypsin were repeatedly normal). No skeletal changes were observed. Clinical examination did not show muscle weakness, and creatine kinase and lactate dehydrogenase levels were in the reference range in different occasions. Endoscopy was performed at 7 months and repeated at 9 months.

Therefore, the clinical picture, the laboratory profile, and the histologic features indicated an inherited disorder of fat malabsorption.

She started a high-calorie (131 kcal/kg), low-lipid diet (28.5%) with an elevated median-chain TG to long-chain TG ratio at 8 months to cope with poor growth and steatorrhea.

In the first 3 months after starting the diet, lipid absorption and growth centile in the patient improved from less than the third centile of weight (5200 g) and height to reach the third in weight (7000 g) and the 10th in height. These centiles remained unchanged throughout the 14 months of follow-up, also because of poor family compliance. A more recent clinical evaluation carried out at the age of 4 years still confirmed poor growth with a body mass index of 15.9 (Table 1).

At 6 and 12 months after having started the diet, 2 repeated 72-hour fecal fat collections showed no steatorrhea.

Follow-up showed a modest slowing of neuromotor development, with no neurologic or cognitive difficulties. Hematochemical parameters showed persistently low levels of hemoglobin and elevated aspartate aminotransferase and alanine aminotransferase, which were 2 to 2.5 times higher than normal values, with an echographic pattern of steatosis. Despite administration of liposoluble vitamins, vitamin E was persistently low (Table 1).

2.1. Polymerase chain reaction amplification of genomic DNA and mutation detection

Blood samples were available from the parents and the affected child and were drawn while fasting. The informed consent for genetic analysis was obtained.

Genomic DNA was purified from blood samples using a commercial kit (Wizard Genomic DNA Purification Kit; Promega, Milan, Italy) and stored in Tris-EDTA buffer. The Sara2 gene was polymerase chain reaction (PCR) amplified using the primers previously described [6]. The PCR products were then purified using the Wizard PCR Preps DNA Purification System kit (Promega, Madison, WI). Direct sequencing of the amplified and purified amplicons was performed using a Cycle Sequencing Termination kit (ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kits, Version 1.1) in an ABI Prism 310 apparatus (Applied Biosystems, Foster City, CA).

3. Results

Clinical and biochemical data of the subjects studied are summarized in Table 1. The proband shows low levels of lipids

Table 1
Clinical and biochemical data of the subjects studied

Subject	Age (y)	Sex	BMI (kg/m^2)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	Apo B (mg/dL)	Apo A-I (mg/dL)	Vitamin E[a] (μg/mL)	25-OH[a] vitamin D (ng/mL)	1 25 (OH)2[a] vitamin D (pg/mL)
I-1	40	M	27.2	250	265	48	149	129	146	NA	NA	NA
I-2	21	F	31.6	189	138	76	85	85	185	10.8	NA	NA
II-1	4	F	15.9	56	68	14	28	39	40	0.5	66.4	5.1

BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C; low-density lipoprotein cholesterol; NA, not available.

[a] Vitamin E reference values: 5 to 16 μg/mL; 25-OH vitamin D reference values: 9.0 to 37.6 ng/mL; 1 25 (OH)2 vitamin D reference values: 19.9 to 67.0 pg/mL.

and low levels of serum vitamin E and D; vitamin K–dependent blood clotting studies show a slight reduction of factor II and factor X (64.0% and 53.1%, respectively), whereas factor VII and factor IX were in the lower limit of the reference range (77.2% and 61.5%, respectively). Her mother is obese with a normal lipid profile and normal serum vitamin E levels. Indeed, the TC and TG levels of the mother's proband have shown moderate fluctuation with values as low as 132 and 90 mg/dL, respectively. The proband's father is overweight and has a moderate hypertriglyceridemia and hypercholesterolemia.

Biopsy of the small intestine of the proband showed normal villi and crypt zone with no increase in intraepithelial lymphocytes. A diffused enterocyte vacuolization with large cytosolic lipid droplets was evident in both biopsies, confirmed by electronic microscopy (Fig. 1).

Fat absorption was assessed during follow-up by means of quantitative determination of fecal fat collected over 72 hours and monitoring lipid intake with a 3-day dietary recall both in the proband and in her mother. Coefficient of fat absorption was calculated as follows:

$$\frac{\text{Dietary fat g/d} - \text{fecal fat g/d}}{\text{Dietary fat g/d}} \times 100\%$$

Coefficient of fat absorption was normal (98%-99%) both in the proband and in her mother, but fat concentration in the stool of the proband resulted twice that of her mother.

Direct sequence analysis of the whole Sara2 gene in the proband allowed for the identification of a 2-nucleotide homozygous deletion in exon 3 leading to a premature stop codon (c.75-76 del TG-L28fsX34), previously described in a white Canadian pedigree [6]. The father was found to be heterozygous for the same mutation, whereas the mother, despite the absence of any clinical signs of the disease, was found to be homozygous for the mutation (Fig. 2). The direct sequence was replicated twice in 2 blood samples drawn at 2 different times to rule out any mistakes due to specimen collection and/or handling processes.

4. Discussion

Chylomicron retention disease is a rare recessive disorder that seems to occur predominantly in certain geographic areas, such as North Africa and Canada [4].

Subjects with this disorder exhibit the clinical manifestations initially described by Anderson and colleagues [9] that consist of a malabsorption syndrome with steatorrhea and growth retardation. Endoscopy shows a white stippling-like hoar frosting covering the mucosal surface of the small intestine. In the comprehensive study performed by Dannoura et al [4], chylomicron-like particles were observed in membrane-bound compartments, reminiscent of dilated, vesiculated channels of the smooth ER, and in huge membrane-bound compartments.

Recently, Jones et al [6] established that Sar1b was defective in CMRD, Anderson disease, and CMRD–Marinesco-Sjogren syndrome by sequencing *SARA2*, which encodes Sar1b.

Missense mutations of *SARA2* represent the most common cause of CMRD; and these mutations were found in the Sar1 β-sheets region and are predicted to perturb the geometry of the guanosine diphosphate (GDP) and guanosine triphosphate (GTP) binding site of Sar1b.

Only 2 families with CMRD harbored frameshift mutations. One of these occurred on 1 allele in 1 Canadian patient and would be expected to stop the translation of Sar1b 34 amino acids into the protein (c.75-76 del TG-

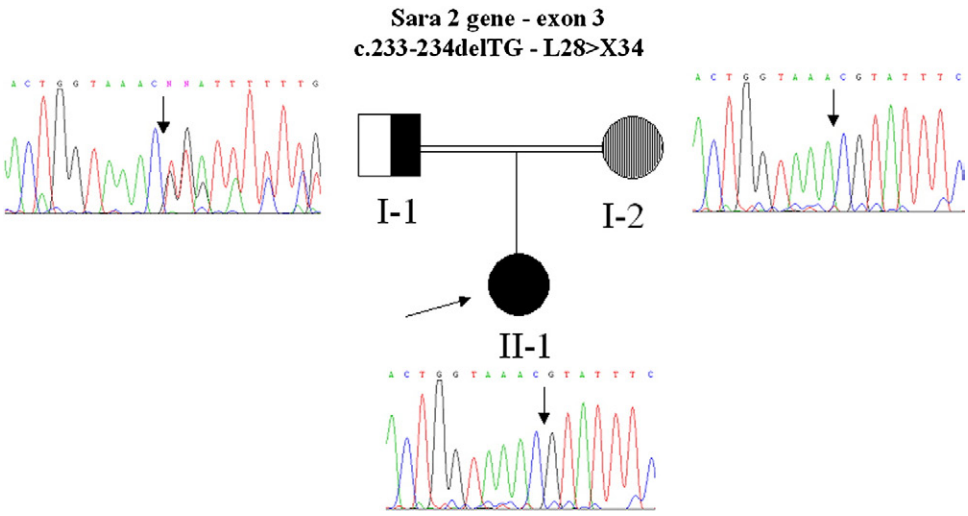

Fig. 1. Pedigree of family with CMRD; plasma lipid concentration and analysis of the Sara 2 gene. Pedigree of family with CMRD. Proband (subject II-1) is indicated with an arrow and full symbol indicating she is clinically affected. Subject I-2 (dashed symbol) is homozygous but clinically diagnosed as non-CMRD. Subject I-1 (half-filled symbol) is heterozygous. The panel also shows the partial sequence of exon 3; the arrow indicates the TG nucleotides deletion causing the frameshift mutation.

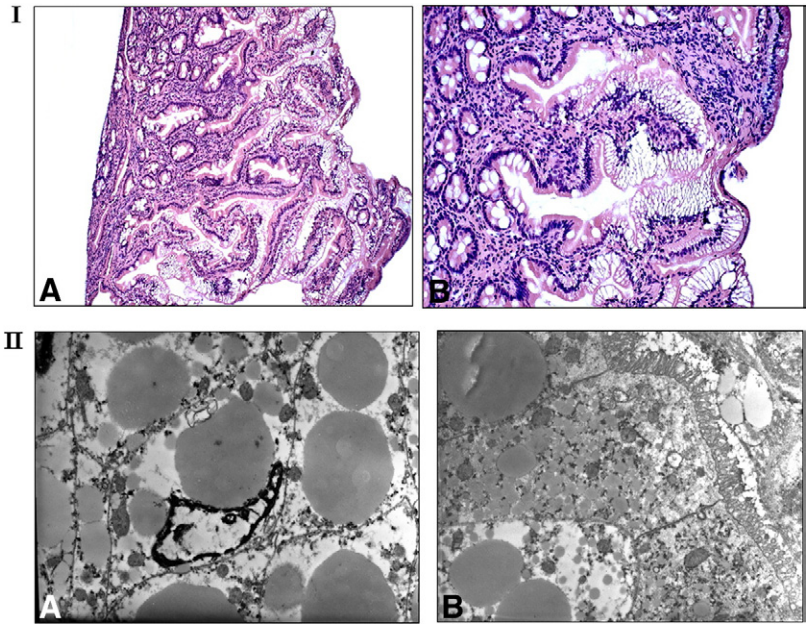

Fig. 2. Jejunal biopsies of the CMRD affected subject. Panel IA and IB, Hematoxylin-eosin staining showing diffuse enterocytes vacuolization with large cytosolic lipid droplets. Panel II, Electronic microscopy. Few basal epithelial cells with unspecific degenerative changes and lipid-containing vacuoles (×5.400) (A). Low magnification of epithelial cells showing a cytoplasmic accumulation of lipid vacuoles and shortening superficial microvilli that are also much sparser; the terminal web is poorly formed (×5.400) (B).

L28fsX34). The second disease allele in this patient carries a missense mutation (D37N) and had been inherited through the paternal line. This patient was the only individual with a compound heterozygote genotype in the cohort of families studied by Jones et al [6].

Herein we describe a case of CMRD in a child harboring the frameshift mutation c.75-76 del TG-L28fsX34 of the Sara2 gene [6]. In our case, the molecular defect gene was present in homozygosis; as expected, the father was heterozygous for the same mutation, whereas, interestingly and unexpectedly, although the mother was found to be homozygous, she was symptom free for CMRD. Her lipid profile was normal with mild fluctuation of TC and TG plasma levels; moreover, she does not show clinical and biochemical signs of malabsorption. She refused to undergo endoscopy and intestinal biopsies, so we lack information on the intestinal mucosa in this subject. In the absence of data on the mother's intestinal biopsy, it is not possible to ascertain whether she is phenotypically normal. In fact, the presence of obesity could affect her lipid phenotype.

As aforementioned, this mutation leads to a truncated short Sar1b protein; and this would suggest an impact on the structural integrity of the protein. However, it is still unknown whether the mutant Sar1b protein retains any affinity for GDP, GTP, or guanosine triphosphatase (GTPase) activity.

Charcosset et al [7] recently well described a group of subjects with various mutations in the *SARA2* gene, and they observed a lack of genotype-phenotype correlation among subjects with Anderson disease carrying different mutations of *SARA2*. These data suggest that Anderson disease might represent a more complex trait rather than a simple autosomal recessive disorder and that modifier genes might be involved in the ER-to-Golgi transport.

Recently it has been shown by Siddiqi [10] that primary hepatocytes use a highly specialized mechanism to transport nascent very low-density lipoprotein from the hepatic ER to the Golgi and that Sar1 is required for effective trafficking of very low-density lipoprotein. Low levels or mild fluctuation of fasting plasma TG observed in both homozygous individuals in the absence of a remarkable steatorrhea could be explained by a failure of this mechanism.

Moreover, it has been demonstrated that a cooperative interaction between COPII proteins and other independent COPII protein complexes in chylomicron transport exists [11] and that mutations and polymorphisms in all these proteins might affect the functionality of this "network" and explain phenotypical differences observed in these and other patients.

The analysis of the polymorphisms of different proteins involved in this pathway may provide a better understanding of variable expression in Anderson disease in subjects carrying the same molecular defect in Sara2 gene and be an interesting way to elucidate lipid trafficking in normal enterocytes.

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