Abetalipoproteinemia in an infant with severe clinical phenotype and a novel mutation

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Abetalipoproteinemia (ABL) is a rare autosomal disorder characterized by extremely low levels of plasma lipids and apolipoprotein B (apoB) with a variable phenotype. Mutations in the *MTP* gene encoding the microsomal triglyceride transfer protein (MTP) cause the disease. A five-month-old boy, born from consanguineous parents, with chronic diarrhea and severe malnutrition had extremely low plasma lipids and apoB levels suggesting the diagnosis of ABL. He was not responsive to treatment with low-fat diet and fat-soluble vitamins and died at 13 months of age with severe malnutrition. Analysis of the *MTP* gene showed that he was homozygous for a two nucleotide deletion in exon 4 (c.398-399delAA) expected to cause a frameshift in the mRNA leading to a premature termination codon. The normolipidemic proband's parents were found to be heterozygous for the mutation. This observation underscores that in some cases, ABL can be extremely severe from early post-natal life and poorly responsive to treatment.

Key words: abetalipoproteinemia, MTP gene, infancy.

Abetalipoproteinemia (ABL, OMIM 200100) is a very rare (<1:100,000) autosomal recessive metabolic disorder that results from mutations in the gene encoding microsomal triglyceride transfer protein (MTP). MTP catalyzes the transfer of lipids onto apolipoprotein B (apoB), the major structural protein component of very low density lipoproteins (VLDL) and chylomicrons in the liver and intestine, respectively[1]. In the absence of MTP, apoB cannot be properly lipidated and undergoes pre-secretory degradation, preventing the assembly of chylomicrons and VLDL. The defective secretion of chylomicrons and VLDL causes lipid accumulation in the cytoplasm of intestinal epithelial cells and hepatocytes[1,2].

Microsomal triglyceride transfer protein deficiency in ABL results in almost undetectable concentrations of plasma apoB-containing lipoproteins- chylomicrons, VLDL, and low-density lipoproteins (LDL)- and exceptionally low plasma concentrations of cholesterol and triglyceride[1,3,4]. Affected patients may express a wide range of clinical features. In early childhood, typical clinical manifestations of ABL are intestinal fat malabsorption and failure to thrive. In later childhood or adolescence, ABL patients may develop ataxic neuropathy and retinopathy[1,3]. The latter clinical manifestations are partly due to deficiency of fat-soluble vitamins, specifically vitamin E, as well as to other factors such as polyunsaturated fatty acid deficiency and oxidative stress[5,6]. Other manifestations of ABL include fatty liver, acanthocytosis and anemia[1,7].

In the present case report, we describe the clinical and biochemical characteristics of a patient of Turkish ancestry with a severe form of hypobetalipoproteinemia who was found to be homozygous for a novel mutation in the *MTP* gene.

Case Report

The proband was a five-month-old boy referred to the hospital because of chronic diarrhea, dehydration and severe malnutrition. He was

the fifth child born from a consanguineous marriage (his parents were first cousins). His eldest brother had died at seven months of age because of diarrhea. The proband was born at 37 weeks of gestation after an uncomplicated pregnancy with a birthweight of 3300 g. He was breastfed from birth and received formula and cow's milk after three months of age. Diarrhea, vomiting and abdominal distention were reported since two months of age when he weighed 5000 g. Physical examination revealed a pale and cachectic baby with a weight of 3180 g and height of 55 cm (both <3rd percentile). He also had oral moniliasis, abdominal distention, and inguinal hernia without any dysmorphic feature. No ophthalmic or neuromuscular abnormalities were found. The laboratory data showed mild anemia (hemoglobin 10.8 g/dl) with a slightly elevated reticulocyte count of 3.6% (normal <2%), while a blood film showed polychromasia and poikilocytosis. The results of coagulation tests, plasma protein assay, electrolytes and sweat chloride test were normal. Serum transaminases were mildly elevated [aspartate aminotransferase-AST: 105 U/L (normal range: 0-40), alanine aminotransferase-ALT: 112 U/L (normal range: 0-33)]. Abdominal ultrasound examination was normal. Blood tests for immunologic deficiency including lymphocyte subsets and immunoglobulins were within normal limits. Tests for food allergy including total immunoglobulin E, epidermal skin tests and milk-specific RAST did not show any apparent evidence for food allergy. Stool specimens for culture and parasite examination, including amoeba, were negative. No steatorrhea was present. He was found to have low levels of serum vitamin E (0.5 mg/dl, normal: 0.8-1.5 mg/dl), vitamin A (17.6 µg/dl, normal: 30-60 µg/dl) and carotene (0.25 µg/ml, normal: 0.91-2.34 µg/ml). The plasma lipid profile demonstrated: total cholesterol (TC) 44 mg/dl, triglycerides (TG) 1 mg/dl, and VLDL-C and LDL-C below detection limits.

Lipoprotein electrophoresis confirmed low beta (6%, normal: 32-58%) and pre-beta (2.4% normal: 9 -37%) lipoprotein levels. The proband's parents were healthy and had normal plasma lipid profile (TC= 193 and 197 mg/dl; TG= 121 and 109 mg/dl; LDL-C= 127 and 113 mg/dl; apoB= 87 and 72 mg/dl in the father and mother, respectively). The clinical and biochemical features of the patient, as well as the normal plasma lipid concentrations of his parents, suggested the clinical diagnosis of ABL. He was treated with high oral doses of fat- soluble vitamins, a medium-chain TG-containing formula and fat restriction. The proband was followed up for three months without any significant weight gain. At eight months of age he started treatment with a fat-free diet (Basic F® formula) supplemented with essential fatty acids. Despite these dietary interventions, he did not gain weight (weight was 3050 g), had mildly elevated transaminases and extremely low levels of plasma lipids. He died during this study at 13 months of age.

The direct sequencing of the *MTP* gene (GenBank accession number NM_000253.2) showed that the proband was homozygous for a two nucleotide deletion in exon 4 (c.398-399delAA) expected to cause a frameshift in the mRNA leading to the insertion of seven novel amino acids and the occurrence of a premature termination codon. The predicted translation product of this abnormal mRNA is a peptide of 139 amino acids (p.V132fsN140X), as opposed to the 894 amino acids of the wild-type protein (GenBank accession number NP_000244.2) (Fig. 1). The proband's parents were found to be heterozygous for the mutation. Informed consent was obtained from all adult subjects and in the case of children from their parents. The study was approved by the Ethics Committees of each participating institution and is in accordance with ethical standards as formulated in the Helsinki Declaration of 1975 (revised in 1983).

Discussion

Primary monogenic hypobetalipoproteinemia consists of a group of inherited diseases: ABL and chylomicron retention disease (CMRD, OMIM 246700), with a recessive transmission, and familial hypobetalipoproteinemia (FHBL, OMIM 107730), with a co-dominant transmission[1].

This case report describes the clinical and molecular characterization of a Turkish child with ABL, the most severe form of the primary monogenic hypobetalipoproteinemias. Less than 50 cases of ABL have been reported worldwide, and very few cases have been reported from Turkey[8,9].

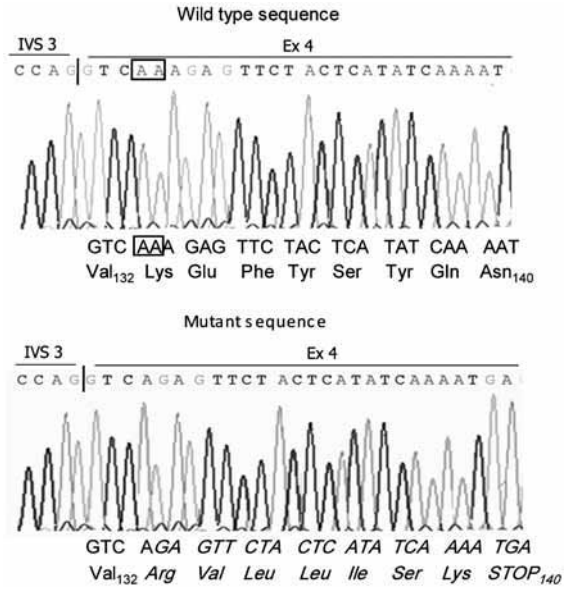

Fig. 1: Partial nucleotide sequence of exon 4 of the MTP gene in the patient. The electropherograms from a control subject (top) and the patient (bottom) show that the patient was homozygous for a two nucleotide deletion (boxed) in exon 4, which results in frameshift leading to the insertion of a premature termination codon in mRNA. The predicted translation product of this abnormal mRNA is a short peptide of 139 amino acids containing seven novel amino acids (indicated in italics) at the C-terminal end (p.V132fsN140X). MTP gene mutation is described according to mutation nomenclature (http://www.hgvs.org/munomen/). Amino acid sequence changes in MTP protein are described according to the NCBI reference sequence (GenBank accession number NP_000244.2).

All reported patients with ABL have gastrointestinal manifestations including diarrhea, steatorrhea, chronic fat malabsorption, oral fat intolerance, and defective absorption of fat- soluble vitamins, leading to failure to thrive and fat-soluble vitamin deficiency. In addition, most patients have retinitis pigmentosa, spinocerebellar ataxia and myopathy later in childhood because of fat-soluble vitamin deficiency[7]. Fatty liver has been reported in few cases. Our patient did not have neurological or muscular manifestations, which usually are long-term effects of the defective secretion of apoB-containing lipoproteins, and as a consequence, the defect in plasma transport and delivery of fat-soluble vitamins to tissues. He presented in the early post-natal period with severe gastrointestinal manifestations, which were refractory to treatment, and unfortunately died probably because of complications of severe malnutrition. The reasons for this extremely severe phenotype are not clear. Most of the ABL patients share a common biochemical defect that is the complete absence of MTP function, as most mutations in the *MTP* gene result in truncated MTP proteins devoid of function[7,10-22]. Because of complete MTP deficiency, these patients are expected to share a similar clinical phenotype, which is often not the case. It is possible that the variability and severity of clinical presentation are related to the residual capacity to absorb lipids by the intestine via an MTP-independent pathway, which allows a sufficient lipid intake to avoid severe diarrhea, growth retardation and fat-soluble vitamin deficiency and possibly a better response to dietary treatment[23]. This hypothesis implies that there may be alternative minor pathways independent of chylomicron formation, which might ensure a minimal absorption of lipids. Since the intestine is a site of synthesis of apoA-I and apoA-IV, it is possible that these apolipoproteins are used as protein constituents for the assembly of lipoprotein particles capable of transporting lipids from the enterocytes to the portal vein or the lymphatic system. However, the paucity of data on reported cases of ABL makes it difficult to define the natural history of the disease and the genotype-phenotype correlations.

From the molecular point of view, the novel *MTP* mutation found in the patient is a two nucleotide deletion in exon 4, which involves a region rich in short direct repeats (Fig. 1) thought to be a hot spot for minute deletions/ insertions. Usually these minute nucleotide deletions and/or insertions occur in genomic regions rich in di-tri-nucleotide repeats; they are due to back or forward slippage of the newly synthesized DNA strand during DNA duplication. The mutation in exon 4 is the cause of a frameshift leading to a premature termination codon. The predicted translation product of this mutant allele is a truncated protein of 139 amino acids which, if synthesized at all, is devoid of function. It is likely that *in vivo* this truncated protein is not synthesized (or is produced in small amounts) as mRNA containing premature termination codons are subjected to rapid degradation (nonsense-mediated mRNA decay). In any case, the truncated MTP protein (p.V132fsN140X) lacks two key domains: i) the middle α-helical

domain (residues 298-603) that mediates the interaction of MTP with PDI and apoB, and ii) the C-terminal domain (residues 604-894) that mediates the lipid-binding and transfer catalytic activity of MTP[2]. The complete loss of MTP function is expected to prevent the initial lipidation of apoB in the liver and intestine and cause a complete block in the secretion of apoB-containing lipoproteins.

The identification of the *MTP* gene mutation will allow us to screen other family members for the mutation and offers the possibility of a pre-natal diagnosis if required.

In conclusion, this observation underscores the importance of the study of the lipid profile in newborns who show poor weight gain and have unexplained diarrhea, especially if born from consanguineous parents. The presence of severe hypobetalipoproteinemia in a child and a normal lipid profile in his/her parents suggests the diagnosis of ABL and guides the laboratory to analysis of the *MTP* gene. If both parents have hypobetalipoproteinemia, one should first consider the hypothesis that the child has homozygous FHBL, a condition that suggests the involvement of other candidate genes (i.e. *APOB* or *PCSK9*)[24].

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