

## Identification of a novel mutation of *MTP* gene in a patient with abetalipoproteinemia

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### ABSTRACT

Abetalipoproteinemia (ABL), or Bassen-Kornzweig syndrome, is a rare autosomal recessive disorder of lipoprotein metabolism, characterized by fat malabsorption, hypocholesterolemia, retinitis pigmentosa, progressive neuropathy and acanthocytosis from early infancy. We describe the clinical and molecular characterization of a 6-month-old infant born of consanguineous, apparently healthy parents from Iran. The patient was hospitalized because of failure to thrive, greasy stool and vomiting. The patient's serum lipid profile, the clinical phenotype and the duodenal histology suggested the clinical diagnosis of ABL. The *MTP* gene analysis by direct sequencing revealed a novel homozygous mutation (c.1586 A > G-H529R). The parents were heterozygotes for the same mutation and interestingly the father showed a lipid profile characterized by a slight reduction of total and LDL-cholesterol plasma levels.

**Key words.** Abetalipoproteinemia. ApoB-containing lipoproteins. Hypocholesterolemia. *MTP* gene mutations.

### INTRODUCTION

Abetalipoproteinemia (ABL), also known as Bassen-Kornzweig syndrome (OMIM#200100), is a rare autosomal recessive disorder characterized by extremely low levels of apoB-containing lipoproteins, fat malabsorption, fat-soluble vitamins deficiency and acanthocytosis in infancy.<sup>1</sup> Deficiency of fat-soluble vitamins due to fat malabsorption could lead to a number of variable manifestations, including spinocerebellar degeneration, coagulopathy, and pigmented retinopathy.<sup>1</sup> Plasma total cholesterol (TC) and triglyceride (TG) levels are extremely low and apoB-containing lipoproteins are nearly absent in plasma. Mutations in the gene encoding the large subunit of microsomal triglyceride transfer protein (*MTP*) gene (OMIM\*157147) are responsible for the phenotype.<sup>2,3</sup> *MTP* gene encodes a protein which is required

for the assembly and secretion of apo B-containing lipoproteins in the liver and intestine;<sup>4,5</sup> in the presence of *MTP* deficiency, apo B cannot be properly lipidated and undergoes rapid intracellular degradation and for this reason apo B-containing lipoproteins are almost undetectable in plasma. It seems that there is no race preference for abetalipoproteinemia or familial hypobetalipoproteinemia, whilst the affected cases have been reported from every continent. However, a conserved haplotype and a common *MTP* mutation (p.G865X0 with a carrier frequency of 1:131 in Ashkenazi Jewish population has been reported.<sup>6</sup> We describe here the clinical phenotype and the molecular genetics in an ABL Iranian patient carrier of a novel mutation of *MTP* gene.

### CASE REPORT

A 6-month-old female infant born to consanguineous apparently healthy parents was admitted to the Children's Medical Center, Pediatrics Center of Excellence in Iran, because of failure to thrive, greasy stool and vomiting. On physical examination, weight was 4,850 g, height was 63 cm and head circumference was 38.5 cm. Her birth weight, height

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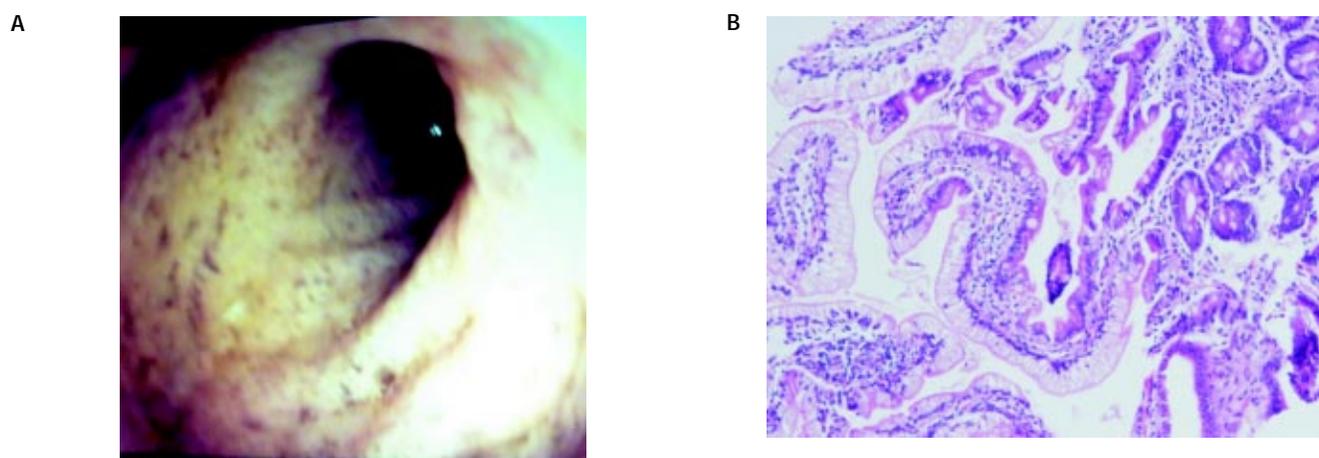
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and head circumference were 3,400 g, 49 cm and 34.5 cm, respectively. Low levels of TC, TG, high density lipoprotein (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were detected, whilst aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase plasma levels were increased. Peripheral blood smear showed many acanthocytes. Stool examination revealed more than 100 fat droplets by Sudan III stain test. Sweat test, thyroid function test and glucose metabolism were normal. Fundoscopic exa-

mination was normal and abdominal sonography showed homogeneous hyperechogenic pattern of liver (fatty liver). Upper endoscopic evaluation of the small bowel showed yellow discoloration of the small intestinal mucosal surface (Figure 1A).

#### PCR amplification of genomic dna and mutation detection

Blood Samples from the parents and the affected child were drawn while fasting. Informed written



**Figure 1** A. Endoscopic view of yellow discoloration in proximal duodenum. B. Fat-laden enterocytes located in the upper portion of the villus by light microscopy.

**Supplemental Table 1.** Primers used for sequencing the *MTP* gene.

|                 | Forward Primer          | Reverse Primer            | Annealing Temp (°C) | PCR Product size |
|-----------------|-------------------------|---------------------------|---------------------|------------------|
| Promoter Region | GGCTTGCTAGTGTGCTAATGA   | TGTGGGAGGGTAGTAAGGATTC    | 60                  | 276              |
| Exon 1          | GCAGCCCACCTACGTTTAAT    | CGTATCTGCCTCCAACCTCCA     | 60                  | 313              |
| Exon 2          | GCACTGATGGTGAGACAGCA    | GCTGTCCAATGGTACACTTCTC    | 60                  | 305              |
| Exon 3          | GTGGCCAACTCTTTCTGTTC    | GAAGTTGGAGAAAAGTTGTGG     | 60                  | 288              |
| Exon 4          | GAATCTGACCTTGCTGACAC    | AGGCAAATGCGACTTCACTAC     | 60                  | 331              |
| Exon 5          | CCTCAATTTTCAAGCCACTTC   | CATCCCTCATTACTAACACCTACAA | 60                  | 390              |
| Exon 6          | GGGGAAGCTGTTGTAGACTG    | ATGACTACACGCGCACACAC      | 62                  | 352              |
| Exon 7          | TATCACAGTTTCAAAGACATGGC | TGAGTTGATGCTTTCACTGGA     | 60                  | 363              |
| Exon 8          | CTTTCGCTTCAGACACAAGG    | TTCTGACAGACAACCCACACA     | 60                  | 305              |
| Exon 9          | AGTATTCTTCCCCTAAACATTG  | GCCTTCATTCAAGTCTAAGAAACT  | 60                  | 313              |
| Exon 10         | TTCTGAGTTTGAATCTAGAAACA | ATGGACAATGGTGTAGAATGATG   | 60                  | 285              |
| Exon 11         | AAGCCAGTCTCACCCAAGTC    | GCATTTGACTCATGTTTCAGCA    | 60                  | 352              |
| Exon 12         | GCTATTCTGCTGAAATGTCC    | TCGGTACCCCATGAACACAC      | 60                  | 344              |
| Exon 13         | CTCTTGAAAGGCATGAGGA     | GTTGTGGGTATGGAAGGAG       | 60                  | 268              |
| Exon 14         | CCACAACCTAGCATTGCTGG    | ACCTCAGCCTGATTTCTTGC      | 60                  | 235              |
| Exon 15         | ATTTACAGAGCAGGCAGGGA    | ACGTGCCTGGCGTATTTTAG      | 60                  | 338              |
| Exon 16         | AGGTCAAATGTGCCATTGGA    | TCCCAAACATATTTGCGAAGT     | 60                  | 284              |
| Exon 17         | TGGCATCATACGTTTCAGACC   | CTGAATGCAAGTTCTGGTG       | 60                  | 333              |
| Exon 18         | AACTTGGCTGGAGAGGTATAGG  | AGTCATGCCACATTGTGTCC      | 60                  | 305              |

consent for genetic investigations was obtained from parents.

Genomic DNA was extracted from peripheral blood leukocytes by standard procedure (Wizard Genomic DNA Purification Kit, Promega). The coding regions including the intron/exon boundaries (reference sequence NM\_000253) of *MTP* gene were amplified using the primers listed in Supplemental Table 1. The amplification conditions for all exons were 95 °C for 3 minutes (hot start), 95 °C for 60 seconds, the specific annealing temperature for 60 seconds, and 72 °C for 60 seconds for 35 cycles; a unique extension step at 72 °C for 7 minutes was performed.

PCR products were then purified using the Wizard PCR Preps DNA Purification System kit (Promega, Madison, WI, USA). 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, USA).

The proband (the first affected family member who seeks medical attention for a genetic disorder) and his parents were also genotyped for  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  polymorphisms in *APOE* gene as previously described.<sup>7</sup>

## RESULTS

Patient laboratory data are summarized in Table 1; the proband shows remarkable low plasma levels of TC, TG and apoB containing particles.

Her mother showed a normal lipid profile (TC 215 mg/dL, TG 93 mg/dL, HDL-C 47 mg/dL, LDL-C 123 mg/dL and VLDL-C 19 mg/dL), while her father showed a slightly reduction of plasma lipids (TC 134 mg/dL, TG 47 mg/dL, HDL-C 47 mg/dL; LDL-C 63 mg/dL and VLDL-C 9 mg/dL).

The proband's duodenal biopsy was analyzed by light microscopy which showed fat-laden enterocytes, located in the upper portion of the villi (Figure 1B).

**ApoE genotypes were  $\epsilon 3/\epsilon 3$  for all subjects.**

Direct sequence analysis of the whole *MTP* gene in the proband allowed the identification of an A > G homozygous substitution in exon 12 of *MTP* gene, which converts a Histidine into an Arginine (c.1586 A > G-H529R). The presence of the mutation in exon 12 (Figure 2) was confirmed in 3 independent

PCR amplifications and direct sequencing. The proband's parents were found to carry the same mutation in the heterozygosity (Figure 2).

Computational analysis using the program PolyPhen ([www.bork.embl-heidelberg.de/PolyPhen/](http://www.bork.embl-heidelberg.de/PolyPhen/)), which predicts effects of amino acid changes on protein function,<sup>8</sup> indicates that the H529R variation has a "damaging effect", given the PSIC (position-specific independent counts) score difference > 2.

## DISCUSSION

ABL is a rare autosomal recessive occurs in less than 1 in one million persons characterized by the absence of plasma apo B-containing lipoproteins. ABL is caused by *MTP* gene frameshift, non-sense and splice site mutations which are responsible for truncated forms of MTP devoid of function.<sup>1</sup> Non conservative missense mutations of *MTP* are also associated to the disorder.<sup>1,9</sup>

In addition to abetalipoproteinemia, *MTP* gene mutations and its variations could be associated with central obesity, elevated liver enzymes, and alcoholic fatty liver disease. The study of 588 Korean subjects showed that the polymorphism I128T (a change of I to T at the position of 128) of the gene was significantly related to alcoholic fatty liver.<sup>10</sup> Polymorphisms in the promoter region of the *MTP* gene may also be associated with the development of atherosclerosis and cardiovascular diseases. The study of 101 Japanese people in Japan showed that those with the -G493T polymorphism in the promoter region had elevated levels of low-density lipoprotein and triglycerides.<sup>11</sup> This polymorphism has been related to increased levels of inflammatory parameters and higher risk of coronary heart disease.<sup>12</sup> Another interesting linkage was noticed among chronic hepatitis C (HCV) genotype 3-infected patients with this polymorphism (-G493T). These patients have been found to be more likely to develop fatty liver accumulation.<sup>13</sup> In addition, the study on 433 Austrian patients revealed that the polymorphism may be connected to the pathogenesis of peripheral arterial disease.<sup>14</sup> However, study on *MTP* gene mutations and polymorphisms have not been performed in our country so far.

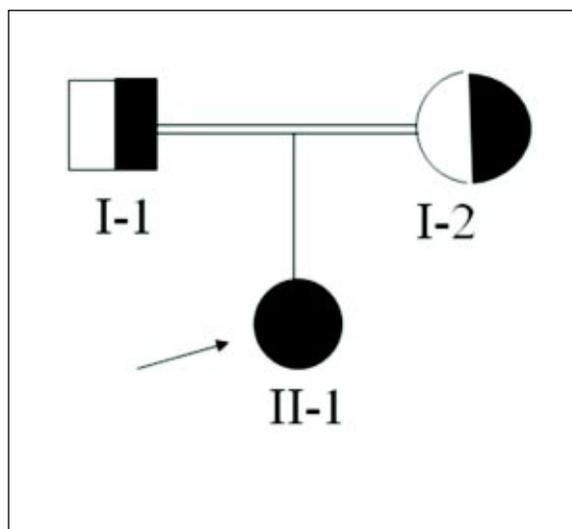
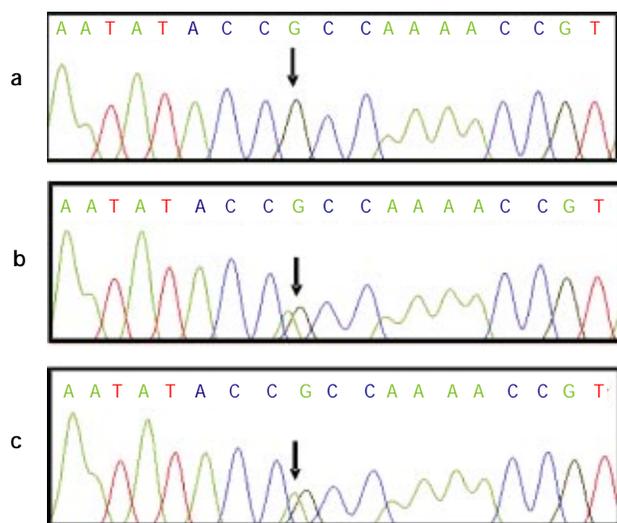
It has recently been demonstrated that *MTP* is also a central regulator of CD1 function. Importantly, CD1 dysfunction in ABL is caused specifically by deficiency in *MTP* and not by its downstream effects on the metabolism of APOB-containing lipoprotein particles, as demonstrated by unimpaired

CD1 function in patients with FHBL caused by heterozygous and compound heterozygous mutations in APOB.<sup>15</sup>

In this report we describe a novel non conservative missense mutation (H529R) of MTP causing an ABL phenotype in a 6-month-old female infant from Iran, born to consanguineous parents. The proband is a carrier of the H529R mu-

tation in homozygosity and as expected the proband's parents are heterozygotes for the same mutation.

H529R is a non-conservative amino acid substitution which is located in a highly conserved region of MTP protein (NiceProtView of Swiss-Prot: P55157) and belongs to a domain of MTP which interacts with apo B.<sup>16</sup> Based on bioinformatic analysis it



**Figure 2.** Pedigree of family with ABL and analysis of the MTP gene (reference sequence NM\_000253). Proband (subject II-1) is indicated with an arrow and full symbol indicating she is clinically affected. The chromatograms show the partial sequence of exon 12 in the proband (a), the proband's father (b) and the proband's mother (c). The arrow indicate the c. 1586 A>G mutation (H529R).

**Table 1.** Laboratory results of the proband with ABL phenotype.

| Test                                 | Results                       | Normal Range                      |
|--------------------------------------|-------------------------------|-----------------------------------|
| White blood cell count               | 10,940/ $\mu$ L               | 6,000-15,000/ $\mu$ L             |
| Neutrophils                          | 23%                           | 40-80%                            |
| Lymphocyte                           | 75%                           | 20-60%                            |
| Hemoglobin                           | 10.8 mg/dL                    | 10.5-14 mg/dL                     |
| Platelets                            | 376,000/ $\mu$ L              | 150,000-450,000/ $\mu$ L          |
| Red blood cell count                 | 4.2x10 <sup>6</sup> / $\mu$ L | 3.8-5.5x10 <sup>6</sup> / $\mu$ L |
| Aspartate aminotransferase (AST)     | 104 IU/L                      | 15-55 IU/L                        |
| Alanine aminotransferase (ALT)       | 89 IU/L                       | 5-45 IU/L                         |
| Prothrombin time                     | 13 sec                        | 12-14 sec                         |
| International normalized ratio (INR) | 1.1                           | 0.9-1.2                           |
| Partial thrombine time               | 33 sec                        | 30-40 sec                         |
| Gamma-glutamyl transferase (GGT)     | 18 IU/L                       | 4-60 IU/L                         |
| Total serum protein                  | 5.3 g/dL                      | 4.6-7.4 g/dL                      |
| Albumin                              | 4.9 g/dL                      | 3.9-5 g/dL                        |
| Alkaline phosphatase                 | 762 IU/L                      | 145-420 IU/L                      |
| Cholesterol                          | 22 mg/dL                      | 50-220 mg/dL                      |
| Triglyceride (TG)                    | 13 mg/dL                      | 70-200 mg/dL                      |
| Low density lipoprotein (LDL)        | 12 mg/dL                      | <130 mg/dL                        |
| High density lipoprotein (HDL)       | 15 mg/dL                      | >35 mg/dL                         |
| Very low density lipoprotein (VLDL)  | 3 mg/dL                       | up to 40 mg/dL                    |

could be suggested that this amino acid substitution might alter MTP function.

Patients with homozygous ABL experience multi-system manifestations and the diagnosis is usually made in infancy because of failure to thrive, fat malabsorption, acanthocytosis, and progressive degenerative neurologic disease. Malabsorption of fats and fat-soluble vitamins is responsible for vitamin E deficiency that could lead to neuromuscular abnormalities and loss of deep tendon reflexes. However our proband was still free of any neurological manifestation.

One of the most striking laboratory feature seen in patients with ABL is the absence of plasma apo B-containing lipoproteins. Plasma TG levels are usually low (less than 10 mg/dL), whereas TC level ranges from 25 to 40 mg/dL as also seen in our case.

The ABL phenotype is similar to homozygous familial hypobetalipoproteinemia (hoFHBL, OMIM#107730) a disorder caused most frequently by mutations in APOB gene.<sup>17</sup> Due to the different inheritance mode, ABL heterozygous parents usually have normal plasma lipoprotein profiles, while heterozygous FHBL parents showed low TC and LDL-C plasma levels.<sup>17</sup> However it has been suggested that some *MTP* gene missense mutations in heterozygosity may result in lipoprotein phenotype similar to that seen in individuals with heterozygous FHBL.<sup>9</sup> This mutation caused severe and complete manifestations of this syndrome which seems resistance to treatment in proband; however, in spite of other previously reported mutations, her father who is heterozygote for the H529R mutation showed a slight reduction of plasma TC, TG and LDL-C levels (134 mg/dL, 47 mg/dL and 63 mg/dL, respectively), while the mother had a normal lipid profile suggesting that other factors may contribute to the phenotype expression.

Our patient presented the typical hematologic and gastrointestinal features of ABL such as acanthocytosis and malabsorption with fat-laden enterocytes in the upper portion of villi. Fat droplets within the cytoplasmic compartment of the enterocyte may be confirmed by electron microscopy.<sup>18</sup>

The diagnosis of ABL should be promptly made in children with malabsorption, acanthocytosis and hypocholesterolemia, since appropriate management can prevent later in life complications. ABL patients are treated by a low-fat diet (~ 15 g/d) in order to improve the fat malabsorption and fat-soluble vitamins supplementation (high dose

for vitamin E) should be monitored periodically. Sufficient serum levels of vitamins A and K can be achieved by supplementation of moderate oral doses; since absorption of tocopherol is severely impaired, massive dose (~ 2,000 mg in infants) of vitamin E are required.<sup>19</sup>

In conclusion we have described a novel missense mutation in *MTP* gene, the H529R, in an Iranian child with an ABL phenotype.

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