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Laboratory Studies

Clinical features and molecular genetics of two Tunisian families with abetalipoproteinemia

Monia Benhamed Hammer [a,b,*], Ghada El Euch-Fayache [a], Houda Nehdi [a], Moncef Feki [c], Wieme Maamouri-Hicheri [a], Fayçal Hentati [a], Rim Amouri [a]

[a] Department of Molecular Neurobiology and Neuropathology, National Institute of Neurology, La Rabta, Tunis, Tunisia
[b] National Institute on Aging, Porter Neuroscience Research Center, National Institutes of Health, Building 35, Room 1A1010, Bethesda, MD 20892, USA
[c] Laboratory of Biochemistry, Rabta Hospital, Tunis, Tunisia

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ABSTRACT

Abetalipoproteinemia (ABL) is a rare monogenic disease characterized by very low plasma levels of cholesterol and triglyceride and almost complete absence of apolipoprotein B (apoB)-containing lipoproteins. Typically, patients present with failure to thrive, acanthocytosis, pigmented retinopathy and neurological features. It has been shown that ABL results from mutations in the gene encoding the microsomal triglyceride transfer protein (*MTTP*). Sanger sequencing of *MTTP* was performed for two unrelated consanguineous Tunisian families with two affected individuals each, presenting a more severe ABL phenotype than previously reported in the literature. The patients were found to be homozygous for two novel mutations. In the first family, a nonsense mutation, c.2313T > A, leading to a truncated protein (p.Y771X) was identified. In the second family, a splice mutation, IVS 9 + 2T > G, was found. These mutations are believed to abolish the assembly and secretion of apoB-containing lipoproteins.

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1. Introduction

Abetalipoproteinemia (ABL, Online Mendelian Inheritance in Man #200100) is a rare autosomal recessive disorder affecting lipoprotein metabolism.[1] Infants with this condition are asymptomatic at birth, but digestive signs such as diarrhea, vomiting and abdominal swelling appear with the ingestion of milk due to its high content of fat.[2] In the longer term, lipid malabsorption leads to below average gains in height and weight. Biochemically, the condition is characterized by extremely low levels of cholesterol and triglycerides with almost complete absence of plasma apolipoprotein B (apo-B) containing lipoproteins-chylomicrons, low density lipoproteins (LDL) and very low density lipoproteins (VLDL).[3] In addition, chronic malabsorption leads to lipid-soluble vitamin deficiencies.[4] Other manifestations include neurological symptoms such as spinocerebellar dysfunction, retinopathy and acanthocytosis.[5]

ABL is caused by mutations in the *MTTP* gene located on the long arm of chromosome 4, which encodes the microsomal triglyceride transfer protein (MTTP).[6,7] Mature MTTP has a molecular mass of 97 kDa and contains 894 amino acids including an 18 amino acid signal peptide.[7] It is a heterodimer composed of protein disulfide-isomerase and a large 97 kDa subunit that confers lipid transfer activity to the complex.[8] This complex is required for assembly of VLDL in the liver and chylomicrons in the intestine, where it transports triglycerides, cholesteryl ester, and phospholipids from the endoplasmic reticulum membrane to developing lipoproteins within the lumen of the endoplasmic reticulum.[4]

In the present study we describe the clinical and genetic aspects of four patients belonging to two unrelated Tunisian families with ABL.

2. Materials and methods

2.1. Patients

This work was approved by the local ethics committee, and patients gave their informed consent. Four patients belonging to two unrelated consanguineous families from Tunisian ancestry were assessed (Fig. 1). The International Cooperative Ataxia Rating Scale (ICARS) was used to assess the intensity of the symptoms.[9] This 100 point semi-quantitative scale involves a compartmentalized quantification of postural and stance disorders, limb ataxia, dysarthria and oculomotor disorders. Lower scores on this scale imply milder symptoms.

2.2. Lipid and vitamin analysis

Plasma total cholesterol and triglycerides were measured by routine methods. Vitamins A and E were assessed by high

* Corresponding author. Tel.: +1 240 485 4410; fax: +1 301 451 5466.
E-mail address: benhamadm@mail.nih.gov (M.B. Hammer).

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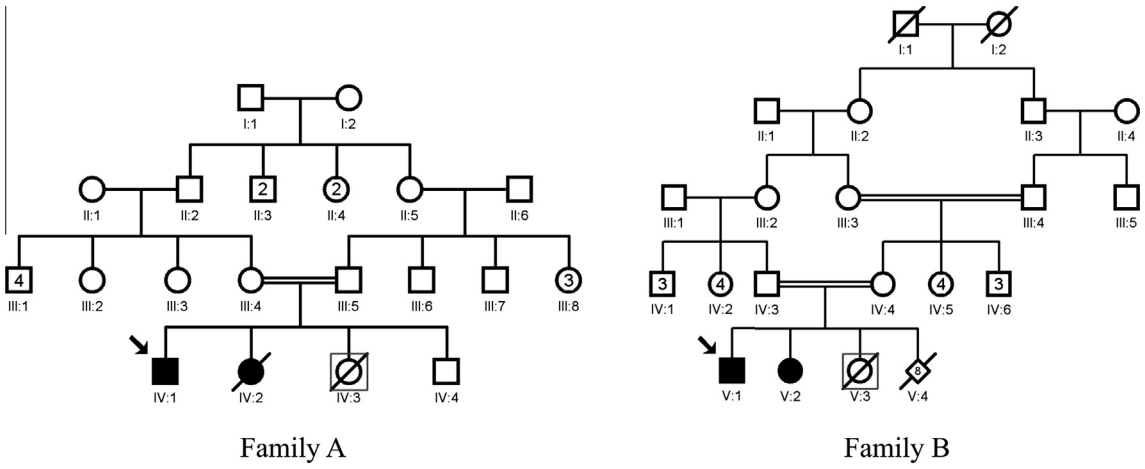

Fig. 1. Pedigrees of the two Tunisian families with abetalipoproteinemia.

performance liquid chromatography. Separation of plasma lipoproteins and analysis of apolipoproteins were performed as previously described.[10]

2.3. Genetic analysis

Genomic DNA was isolated from white blood cells according to standard protocols. Genomic sequencing of *MTTP* was first performed on the proband's DNA. The 18 exons of *MTTP*, including exon-intron boundaries, were amplified by polymerase chain reaction (PCR). Primer sequences were kindly provided by Tamar Ben-Yosef. PCR-amplified products were agarose gel purified and directly sequenced using the BigDye Terminator cycle sequencing kit on an ABI-3130 Genetic Analyzer (Life Technologies Corp., Carlsbad, CA, USA). The mutations found in the proband were subsequently screened in available family members.

3. Results

3.1. Clinical study

3.1.1. Patient A.IV.1

A 21-year-old male initially presented with chronic diarrhea and difficulty walking at 12 months of age. Neurological examination at that time showed a static and kinetic cerebellar syndrome with dyarthria and nystagmus (ICARS score = 24/100), deep sensory disturbances, absent tendon reflexes, pes cavus, hammer toes (Fig. 2), Achilles tendon shortening, and scoliosis. When the patient was 16 years old, nerve conduction studies (NCS) were consistent with sensory axonal neuropathy (peroneal nerve amplitude = 3 μV; sural amplitude = 10 μV). Follow-up NCS performed when the patient was 20 showed severe sensori motor axonal neuropathy. Nerve and muscle biopsies at age 16 indicated a discrete neurogenic atrophy in the muscle and a reduction in large myelinated fibers in the nerve. Fundoscopy revealed retinitis pigmentosa and there was P100 wave latency on visual evoked potential testing. Somatosensory evoked potentials were consistent with damage to the dorsal column–medial lemniscus pathways. Auditory evoked potentials were abnormal. MRI of the brain and spinal cord were normal. The patient failed to thrive (size = −3 standard deviations [SD], weight = −4 SD). The high percentage of acanthocytes (Fig. 3), lipid profile and vitamin E and A levels (Table 1) led to the diagnosis of ABL. Treatment with vitamin E and injectable vitamin K was prescribed.

3.1.2. Patient A.IV.2

The sister of A. IV.1. She was cyanosed and hypotonic at birth. She acquired the ability to sit by the age of three and suffered from episodic diarrhea. The clinical examination at the age of four showed a severe axial hypotonia, a motor weakness of the four limbs with a diffuse amyotrophy and multiple articular deformations (flexion of the elbows, flat feet, retraction of the pelvic and scapular girdles and of the Achilles tendon). Deep tendon reflexes were absent. NCS were normal and muscle biopsy was abnormal. The diagnosis of ABL was suspected after the diagnosis of the disease in her brother. The lipid profile and vitamin levels were consistent with ABL (Table 1). She died at the age of four.

3.1.3. Patient B.V.1

This patient suffered from chronic diarrhea since birth. At the age of five, he had difficulty with fine motor skills. At age of 14, his gait was abnormal. Neurological examination revealed a moderate static and kinetic cerebellar syndrome with nasal voice (ICARS score = 28/100), absent deep tendon reflexes, foot

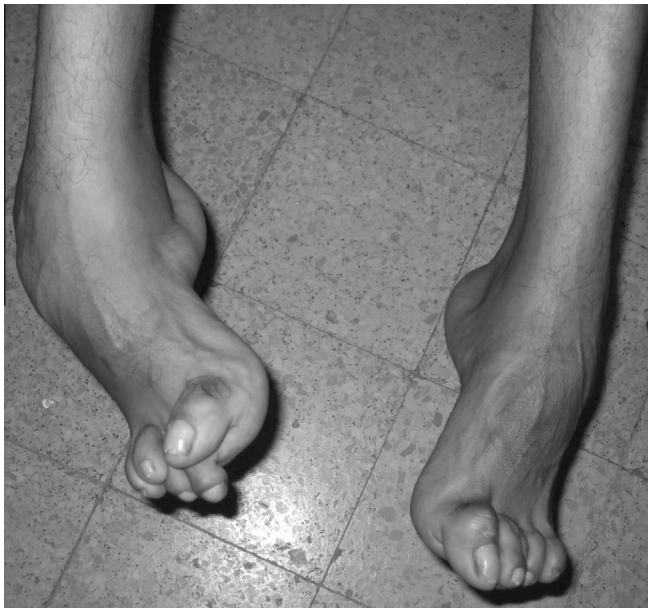

Fig. 2. Photograph showing the pes cavus in Patient A.IV.1.