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Novel mutations of ABCA1 transporter in patients with Tangier disease and familial HDL deficiency

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ABSTRACT

The objective of the study was the characterization of *ABCA1* gene mutations in 10 patients with extremely low HDL-cholesterol. Five patients (aged 6 months to 76 years) presented with splenomegaly and thrombocytopenia suggesting the diagnosis of Tangier disease (TD). Three of them were homozygous for novel mutations either in intron (c.4465-34A>G) or in exons (c.4376delT and c.5449C>T), predicted to encode truncated proteins. One patient was compound heterozygous for a nucleotide insertion (c.1758_1759insG), resulting in a truncated protein and for a nucleotide substitution c.4799A>G, resulting in a missense mutation (p.H1600R). The last TD patient, found to be heterozygous for a known mutation (p.D1009Y), had a complete defect in ABCA1-mediated cholesterol efflux in fibroblasts, suggesting the presence of a second undetected mutant allele.

Among the other patients, four were asymptomatic, but one, with multiple risk factors, had severe peripheral artery disease. Three of these patients were heterozygous for known mutations (p.R130K + p.N1800H, p.R1068C, p.N1800H), while two were carriers of novel mutations (c.1195-27G>A and c.396_397insA), predicted to encode truncated proteins.

The pathogenic effect of the two intronic mutations (c. 1195-27G>A and c.4465-34A>G) was demonstrated by the analysis of the transcripts of splicing reporter mutant minigenes expressed in COS-1 cells. Both mutations activated an intronic acceptor splice site which resulted in a partial intron retention in mature mRNA with the production of truncated proteins.

This study confirms the allelic heterogeneity of TD and suggests that the diagnosis of TD must be considered in patients with an unexplained splenomegaly, associated with thrombocytopenia and hypocholesterolemia.

1. Introduction

Tangier disease (OMIM #205400) and familial HDL deficiency (OMIM #604091) are two disorders of lipoprotein metabolism characterized by reduced plasma levels of high density lipoprotein-cholesterol (HDL-C) due to loss of function (LOF) mutations of the ABCA1 transporter [1–3]. The membrane transporter ABCA1 plays a key role in the first step of reverse cholesterol transport (RCT), that is the efflux of free cholesterol (FC) from the cell membrane to an extracellular acceptor represented by

Abbreviations: CE, Cholesteryl esters; TG, Triglyceride; HDL, High density lipoproteins; LDL, Low density lipoproteins; ApoA-I, Apolipoprotein A-I; apoB, Apolipoprotein B; ABCA1, ATP-binding cassette transporter 1.

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lipid-poor Apolipoprotein A-I (ApoA-I) [4–6]. ABCA1 deficiency impairs FC efflux from cells, leading to intracellular accumulation of cholesteryl esters (CE), prevents the conversion of the lipid-poor ApoA-I particles into pre-β-HDL and causes a rapid catabolism of the poorly lipidated ApoA-I, primarily by the kidney [4–6]. Tangier disease (TD) is a recessive disorder characterized by extremely low levels of HDL-C and ApoA-I and the accumulation of cholesteryl esters (CE) in macrophage rich tissues such as the tonsils which acquire a typical yellow-orange color. Some TD patients have hepato-splenomegaly, anemia, thrombocytopenia, peripheral neuropathy and corneal opacification [1–3]. TD patients are expected to have mutations in both alleles of the *ABCA1* gene [7]. Heterozygous carriers of *ABCA1* mutations display an intermediate phenotype of low HDL-C (designated familial HDL deficiency) and ~50% reduction in ABCA1-mediated cell cholesterol efflux [8,9]. Population studies have indicated that carriers of *ABCA1* mutations are found among subjects with lowest HDL levels [10–12]. The frequency of heterozygous carriers of LOF ABCA1 mutations has been estimated to be approximately 3:1000 [12].

More than 150 *ABCA1* mutations have been identified in patients with TD or FHD [http://www.hgmd.cf.ac.uk/ac/gene.php?gene=ABCA1]. The majority are missense mutations while the others are nonsense or frameshift mutations. Surprisingly for a large gene such as *ABCA1*, only few intronic mutations affecting mRNA splicing have been reported so far in TD or FHD patients.

In this work we describe ten patients with low plasma HDL-C and ApoA-I referred to lipid clinics for molecular diagnosis. Five patients presented with some clinical manifestations of TD; one patient had a generalized atherosclerosis affecting multiple arterial districts, while the others were asymptomatic. The analysis of the *ABCA1* gene led to the identification of seven novel mutations, including two intronic mutations affecting mRNA splicing.

2. Methods

2.1. Study participants

The subjects investigated in this study belong to a group of patients referred to the lipid clinics for the presence of a severe hypoalphalipoproteinemia (plasma HDL-C<5th percentile, 0.75 mmol/L), associated or not with clinical manifestations suggesting the diagnosis of TD.

Informed written consent was obtained from all patients or in case of children from their parents.

Following ethical guidelines, all samples were obtained for analysis and storage with the patients' written informed consent. The study protocol was approved by the institutional human investigation committee of each participating institution.

2.1.1. Patient # Mo-1

This patient was a 76 year-old Caucasian female referred to the lipid clinic for low total cholesterol, exceedingly low HDL-C (Table 1) and thrombocytopenia (platelet count: 106×10^9/L). She did not have any specific symptoms; her past medical history included bilateral Dupuytren's contractures, cataract, glaucoma, hypothyroidism, osteoporosis and controlled type 2 diabetes. At the clinical examination she had extensive bilateral Dupuytren's contractures, a mild degree of corneal clouding, a palpable splenic tip and a peripheral sensory neuropathy. Her tonsils were not enlarged. She was a non-smoker. Ultrasound imaging of the abdomen showed enlarged spleen of 15 cm in size and normal liver, kidneys and pancreas. At the coronary angiogram clear coronary arteries and normal ventricular function were shown. She was found to have mitral and tricuspid valve regurgitation for which she underwent surgical repair. There was no family history of ischemic heart disease, peripheral vascular disease or hypocholesterolemia.

2.1.2. Patient # Mo-2

This patient was a 33 year-old Caucasian male with motor neuropathy, splenomegaly and thrombocytopenia (platelet count: 62×10^9/L). His medical history included tonsillectomy at the age of 5, recurrent nose bleeding and relapsing Graves' disease. He was a non smoker. At the clinical examination he showed mild bilateral blepharoptosis and some somatic features consistent with Noonan syndrome (hypertelorism, low set ears and wide spaced nipples). Other positive systemic findings were palpable spleen and multiple freckles of 1–5 mm over his trunk and limbs. He developed chest pain after 10 min of exercise. At the coronary angiogram he showed a single vessel disease affecting a small side branch of the circumflex artery. Echocardiogram showed good ventricular function and normal heart valves. At the bone marrow biopsy an increased number of foamy macrophages were found. His plasma lipid profile was characterized by very low levels of HDL-C and ApoA-I (Table 1).

His twin brother presented to the clinic at the age of 35 for further investigations of a condition of thrombocytopenia (platelet count: 79×10^9/L). He had a history of clinical diagnosis of Noonan syndrome, asthma, surgical procedures for coarctation of the aorta, grommet insertion, undescended testicles and recurrent nose bleeding. He was a non-smoker and drank a moderate amount of alcohol. His general health was good with normal exercise tolerance. At the age of 37, he suffered from a sudden onset of chest pain after an asthma attack and died. Post mortem examination showed that his left anterior descending artery was occluded by atherosclerosis at a distance of 20 mm from its point of origin. The rest of the vasculature showed minimal atherosclerotic lesions. Liver and spleen were enlarged. Microscopic examination of the bone marrow showed actively phagocytizing foamy macrophages. His plasma lipid profile showed TC 2.7 mmol/L and triglycerides 2.4 mmol/L; HDL-C and apolipoprotein levels were not determined at that time. His routine biochemistry results were normal.

2.1.3. Patient # Mo-3

This patient was a 6 year-old Caucasian female born from consanguineous marriage (her parents were first cousins). Her medical history included hepato-splenomegaly, anemia (Hb: 100 g/L) and thrombocytopenia (platelet count: 90×10^9/L). At the bone marrow biopsy a high number of lipid-laden macrophages were observed. Her plasma lipid profile showed an exceedingly low level of HDL-C while ApoA-I was undetectable (Table 1).

2.1.4. Patient # Mo-4

This patient was a 32 year-old Caucasian male, born from consanguineous parents, who at the age of 23 was found to have a moderate hepato-splenomegaly and thrombocytopenia (platelet count: 81×10^9/L). Bone marrow biopsy indicated the presence of numerous lipid laden macrophages. The presence of enlarged and yellow-orange tonsils and the extremely low levels of HDL-C and ApoA-I (Table 1) suggested the diagnosis of Tangier disease. He did not smoke and did regular physical exercise. Neurological and ocular examinations were negative. The carotid Doppler ultrasound revealed an intima-media thickness of 0.7 mm bilaterally, without evidence of plaques. ECG, echocardiogram and cardiac stress test were negative.

2.1.5. Patient # Mo-5

This patient was a 6 month-old Caucasian male, born from apparently non-consanguineous parents. He was admitted to the hospital for the presence of thrombocytopenia (platelet count: 65×10^9/L). On physical examination, he was found to have mild hepato-splenomegaly which was confirmed by ultrasound examination. His plasma lipid profile revealed a very low level of HDL-C; ApoA-I was undetectable (Table 1).

2.1.6. Patient # Mo-6

This patient was a 69 year-old Caucasian female, referred to the hospital for drug resistant hypertension with no overt evidence of