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Case Report: A Novel Mutation in the *ABCA1* Gene, Resulting in a Compound Heterozygote with Tangier Disease

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Abstract

Background: Tangier Disease (TD) is a rare autosomal recessive disorder characterized by significantly reduced levels of plasma HDL-C, typically leading to an accumulation of cholesterol esters in various tissues. The clinical phenotype and degree of TD is highly variable.

Case Summary: Here in we present a case study of a 50-year old female Caucasian patient with features typical of TD, and her relatives.

Genetic analyses were performed using next generation sequencing. Peripheral Blood Mononuclear Cells (PBMCs) of the index patient, her children and four healthy volunteers were isolated to analyze cholesterol efflux capacity. *ABCA1* protein expression was visualized using immunoblot analysis.

Genetic analysis of the index patient revealed a compound heterozygosity for a novel mutation (c.1776delC) as well as a known mutation (c.1824delG) affecting the *ABCA1* gene, associated with TD. The offspring, both heterozygous for c.1824delG, displayed impaired cholesterol efflux without clinical features of TD.

Conclusion: This case-study expands the knowledge on mutations causing TD and confirms an impaired cholesterol efflux even in heterozygous individuals, underlining the importance of extended screening of relatives who may not directly present with clinical features typical of TD.

Keywords: *ABCA1*; Tangier disease; Cholesterol efflux; Reverse cholesterol transport; Novel mutation

Introduction

Tangier Disease (TD) is a rare autosomal recessive disease characterized by a severe deficiency in High-Density Lipoprotein Cholesterol (HDL-C), resulting in an impaired Reverse Cholesterol Transport (RCT) and a respective accumulation of cholesteryl esters in various tissues. The disease was first described and observed in 1961 in kind red living on Tangier Island, Virginia [1-3]. Yet, more than 170 cases have been reported in literature, emphasizing that the clinical expression of TD may be highly variable [4]. We here in report on a novel *ABCA1* mutation in a 50-year old female presenting with features typical of TD, and on a known ABCA1 mutation in both her offspring, associated with a normal clinical, but abnormal laboratory phenotype.

Material and Methods

Ethic statement

Written informed consent was obtained for the publication of this case. Informed consent was provided by the subjects. The ethics committee of the Medical University of Innsbruck waived the need for ethics approval for the collection, analysis and publication of the obtained and anonymized data for this clinical case-series.

Mutational analysis of the *ABCA1* gene

DNA was isolated from patients' EDTA-blood samples using a QIA Symphony kit. Target genes were amplified using Nextera® Rapid Capture (TruSight™ One Panel, Illumina) and next generation sequencing (NextSeq Illumina) was performed. The GRCh37 (hg19) was used as a reference genome sequence. Obtained data was analyzed using the SeqPilot software package including SeqNext from JSI medical systems [5]. To ensure adequate quality control of resulting *ABCA* genes reads, a minimum coverage of 20 reads in forward and reverse as well as flanking intronic sequences from -15 to +5 with a minimum coverage of 10 reads was specified. Unclassified variants were called and considered likely pathogenic and pathogenic variants based on a method proposed previously (classes 3 or 5 according to Plon et al.) [6]. Quantitative read analysis regarding Copy Number Variation (CNV) was determined using SeqNext from JSI medical systems. The adenine of the start codon is c.1 (see Human Genome Variation Society (HGVS) [7]).

Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs of patients and healthy volunteers were isolated using density centrifugation (Biocoll, Biochrom L6115), followed by magnetic cell separation with CD14 microbeads (Miltenyi Biotec 130-05-201). Isolated (CD14 positive) cells were seeded on a poly-L-lysine-coated 12-well plate (7.5×10^5 cells/well) in DMEM, supplemented with 10% fetal calf serum (Biochrom S0615), 1% Glutamine (Thermo Fisher Scientific 25030-081), 1% Penicillin/Streptomycin (Thermo Fisher Scientific 15140122), and 50 ng/ml hMCSF (Reprotech 300-25). Later after 5 days, isolated and differentiated monocyte-derived macrophages were subjected to immunoblots or cholesterol efflux analyses, respectively.

Cholesterol efflux from PBMCs

Experimental procedures were performed according to Demetz et al. [8,9] Tancevski et al. [10], and Duong et al. [11].

Briefly, PBMCs were seeded on a 6-well plate and incubated with 5 µCi/ml [3H]-cholesterol for 24 h in DMEM containing 1% FBS. Then, cells were washed twice with PBS and equilibrated for 16 h to 18 h in DMEM containing 0.2% fatty acid free BSA (Sigma Aldrich, A7030), 0.3 mM cAMP (Sigma Aldrich, C2912), and 1 µg/mlACAT-inhibitor (Sigma Aldrich, S9318). Subsequently, cells were washed twice with PBS and incubated with DMEM supplemented with 10 µg/ml ApoA1 (Sigma Aldrich, A0722), 0.3 mM cAMP, and 1 µg/ml ACAT-inhibitor for 4 h. Supernatant was then collected, cells were washed twice with PBS and harvested in 1 ml 0.1 M NaOH. Both, supernatants and cell lysates were analyzed using a liquid scintillation counter (Perkin Elmer). Percentages of cholesterol efflux were calculated using following formula:

$$\% \text{ efflux} = CPM_{supernatant} \times 100/(CPM_{supernatant} + CPM_{cells})$$

Immunoblot analysis

Immunoblot analyses were performed as described [8]. Anti-ABCA1 antibody was from Novus Biologicals (NB400-105), anti-ACTIN antibody from Sigma Aldrich (A2066). The chemiluminescent reaction was performed using Super Signal West Dura Reagent (Thermo Fischer Scientific, 34076); blots were visualized by the use of a ChemiDoc (BioRad) device.

Fast protein liquid chromatography (FPLC)

Lipoprotein profiles were analyzed by fractionation of pooled plasma using two Superose 6-columns (GE Healthcare 29091596) in series, followed by cholesterol measurement (Roche 42024401).

Results

Case report

A 50-year old female patient (Body-Mass-Index: BMI=24.3) was referred to our clinic due to both elevated plasma triglyceride concentrations (TG=422 mg/dl) and unusually low concentrations of HDL-C (<3 mg/dl) during a routine check-up before an elective surgical intervention. The patient had a history of mild thrombocytopenia, a neuropathy similar to syringomyelia, a severe obstructive sleep apnea syndrome and a goiter with normal Thyrotrophic Hormone (TSH).

The parental medical history was not retrievable; the patient is mother to two healthy children. Physical examination did not show the characteristic orange tonsils, as the patient underwent tonsillectomy in her childhood. Abdominal and carotid ultrasound revealed a mild splenomegaly, cholecystolithiasis and atherosclerotic lesions of the aorta as well as non-stenosing plaques of both carotid arteries. Histological evaluation following elective thyroidectomy revealed areas with aggregation of foam-cells and lipomatous transformation (data not shown). Similarly, histological examination of a surgically resected hamartoma of the chin revealed an elevated lipid content and infiltration with foam cells (Figures 1A-1C).

Further laboratory analyses confirmed low concentrations of plasma HLD-C, decreased plasma concentrations of total cholesterol (<100 mg/dl), reduced plasma low-density lipoprotein cholesterol (LDL-C=27 mg/dl) and low apolipoprotein A1 (ApoA1<10 mg/dl) levels. Her children displayed mildly reduced plasma HDL-C levels, slightly elevated TGs and normal LDL-C as well as total cholesterol. Details are listed in Table 1. Clinical presentation together with changes in plasma cholesterol distribution led to further genetic and functional analyses.

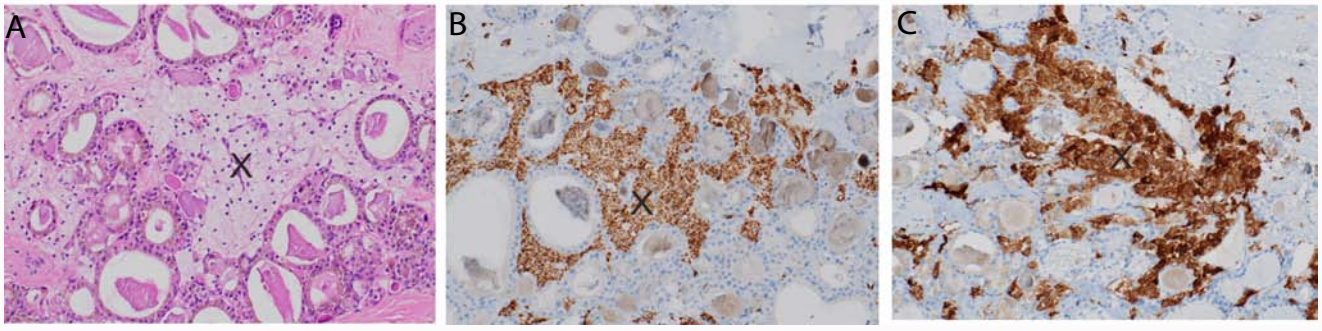

Figure 1: Foam cell accumulation in the index patient ascertained by histological examination of the excised hamartoma. (A) Hematoxylin eosin staining, (B) Immunohistochemical (IHC) staining for CD68, (C) IHC staining with adipophilin; (x) indicates foam cells.

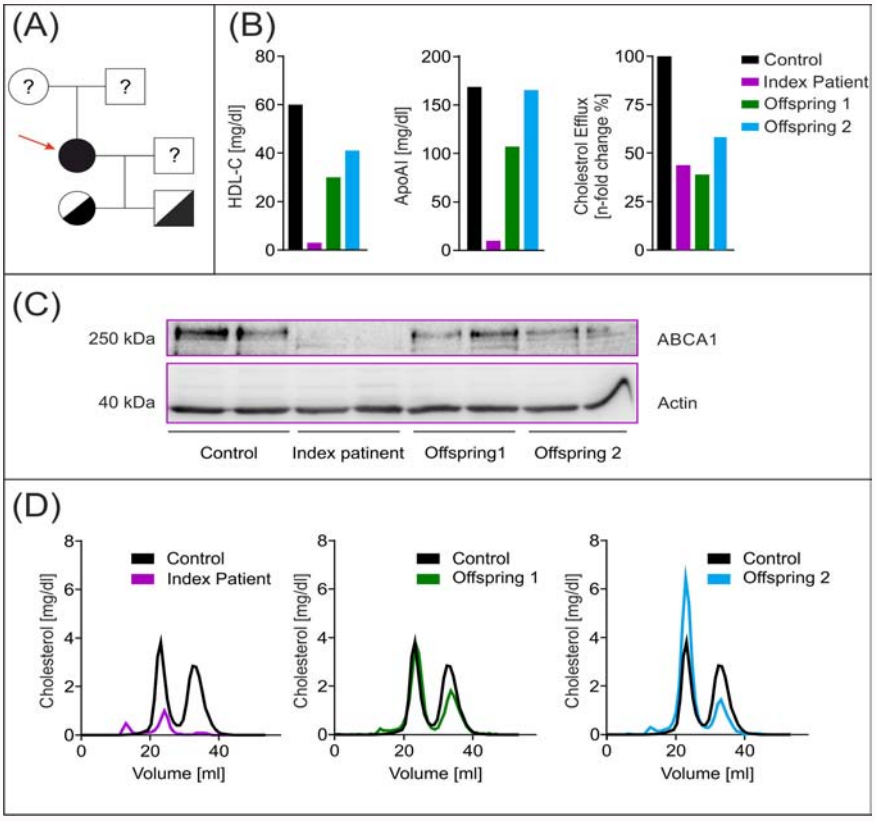

Figure 2: Characterization of the TD patient and her offspring. (A) Pedigree analysis highlighting the index patient (compound heterozygous for c.1776delC and c.1824delG) and her heterozygous, asymptomatic offspring. (B) Plasma HDL-C levels, cholesterol efflux capacity from PBMCs isolated from the index patient (mother), her offspring and healthy controls. (C) Immunoblot analyses of ABCA1 in PBMCs of four healthy controls, the index patient and her offspring. Actin served as loading control. (D) Fast protein liquid chromatography analysis of plasma from index patient and offspring compared with healthy.

Table 1: Plasma cholesterol distribution and lipoprotein components of the index patient and her children. HDL-C, High-Density Lipopoprotein cholesterol; LDL-C, Low-Density Lipoprotein cholesterol; TG, Triglycerides.

Parameter	Mother	Daughter	Son	Normal range
Total cholesterol (mg/dl)	89	178	187	145-199
HDL-C (mg/dl)	<3	41	30	>40
LDL-C (mg/dl)	27	97	147	<129
TG (mg/dl)	422	178	149	40-200
Apolipoprotein A1 (mg/dl)	<10	165	107	108-225
Apolipoprotein B (mg/dl)	91	103	132	60-117

Mutational analysis of the *ABCA1* gene

Comprehensive genetic analysis of the index patient using next generation sequencing revealed compound heterozygous mutations at position c.1776delC and c.1824delG of the *ABCA1* gene. The analyses of her offspring revealed heterozygous mutations at c.1824delG of the *ABCA1* gene. The pedigree analysis highlighting the index patient and her heterozygous, asymptomatic offspring is shown in Figure 2A.

Available literature suggests that c.1776delC may cause a frameshift of the open reading frame at position p.593, leading to a premature stop codon after 15 changed amino acids (p.Phe593Serfs*16), hence inducing nonsense mediated decay. The NHLBI GO Exome Sequencing Project (ESP) detected c.1776delC once in 8,246 alleles of European-American background with unclear putative relevance. Importantly, so far this mutation has not been described in other databases like Leiden Open Variation Database (LOVD), Single Nucleotide Polymorphism Database (dbSNP) or Exome Aggregation Consortium (ExAC).

The probable effect of c.1824delG is a frameshift of the open reading frame at position p.Thr609 as well as a premature stop codon after 26 changed amino acids (p.Thr609Argfs*27). This variant has already been described in the dbSNP database (rs387906413) and listed in ClinVar and Human Gene Mutation Database (HGMD) as a pathogenic variant [12].

Functional cholesterol analyses in peripheral blood mononuclear cells

Peripheral Blood Mononuclear Cells (PBMCs) of the index patient, her children and four healthy volunteers were isolated to analyze cholesterol efflux capacity. ABCA1 protein expression was visualized using immunoblot analysis [8]. Cholesterol efflux experiments were performed according to Demetz et al. [8,9], Tancevski et al. [10], and Duong et al. [11].

The index patient not only showed decreased plasma HDL-C levels (Figure 2B), but also displayed a 50% lower cholesterol efflux capacity as compared to healthy controls (Figure 2B). Both heterozygous children had 39% (daughter) and 58% (son) efflux capacity of healthy controls (100%; Figure 2B). The index patient also presented with almost undetectable ApoA1 concentrations in plasma (<10 mg/dl, Figure 2B). Of note, her daughter presented with mildly reduced ApoA1 concentrations (107 mg/dl), whereas her son had normal plasma ApoA1 concentrations (165 mg/dl) (Figure 2B). Further immunoblot analyses confirmed the absence of *ABCA1* in PBMCs

derived from the index patient. Her offspring had a lower expression of *ABCA1* as compared to healthy controls (Figure 2C). Finally, FPLC analysis of plasma from the index patient and her offspring confirmed pathological cholesterol distribution among different lipoproteins, mainly reflected by reduced HDL-C levels (Figure 2D).

Discussion

Tangier Disease (TD) is an autosomal recessive disorder defined by significantly reduced levels of plasma HDL-C, typically leading to an accumulation of cholesterol esters in tissue. Common clinical features include lipid laden tonsils, intestinal lipid storage, recurrent peripheral neuropathy, hypocholesterolemia, and abnormal chylomicron remnants [13,14]. Patients with TD are expected to have Loss of Function (LOF) mutations in both alleles of the *ABCA1* gene [12,15].

Here we report on a 50-year old Caucasian patient with features typical of TD including splenomegaly and peripheral neuropathy. Laboratory studies showed undetectable plasma HDL-C, decreased total cholesterol and ApoA1 concentrations, elevated plasma triglycerides and thrombocytopenia. Histological findings of a surgically removed hamartoma of the chin showed deposition of fat droplets and infiltration with foam cells. Genetic analysis of the index patient revealed a compound heterozygosity for a novel mutation (c.1776delC) as well as an already known mutation (c.1824delG) affecting the *ABCA1* gene, leading to the above mentioned clinical phenotype typical of TD. The offspring, both heterozygous for the already described c.1824delG, showed no clinical evidence for TD and had normal laboratory findings. Of note, cholesterol efflux from peripheral monocytes was significantly reduced in this family of three, however most markedly in the heterozygous index patient.

The human *ABCA1* gene on chromosome 9q22-q31 contains 50 exons and spans 150 kb [16], and at present, over 200 *ABCA1* mutations have been identified [17]. Variants in the *ABCA1* gene can result in a broad spectrum of biochemical abnormalities, leading to many different clinical phenotypes in TD patients [18].

The exact clinical and biochemical significance of this novel mutation at position c.1776delC remains unclear, as both offspring were heterozygous for the previously described mutation at position c.1824delG. Also, unfortunately, no additional medical information is retrievable from the index patient's parents. Our findings suggest that the novel mutation may lead to a premature stop codon, yielding a non-functional protein reflected by absent *ABCA1*. As a consequence of that, HDL-associated components may be reduced, especially cholesterol and ApoA1, as was observed in the index patient.

Available literature suggests that heterozygous relatives of TD patients may present with an intermediate TD phenotype characterized by aberrant laboratory, but normal clinical findings. Typical laboratory findings may include HDL-C concentrations amounting to 50% of those of healthy age- and sex-matched controls [19]. Of note, impaired cholesterol efflux capacity, as detected in the offspring of our index-patient, might be associated with an increased risk for coronary artery disease even in heterozygous patients [20]. These findings clearly underline the importance of raising clinical awareness towards not only patients presenting with obvious clinical and laboratory aberrations typical of TD, but also towards respective clinically asymptomatic relatives. These patients may present with only mild laboratory aberrations in need of more frequent or thorough follow-up investigations to avoid long-term complications and severe cardiovascular disease.

In summary, this case-study expands the knowledge on mutations causing TD. We were able to characterize the impact of a novel ABCA1mutation on cholesterol efflux capacity, a major pathway in HDL particle synthesis and RCT. The findings of this report suggest that even asymptomatic relatives of patients affected by TD may benefit of thorough work-up and regular follow-up visits to avoid long-term complications.

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