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A novel splicing mutation in the ABCA1 gene, causing Tangier disease and familial HDL deficiency in a large family

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ABSTRACT

Tangier disease is a rare disorder of lipoprotein metabolism that presents with extremely low levels of HDL cholesterol and apoprotein A-I. It is caused by mutations in the ATP-binding cassette transporter A1 (*ABCA1*) gene. Clinical heterogeneity and mutational pattern of Tangier disease are poorly characterized. Moreover, also familial HDL deficiency may be caused by mutations in ABCA1 gene.

ATP-binding cassette transporter A1 *(ABCA1)* gene mutations in a patient with Tangier disease, who presented an uncommon clinical history, and in his family were found and characterized. He was found to be compound heterozygous for two intronic mutations of ABCA1 gene, causing abnormal pre-mRNAs splicing. The novel c.1510-1G > A mutation was located in intron 12 and caused the activation of a cryptic splice site in exon 13, which determined the loss of 22 amino acids of exon 13 with the introduction of a premature stop codon. Five heterozygous carriers of this mutation were also found in proband's family, all presenting reduced HDL cholesterol and ApoAI (0.86 ± 0.16 mmol/L and 92.2 ± 10.9 mg/dL respectively), but not the typical features of Tangier disease, a phenotype compatible with the diagnosis of familial HDL deficiency. The other known mutation c.1195-27G > A was confirmed to cause aberrant retention of 25 nucleotides of intron 10 leading to the insertion of a stop codon after 20 amino acids of exon 11. Heterozygous carriers of this mutation also showed the clinical phenotype of familial HDL deficiency.

Our study extends the catalog of pathogenic intronic mutations affecting *ABCA1* pre-mRNA splicing. In a large family, a clear demonstration that the same mutations may cause Tangier disease (if in compound heterozygosis) or familial HDL deficiency (if in heterozygosis) is provided.

1. Introduction

Tangier disease (TD) (OMIM 205400) is a rare disorder of lipoprotein metabolism. At biochemical level it presents with extremely low levels of HDL cholesterol (HDL-C) and apoprotein ApoA-I. At cellular level, cholesteryl esters accumulate in macrophage-rich tissues [1,2]. TD patients usually present enlarged orange-yellow tonsils, hepatosplenomegaly, lymphoadenopathy, anemia, thrombocytopenia, peripheral neuropathy and corneal opacity [3]. Moreover, TD is often associated with an increased risk of coronary artery disease [4–6].

TD is caused by loss-of-function mutations in the ATP-binding cassette transporter A1 (*ABCA1)* gene, which encodes the membrane transporter ABCA1 [7]. The role of this transporter is critical for the efflux of free cholesterol from peripheral cells. Cholesterol is transferred to lipid-poor Apo A-I particles, which start the reverse cholesterol transport pathway [8,9]. A rapid catabolism of poorly lipidated Apo A-I is the main consequence of ABCA1 deficiency. This mostly happens in the kidney, as the consequence of the impaired

Abbreviations: TD, Tangier disease; FHD, familial HDL deficiency; ABCA1, ATP-binding cassette transporter A1.

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conversion of lipid-poor Apo A-I particles into pre-β HDL [10].

TD is an autosomal recessive disease caused by loss-of-function mutations in both *ABCA1* alleles. Heterozygotes show a phenotype compatible with familial HDL deficiency (FHD), with low HDL-C linked to a reduction of about 50% in the ABCA1-mediated cell cholesterol efflux [11]. The number of *ABCA1* variants identified till now is 236 (http://www.hgmd.cf.ac.uk/ac/index.php. Last access: November 2018), mostly reported in exons encoding the extracellular domain and in ATP binding cassettes, of the 2261 amino acid protein. Among several kinds of mutations found [12,13], only a small number affects intronic sites [11]. Here we characterized a new Italian case of TD associated with compound heterozygosity for 2 intronic mutations causing abnormally spliced *ABCA1* mRNA in a man who presented with an unrecognized bleeding episode.

2. Materials and methods

2.1. TD kindred

The proband was a 40 years-old Caucasian male referred to our outpatient Lipid Clinic for extremely low levels of HDL-C (0.03 mmol/L) associated with mild hypertriglyceridemia (2.28 mmol/L). His medical history revealed tonsillectomy in childhood and thrombocytopenia. For this reason, he performed at the age of 29 a bone-marrow biopsy, which showed 50% foamy cells infiltration and mild hematopoietic hypoplasia. At the age of 30, due to the presence of recurrent abdominal pain, he was diagnosed with intra-abdominal hemorrhage caused by spontaneous spleen rupture requiring splenectomy. He was a past-smoker with a sedentary lifestyle. At physical examination, he was found to have mild hepatomegaly while neurological and ocular evaluations were negative. Laboratory tests showed thrombocytopenia (platelets count 90000/mm^3) but normal liver and kidney function (AST 36 UI/L, ALT 28 UI/L, plasma creatinine 61.6 μmol/L). Electrocardiogram and echocardiogram were unremarkable. Abdominal ultrasound documented liver steatosis (4 points according to Hamaguchi's score) and carotid ultrasound revealed a carotid intima-media thickness of 0.8 mm (over the 75th age and sex-specific percentile for European population) without evidence of atherosclerotic plaques [14].

The proband's pedegree is shown in Fig. 1 (Panel A) and the clinical characteristics of proband and his relatives are reported in Table 1. Consanguinity was not documented in the family. Proband's father had history of thrombocytopenia and proband's mother died at the age of 42 for rupture of a cerebral aneurysm. There was also family history of tonsillectomy (the sister and the twin children) and splenomegaly (the twin children). HDL-C levels in adults were below the 10th and in children below the 5th age – sex specific percentiles for the Italian population [15].

2.2. Determination of plasma lipid values

Blood samples were collected early in the morning after an overnight fast in EDTA-containing tubes. Plasma lipid and apolipoproteins were determined as previously described [16].

2.3. Screening of ABCA1, APOA1, LPL and LCAT genes

General approaches for mutational search, genotype analysis and functional effect of mutations found were already published by us both for other diseases [17–21] and for genes involved in lipid metabolism [16,22,23]. They will be only summarized below. The extraction of genomic DNA from peripheral blood leukocytes was performed by the QIAamp Blood DNA kit (Qiagen, Hilden, Germany). For PCR-amplification and sequencing of promoter, 50 exons and respective exon-intron junctions of *ABCA1* gene, a set of primers already described [24] and four originally designed primers (reported in the S1 Table) were used. A PTC100 thermal cycler (MJ Research, Bruno, Canada) was used with the following parameters: 2′95 °C; 35 cycles 45″ 94 °C, 1′30″ 58 °C, 2′30″ 72 °C; 7′ 72 °C. The total volume of PCR reactions was 15 μL containing 50 ng of genomic DNA, 0.25 units of GoTaq hot start polymerase (Promega, Fitchburg, Wisconsin, USA), 1.5 mM $MgCl_2$, 175 μM of each dNTP (Fermentas, Waltham, Massachusetts, USA), 6 pmols of each primer with a 1x final concentration of manufacturer's buffer. PCR amplicons were purified by thermosensitive alkaline phosphatase (Fermentas, Waltham, Massachusetts, USA) and exonuclease (USB Corporation, Cleveland, Ohio, USA). Sequencing was performed by using the Big Dye Terminator Reaction Kit version 1.1 (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. Sequences were purified by Montage SEQ96 sequencing reaction cleanup kit (Millipore, Billerica, Massachusetts, USA) following manufacturer's instructions. The genetic analyzer ABI PRISM 3130*xl* (Applied Biosystems, Foster City, California, USA) and the software SeqScape (Applied Biosystems, Foster City, California, USA) were used for electropherograms development and analysis.

To rule out mutations in other HDL-related genes, *APOA1*, *LPL* and *LCAT* genes were also sequenced in the proband. The first 2 genes were analyzed by an already published approach [16], the third gene by a Sanger sequencing approach using a specific set of primers (unpublished).

The gene variants were labeled according to the Human Genome Variation Society nomenclature (version 15.11) (http://varnomen.hgvs.org/. The GenBank NM_005502.3 and NP_005493.2 were used for the analysis of, respectively, human ABCA1 cDNA and protein. The *in silico* analysis was performed using Polyphen (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://sift.jcvi.org/). The *in silico* prediction of the effect of intronic variants was performed using ASSEDA (https://splice.uwo.ca), NetGene2 (http://www.cbs.dtu.dk/services/NetGene2) and GeneMark (http://exon.biology.gatech.edu/). The *in silico* prediction of truncated protein was obtained by the ExPASY Translate tool (http://web.expasy.org/translate/).

2.4. Analysis of abnormally spliced ABCA1 mRNAs

Five milliliters of heparinized blood were diluted in PBS (Carlo Erba, Milano, Italy). The mixture was layered on 15 mL of Lymphoprep (Axis-Shield, Norway) and centrifuged at 508 g for 30 min. The mononuclear cell layer at the interphase was collected, washed twice with PBS and centrifuged at 1200 g for 20 min until depletion of thrombocytes.

Total RNA was extracted from mononuclear cells using RNeasy mini kit (Qiagen, Hilden, Germany). After a common DNAse treatment, total RNA was reverse transcribed using iScript cDNA Synthesis kit (Biorad, Hercules, California, USA) in a final volume of 20 μL, according to manufacturer's instructions. *ABCA1* cDNAs of the proband and family members were PCR-amplified by primers shown in the S2 Table using for reactions a PTC100 thermal cycler (MJ Research, Bruno, Canada). PCR amplifications were performed in a total volume of 15 μL with 2.5 μL of reverse transcription mix, 6 pmols of each primer, 0.25 units of GoTaq hot start polymerase (Promega, Fitchburg, Wisconsin, USA), 175 μM of each dNTP (Fermentas, Waltham, Massachusetts, USA), 1.5 mM $MgCl_2$ and 1x manufacturer's buffer. The PCR cycle was the following: 2′95 °C; 35 cycles of 45″ 94 °C, 1′30″ 58 °C, 2′30″ 72 °C followed by 7′ 72 °C. The RT-PCR products were separated in 2% agarose gel electrophoresis, eluted from agarose by the QIAquick gel extraction kit (Qiagen, Hilden, Germany) and sequenced by using the same primers utilized for RT-PCR.

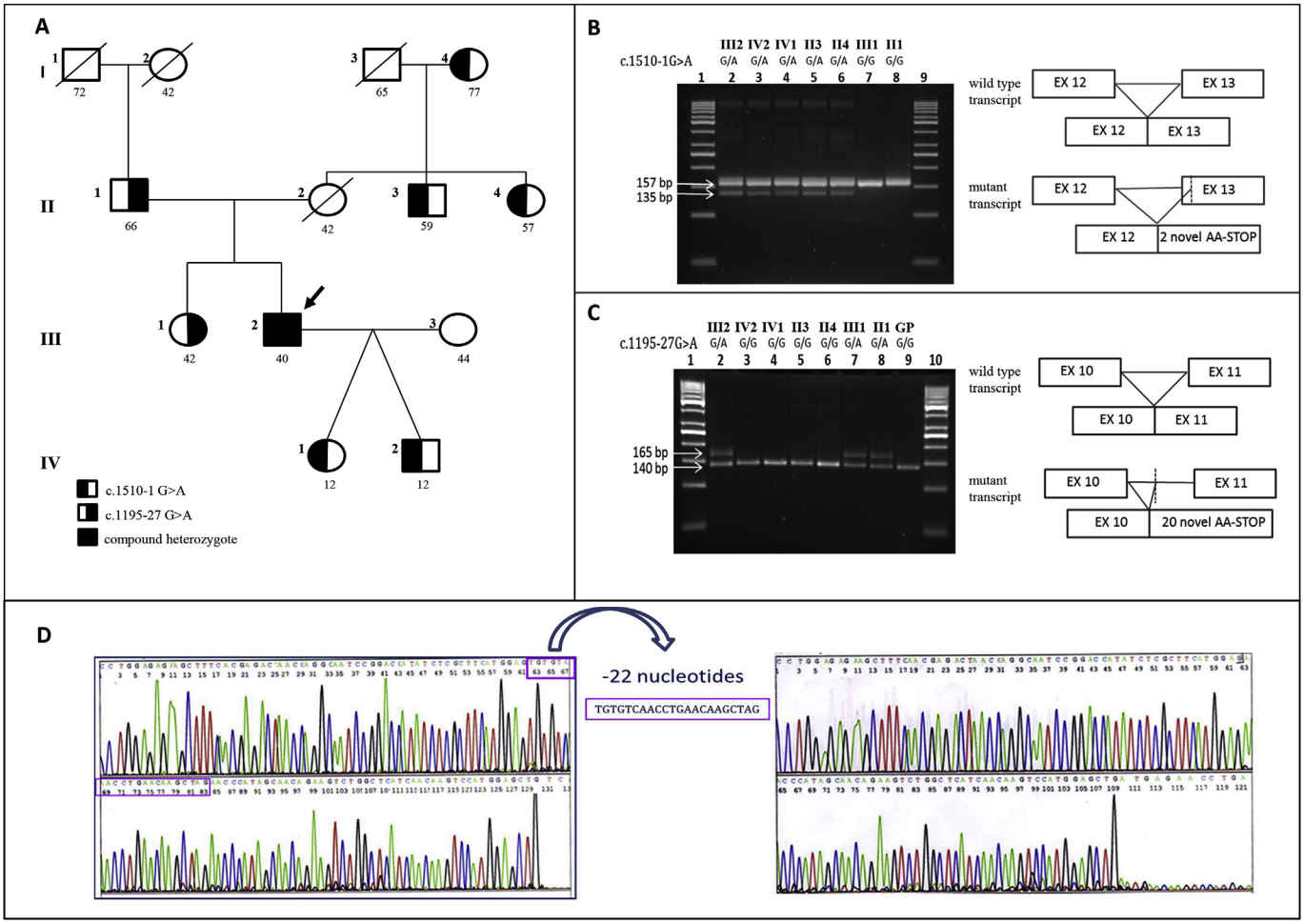

Fig. 1. ***Panel A.*** **Pedigree of TD family.** Squares and circles indicate male and female family members, respectively. Slashes indicate people who were not possible to analyze from genetic point of view. Roman numerals to the left of the pedigree indicate the generation; numerals to the upper left of each symbol indicate the individual family member. Numbers under each symbol refer to kindred's age. ***Panel B.*** **PCR amplification of the *ABCA1* cDNA of the region from exon 12 to exon 13.** Agarose gel electrophoresis of the RT-PCR products are shown on the left: the lower band corresponds to the mutant transcript. Lanes 1 and 9: DNA size markers; lane 2: ABCA1 amplicons in the proband; lanes from 3 to 6: heterozygous subjects for the c.1510-1G > A variant; lanes 7 and 8: subjects without the variant. Schematic representation of the anomalous mRNA splicing caused by the c.1510-1G > A variant is shown on the right. ***Panel C.*** **PCR amplification of the ABCA1 cDNA of the region from exon 10 to exon 11.** Agarose gel electrophoresis of the RT-PCR products are shown on the left: the upper band corresponds to the mutant transcript. Lanes 1 and 10: DNA size markers; lane 2: ABCA1 amplicons in the proband; Lanes from 3 to 6 and 9: subjects without the variant; lanes 7 and 8: heterozygous subjects for the c.1195-27G > A variant. GP indicates a subject from general population. Schematic representation of the anomalous mRNA splicing caused by the c.1195-27G > A variant is shown on the right. ***Panel D.*** **Characterization of the anomalous splicing induced by the c.1510-1G > A mutation.** The cDNA sequence of the exon 12 – exon 13 junction is shown: wild type sequence on the left, mutated sequence on the right.

Table 1
Demographic, clinical and biochemical characteristics of TD family.

Subject	Sex	Age (*years*)	Cholesterol (*mmol/L*)			TG (*mmol/L*)	Lipoprotein (*mg/dL*)		CIMT (*mm*)	Medical history
			TC	LDL	HDL		ApoA1	ApoB		
I.1	M	–	–	–	–	–	–	–	–	Died at age 72 for unknown causes
I.2.	F	–	–	–	–	–	–	–	–	Died at age 42 for unknown causes
I.3	M	–	–	–	–	–	–	–	–	Died at age 65 for unknown causes
I.4	F	77	4.16	2.55	0.98	1.37	103	68	2.5	TEA* left internal carotid artery, subclavian steal syndrome, gallbladder stones
II.1	M	66	4.86	3.60	0.88	0.84	99	90	1.9	Thrombocytopenia, arterial Hypertension
II.2	F	–	–	–	–	–	–	–	–	Died at age 42 for cerebral aneurysm rupture, porphyria
II.3	M	59	3.93	2.70	0.90	0.70	98	68	1.5	Liver steatosis
II.4	F	57	7.31	5.72	1.01	1.28	79	93	1.8	Left subclavian artery angioplasty, arterial Hypertension, hypercholesterolemia
III.1	F	42	3.82	2.50	0.93	0.86	113	64	0.8	Tonsillectomy
III.2	M	40	1.37	0.30	0.03	2.28	0	1	0.8	Tonsillectomy, splenectomy, hepatomegaly, thrombocytopenia
III.3	F	44	5.99	3.87	1.76	0.81	–	–	0.7	Hypercholesterolemia, thyroid nodules
IV.1	F	12	3.44	2.47	0.65	0.70	82	61	–	–
IV.2	M	12	4.57	3.36	0.75	1.02	99	86	–	Tonsillectomy, appendectomy

2.5. Histological analysis

Specimens were prepared for the histologic analysis by fixation in 10% neutral buffered formalin, dehydration through alcohol baths of increasing concentration (from 70% to 100% ethylic alcohol and then xylene) and inclusion in paraffin. Sections of 4 μm thickness were finally prepared and stained with Hematoxylin and Eosin and immunostained with CD68 antibody (clone PG-M1 Dako, Milano, Italy).

3. Results

3.1. Analysis of ABCA1 gene in the TD kindred

ABCA1 gene resequencing showed that proband (subject III-2 in Fig. 1-A) was compound heterozygous for two intronic *ABCA1* mutations: i) the novel c.1510-1G > A in intron 12 and ii) the recently described TD-causing c.1195-27G > A in intron 10 [25]. He was also found to be heterozygous carrier of several variants, all of which were common polymorphisms or resulted to be not damaging by *in silico* prediction analysis as well as by our RT-PCR analysis (data not shown). No mutations were found in *APOAI, LCAT* and *LPL* genes. He showed very low plasma concentration of HDL-C (0.03 mmol/L) and ApoAI (non-measurable levels).

The analysis of the *ABCA1* gene in the proband's relatives revealed that his maternal grandfather, uncle, aunt and the twin children were heterozygotes for the novel c.1510-1G > A mutation, while his father and sister were heterozygotes for the c.1195-27G > A mutation (Fig. 1-B and 1-C). The five heterozygous carriers of the novel intronic variant showed average plasma concentrations of HDL-C and ApoAI of 0.86 ± 0.16 mmol/L and 92.2 ± 10.9 mg/dL, respectively (Table 1). Two of them presented splenomegaly and two reported history of carotid atherosclerosis (Table 1). The heterozygous carriers of the known intronic mutation showed comparably reduced plasma concentrations of HDL-C and ApoAI (0.9 ± 0.04 mmol/L and 106.0 ± 9.9 mg/dL, respectively), in one of them thrombocytopenia was detected (Table 1).

3.2. Analysis of transcripts of ABCA1 gene harboring the splicing variants

To assess the effect of the novel c.1510-1G > A mutation on pre-mRNA splicing, we analyzed *ABCA1* cDNA obtained from peripheral blood mononuclear cells of proband and relatives. The analysis of region encompassing exon 12 and exon 13 demonstrated that family members without the c.1510-1G > A showed a single ABCA1 transcript of the expected size (157 nucleotides) (Fig. 1 Panel B, lanes 7 and 8). Conversely, proband showed two transcripts of 157 nucleotides and of 135 nucleotides, corresponding to the wild-type and mutated allele, respectively (Fig. 1 Panel B, lane 2). All relatives heterozygous tested for this variation (proband's uncle, aunt and twin children), showed a similar anomalous splicing pattern (Fig. 1 panel B, lanes from 3 to 6). The sequence of the mutant transcript of 135 nucleotides showed that the initial 22 nucleotides of exon 3 were missing (Fig. 1 Panel D). Due to the aberrant sequence of spliced exon 3, a translation of 2 amino acids followed by a premature stop codon at position 506 of ABCA1 protein was predicted (Fig. 1 Panel B). This suggests the translation of a not functional truncated ABCA1 (505 aa) protein. We also determined the effect of the known c.1195-27G > A variant. This mutation gave rise to a 165 nucleotide aberrant transcript in the proband (Fig. 1 Panel C, lane 2) and heterozygous relatives (proband's father and sister) (Fig. 1 Panel C, lanes 7 and 8). The sequence of the mutant transcript confirmed the retention of 25 nucleotides of intron 10 of *ABCA1* leading to the insertion of a stop codon after 20 aberrant amino acids (Fig. 1 Panel C) at position 419 of the ABCA1 protein thus resulting in a truncated (418 aa) and dysfunctional protein as recently described [25].

3.3. Histological analysis of the spleen

We had the opportunity to revise the proband's spleen specimen. It was noted that between white pulp lymphoid follicles and red pulp Billroth cords, the splenic parenchyma showed aggregates of medium size cells characterized by a peripheral nucleus (sometimes with a small nucleolus) and a large cytoplasm. In keeping with TD the latter was mostly replaced by empty vacuoles (in haematoxylin and eosin stain Fig. 2 Panel A), suggestive of the fat presence. CD68 stain consistently showed intense immunoreactivity confirming histiocytic differentiation (Fig. 2 Panel B). The lipid histiocytosis was associated with red pulp congestion.

4. Discussion

Here we report the molecular characterization of a patient presenting several typical features of TD, including almost undetectable plasma HDL-C and ApoAI concentrations, hypocholesterolemia, elevated plasma triglycerides, thrombocytopenia, enlarged tonsils (tonsillectomy) and hepatomegaly. It is noteworthy that he did not show signs of premature atherosclerosis. However, the most relevant event in patient's medical history was the splenectomy due to the spontaneous rupture of the spleen. We did not have chance to examine the report of macroscopic evaluation of resected spleen, but we can reasonably assume that the spleen

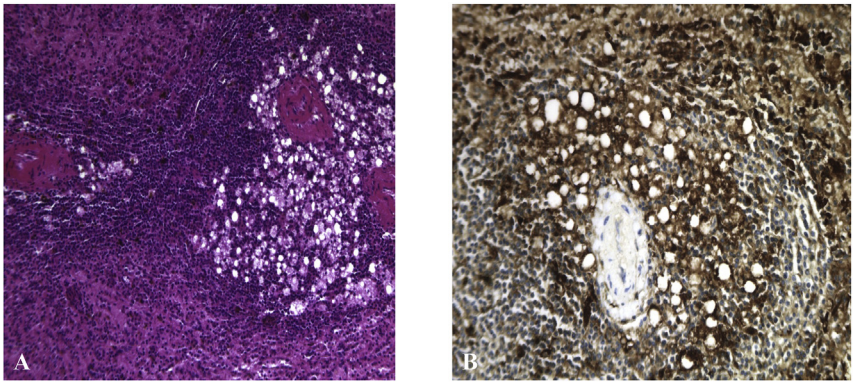

Fig. 2. Microscopic photograph of section of spleen of TD proband. ***Panel A***. A collection of foamy and vacuolated (lipid rich) histiocytes (paraffin embedded tissue; Haematoxylin and Eosin stain; x10 magnification). ***Panel B***. CD68 immunohistochemical stain shows histiocyte differentiation (x20 magnification).

rupture was due to intra-splenic hemorrhage. To this regard, the revision of spleen biopsy revealed the engulfment of red pulp and the presence of foamy histiocytes, highly suggestive for lipid accumulation.

Although infrequent, this does not represent a novel finding in TD, as others have identified bleeding tendency associated with this disorder. In fact, Hooper and co-workers [26] first described the case of a 62-year-old man with TD due to a nonsense mutation in *ABCA1* who presented with severe anemia secondary to a spontaneous splenic hematoma also requiring elective splenectomy. The hemorrhagic diathesis in TD has been mainly attributed to thrombocytopenia, which is thought to be linked to a hematopoietic defect interesting all bone-marrow cell lineages, including megakaryocyte [27]. It has been reported that the lack of ABCA1 increases platelet size, thus suggesting the potential contribution of this transporter in the last steps of megakaryocytopoiesis [28]. Consistently, our patient's bone marrow biopsy revealed a mild hematopoietic hypoplasia and the lack of resolution of thrombocytopenia after splenectomy strongly suggests that defect in platelet maturation not in catabolism (due to hypersplenism) was the most probable cause of reduced platelets counts in our patient. On the other hand, there is evidence indicating that TD may also cause platelet dysfunction, as demonstrated by reduced reactivity to low doses of thrombin or collagen in *abca1* −/− platelets [28]. To explain this abnormality, it has been proposed that the ABCA1 transporter is involved in the calcium-dependent exposure of the anionic PS to the plasma membrane. It is known that this mechanism represents a crucial step in the development of pro-coagulant activity of platelets [29]. In addition, it has been recently shown that in the absence of ABCA1, the platelet generation of TXA2, 12-HETE, and 15-HETE are significantly decreased [28], thus impairing the positive feedback loop needed to amplify platelet aggregation and, in turn, thrombus growth and stability. If these mechanisms might expose TD patients to an exaggerated response to antiplatelet medications or influence their cardiovascular risk is far to be clearly determined.

The molecular analysis demonstrated that our patient was a compound heterozygote for two damaging *ABCA1* intronic mutations, one novel and one previously reported. We investigated their effect by analyzing the RT-PCR transcript size and sequence and found that both variants altered *ABCA*1 pre-mRNA splicing. In particular, the novel c.1510-1G > A intronic variant affected exon 13 transcription, causing the partial loss of 22 amino acids of exon 13 with the consequence of introducing a stop codon. This may predict

Table 2
Demographic, laboratory and clinical characteristics of described TD patients carrying intronic *ABCA1* mutations.

Splicing Mutation	cHe/Ho	Age	Sex	Lipid Profile	Major TD clinical manifestations	CVD and comorbidities	Reference
IVS2+5G>C	cHe	66	Female	TC 2.82 HDL-C 0.08 TG 2.18 Apo-A1 <10	Tonsillectomy	NO CVD, thoraco-abdominal dissection, primary hypogonadism	[32]
IVS7+6C>T IVS31-1 G > C	cHe	41	Male	TC 2.62 HDL-C 0.31 TG 0.63 Apo-A1 70	Absent	Maternal grandfather MI at 35 years	[42]
IVS20-2A > C	Ho	37	Male	TC 1.5 HDL-C 0.10 TG 2.08 Apo-A1 <3.90	Thrombocytopenia, orange tonsils, hepato-splenomegaly	No CVD	[33]
IVS24+1G>C	Ho	43	Male	TC NA HDL-C <0.10 TG 1.96 Apo-A1 NA	NA	MI at 38 years	[34]
IVS25+1G>C	cHe	38	Male	TC 2.3 HDL-C 0 TG 2.00 Apo-A1 1	Splenomegaly, corneal opacities, peripheral neuropathy, tonsillectomy	MI at 38 years, peripheral artery disease	[38]
IVS29-11 T > G	cHe	4	Female	TC 1.29 HDL-C 0.14 TG 1.70 Apo-A1 <0.15	Splenomegaly, thrombocytopenia, yellowish tonsils	No CVD	[35]
IVS31-1A > G	Ho	6	Female	TC 1.60 HDL-C 0.06 TG 1.5 Apo-A1 ND	Thrombocytopenia, hepato-splenomegaly	No CVD	[36]
IVS32-1 G > C	Ho	38	female	TC 3.20 HDL-C <0.13 TG 1.56 Apo-A1 0.70	Relapsing-remitting major neurologic symptoms, corneal opacity	CIMT 0.6 mm [>66th age-sex specific percentile]	[39]
IVS34+1G>A	cHe	53	Female	TC NA HDL-C 0.13 TG 3.57 Apo-A1 1	Thrombocytopenia, orange tonsils, splenomegaly, mixed peripheral neuropathy	MI at 57 years	[40]
IVS35+1G>A	Ho	7	Male	TC 1.89 HDL-C 0.10 TG 1.93 Apo-A1 <5	Hypertropfic tonsils, yellowish pharyngeal deposits	No CVD, Mild dimorphism, developmental and speech delay, abnormal gait, congenital cataracts	[41]
IVS46:delT −39-46	cHe	57	Male	TC 2.97 HDL-C 0.16 TG 1.07 Apo-A1 24	Absent	PTCA at 44	[37]

cHe, compound Heterozygous; Ho, Homozygous; TD, Tangier Disease; CVD, cardiovascular disease; TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; TG, Triglycerides; Apo-A1, Apolipoprotein A1; MI, Myocardial Infarction; NA, data Not Available. TC, HDL-C and TG levels are expressed in mmol/L; Apo-A1 is expressed in mg/dL.

to result in a non-functional truncated ABCA1 protein. This variant was detected at heterozygote state in five family members, which showed moderately reduced plasma levels of HDL-C and ApoAI but not the clinical signs of lipid accumulation, such as hepatosplenomegaly or tonsillar enlargement. Therefore, the resulting phenotype is more suggestive for FHD. It is remarkable that three carriers of this mutation developed extra-coronary atherosclerosis.

The known c.1195-27G > A variant in intron 10 was identified in the paternal proband's side and heterozygous carriers also displayed a FHD-compatible phenotype. This intronic mutation has been already reported in another Italian patient diagnosed with FHD, where HDL-C concentration was much lower than that observed in our heterozygous carriers, being in the range of TD (0.14 mmol/L). The reason of this discrepancy is not clear, even though it might be related to the severe hypertriglyceridemia reported in this subject [25].

A limitation of our study is that we were unable to obtain fibroblasts from the patient and his heterozygous family members to perform cholesterol efflux study and, therefore, to directly measure the functional effect of both novel and known intronic mutations. However, the family segregation of lipid phenotypes was highly suggestive that both *ABCA1* variants were functionally defective.

It has been noted that despite *ABCA1* is a very large gene, only few intronic mutations have been reported in TD, so far. Table 2 lists those already described in the literature. It is noteworthy that these 11 patients showed a very pronounced hypoHDLemia (mean levels of HDL-C 0.12 ± 0.8 mmol/L and ApoAI 13 ± 23 mg/dL) and 36% had thrombocytopenia. Moreover, 30% of them reported history of premature CVD, which is higher if compared to the 15–20% prevalence reported in the literature [4]. Overall, these findings further demonstrate that intronic variants by affecting exon splicing may be causative of genetic low HDL syndromes, thus reinforcing the concept that they should not be neglected. Although the scansion of introns is a time-consuming and expensive effort, it should be always planned for large genes, such as *ABCA1,* at very least when the phenotype is highly suggestive of a disease and exon analysis is not able to reveal any mutation [30]. On the other hand, approaches of mutational search based on next generation sequencing approaches may greatly facilitate the search for a large number of mutations, as also application to other diseases has been demonstrating [31].

We have reported a new case of TD presenting, in addition to the typical features of this disorder, a severe complication represented by spleen rupture. We found that the patient was a compound heterozygote for a novel and a known intronic mutations causing aberrant splicing of *ABCA1* mRNA. His heterozygous family relatives carrying each one of mutated alleles showed a clinical phenotype compatible with the diagnosis of FHD. This study expands the catalog of intronic mutations causative of TD and FHD, further supporting the notion that the search and functional characterization of intronic variants should be convincingly pursued. Moreover, common and distinctive molecular features of TD and FHD are evidenced.

Ethics approval and consent to participate

This study is included in the program “Mappatura genetica delle dislipidemie in Italia” – 13/2011 Cardiovascolare, approved by the Ethics Committee of Sapienza University of Rome on the 14th June 2012.

Availability of data and material

The datasets used and/or analysed during the current study are available from the following authors: Marcello Arca and Marco Lucarelli.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.11.064.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.bbrc.2018.11.064.

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