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GENOTYPE AND PHENOTYPE OF SUBJECTS WITH TANGIER DISEASE DEVELOPING CARDIOVASCULAR DISEASE

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***ABSTRACT* – Objective:** Tangier Disease (TD) is a rare inherited disorder that disrupts high-density lipoprotein (HDL) metabolism due to loss-of-function variants in the ATP-binding cassette subfamily A member 1 *(ABCA1)* gene. TD patients exhibit diverse clinical presentations, with only some individuals developing cardiovascular disease (CVD) despite extremely low HDL levels. To date, no detailed analyses have explored the genetic and phenotypic markers predicting cardiovascular complications in TD patients. This study aims to identify specific traits distinguishing TD patients prone to CVD.

Materials and Methods: We reviewed all documented TD cases with a genetic diagnosis and constructed a clinical and genetic database using terms from the Human Phenotype Ontology database.

Results: Among 73 TD patients with genetic diagnoses reported in the literature, 30 (41%) experienced CVD, while 43 (59%) did not. Patients with CVD exhibited recurring *ABCA1* variants (n=25), absent in those without complications. These variants also showed distinct distributions across *ABCA1* protein domains (X^2=6.0685, p=0.04). Demographic and clinical features strongly associated with cardiovascular risk included older age (53 ± 10 years *vs.* 45 ± 15 years, p=0.04), orange-colored tonsils (X^2=10.374, p=0.001), and hepatomegaly (X^2=6.423, p=0.01). Lipid profiles and gender differences were not significant between groups.

Conclusions: This study demonstrates, for the first time, that both genetic and phenotypic markers distinguish TD patients with cardiovascular complications. Future research is needed to confirm whether these differences reflect distinct disease mechanisms.

Keywords: Tangier disease, Variants, High-density lipoprotein metabolism, Atherosclerosis.

INTRODUCTION

Tangier disease (TD) (OMIM 205400, ORPHA 31150) is an inborn metabolic disease (IMD) caused by a deficiency in High-Density Lipoprotein (HDL) metabolism[1]. The prevalence of this disease has been estimated at 1:640.000[2].

The disease was first identified in a proband from Tangier Island, located in Chesapeake Bay, (VA, USA), an isolated community that remained geographically secluded for centuries[3].

By the late 1990s, researchers established that TD results from loss-of-function variants in the *ABCA1* gene[4-6]. Individuals carrying homozygous or compound heterozygous loss-of-function variants in *ABCA1* exhibit extremely low HDL levels and an accumulation of cholesteryl and retinyl

esters, along with carotenoids, in non-adipose tissues. Clinically, TD may present with multi-systemic manifestations, including hepatosplenomegaly, anemia, thrombocytopenia, peripheral neuropathy, and corneal opacifications. Cardiovascular disease (CVD) is also observed in some, but not all, patients[7].

Genome-wide studies have identified polymorphisms in *ABCA1* loci as significant determinants of HDL cholesterol levels in the general population[8]. However, whether these common polymorphisms in *ABCA1* associate with altered risk of CVD is less clear.

To date, no analysis has specifically focused on *ABCA1* variants associated with cardiovascular complications in TD patients. Investigating these variants offers a unique opportunity to explore the genetic mechanisms underlying *ABCA1*'s impact on cardiovascular risk, particularly as all identified variants result in a loss of gene function.

Thus, the purpose of this study is to assess the prevalence and localization of *ABCA1* variants, and the clinical phenotypes specifically associated with the cardiovascular complications in all TD subjects reported in the literature.

MATERIALS AND METHODS

Literature Search

We applied the PRISMA methodology in PubMed, Scopus, and Web of Science libraries until September 2024 using the following predefined term: "Tangier Disease". We could retrieve a total of 765 articles. We selected only original articles (568) and, among them, we excluded reports lacking complete and adequate information on clinical and genetics data, which left a total of 100 papers available for this review (**Supplementary Figure 1**).

TD Subject Database Creation

We collected from the literature all the cases of TD (n=128) with a reported genetic cause of the disease, and we built the clinical and genetic database using the terms associated with TD in the Human Phenotype Ontology database.

Among the included cases, a specific description of the presence or not of a CVD, defined as carotid artery stenosis or accelerated atherosclerosis or coronary artery stenosis or left ventricular hypertrophy, was reported in 73 cases.

Statistical Analysis

Frequency of categorical variables was analyzed with Pearson's χ^2. Quantitative variables were compared using T-test. Significance was set at p<5*10-2. SPSS for Mac (vers. 29 for Mac, IBM-SPSS Bologna, Italy) and GraphPad, Prism (vers. 8.1.3 for Mac, GraphPad Software, La Jolla, CA, USA) were used for the statistical analyses.

RESULTS

Variants of ABCA1 associated with cardiovascular manifestations in TD cases

Among the 128 cases that we collected from the literature, a total of 79 different genotypes were identified. The most frequent were c.1764delG (6 cases), c.2033C>A (4), c.5094C>A, and c.219insT (3 cases), respectively. In 67% of patients the disease was caused by a homozygous variant and homozygous subjects were significantly younger (p=0.016). Among them, 45% of cases (n=30) developed a CVD and 23% (n=15) died after it. By selecting only subjects with a CVD, we found 31 variants of *ABCA1* gene,

of which 25 known to cause a change in the protein-coding sequence (Table 1 and Figure 1, panel A). Most of them were specifically associated with the CV phenotype: only 6 variants (p.N935H, p.H1600R, p.R1270X, p.R587A, p.W1699C, p.Y1698X) were detected in patients with or without a CVD. All published cases carrying the homozygous c.1764delG (p.L548X) variant suffered from a CVD. In particular, this variant appears specifically associated with CVD since it was not reported in patients who did not develop a CVD.

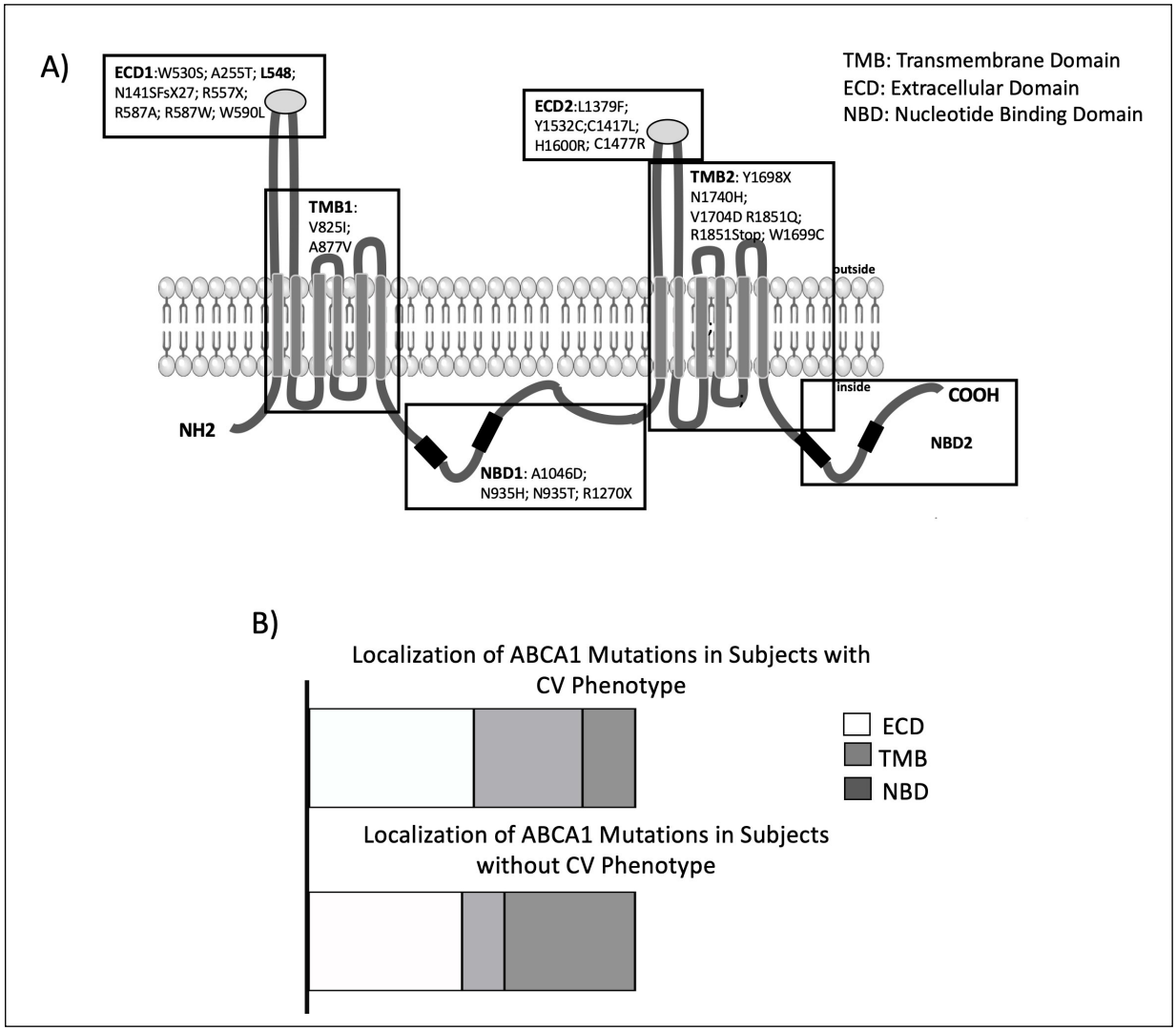

Figure 1. Localization of the ABCA1 variants associated with CV disease in TD subjects. The majority of ABCA1 variants in TD cases with CV phenotype occurs in the large extracellular loops, while the areas where ATP is hydrolysed (NBDs) are apparently the less mutated in subjects with CV disease (Panel A). Comparison of the variant localization on ABCA1 protein domains associated with (Panel B, upper) or without CV disease (Panel B, bottom).

Functional mapping of ABCA1 variant associated with cardiovascular manifestations in TD cases

To understand whether variants associated with the CV phenotype specifically map on functional regions of *ABCA1*, we assessed their distribution on the different *ABCA1* protein domains.

ABCA1 is composed of two transmembrane domains (TMDs), two nucleotide-binding domains (NBDs), and two extracellular domains (ECDs)[9]. The two large extracellular domains are responsible for the binding of apo A-I[8], the two nucleotide-binding domains function as ATP hydrolases providing the energy for substrate transport and the two large transmembrane domains (TMDs) contain the substrate-binding site[10].

Table 1. Variants of *ABCA1* associated with CV disease in TD subjects.

Aminoacid Change	DNA Change
A1046D	C3137A
A255T	G1158A
A877V	C2750T
C1417L	T4369C
C1477R	G3738C
H1600R	1758_1759 insGA4799G
L1379F	C4425T
L548	G1764
N141SFsX27	NA
N1740H	A5338C
N935H	A3198C
N935T	A3198G
R1270X	C3808T
R1851Q	G5947A
R1851Stop	C5946T
R557X	NA
R587A	1758_1759insG
R587W	C1699T
V1704D	T5401A
V825I	NA
W1699C	A5097G
W530S	G1709C
W590L	NA
Y1532C	A4595G
Y1698X	C5094A

Of all published *ABCA1* variants reported in TD cases with a CVD, 50% mapped in the extracellular domain (ECD1 and 2) regions, with a 33% in ECD1 and 17% in ECD2, respectively (Figure 1 Panels A and B). A total of 34% mapped on the transmembrane domains (9% on TMB1 and 25% on TMB2); 16% were on the nucleotide-binding domain 1 (NBD1) and no variant on the NBD2 (C-term).

Looking at the distribution of the variants detected in subjects who did not develop a CVD, the extracellular distribution rate is maintained (30% in ECD1 and 17% in ECD2), while, intracellularly, the rate in NBD1 (33%) and NBD2 (7%) increases to 40%, and in TMB1 and 2 decreases to 13% (0% and 13%, respectively) (Figure 1 Panel B). The localization pattern of variants was significantly different between subjects with and without a CVD (X^2=6.0685, p=0.048).

Other factors associated with cardiovascular manifestations in TD cases

To understand whether other features may be able to distinguish TD cases with or without a CVD, we compared clinical and demographics characteristics of the subject included in the study.

By this analysis, we found that the group of patients with CVD was older (53±10y) than the non-CVD patients (45±15y, p=0.04). Considering the multi systemic manifestations of the disease (Table 2) and comparing subjects with or without CVD, we found that the presence of CVD is associated with the

Table 2. The Human Phenotype Ontology terms associated or not to cardiovascular manifestations in TD patients.

HPO_TERM_NAME	Category	Association with Cardiovascular Phenotype
Anemia	Blood and blood-forming tissues	Yes
Thrombocytopenia	Blood and blood-forming tissues	Yes
Hepatosplenomegaly	Digestive System	Yes
Chronic noninfectious lymphadenopathy	Immunology	Yes
Orange discolored tonsils	Immunology	Yes
Hypertriglyceridemia	Metabolism/Laboratory abnormality	No
Hypocholesterolemia	Metabolism/Laboratory abnormality	No
Impaired thermal sensitivity	Constitutional Symptom	NA*
Abdominal pain	Constitutional Symptom	No
Corneal opacity	Eye	Yes
Ectropion	Head and neck	NA*
Distal muscle weakness	Musculature	NA*
Facial diplegia	Musculature	NA*
Syringomyelia	Nervous System	No
Peripheral axonal neuropathy	Nervous System	No
Progressive peripheral neuropathy	Nervous System	No
Nail dystrophy	Skin, Hair, and Nails	No
Dry skin	Skin, Hair, and Nails	No

*Data not available in analyzed TD subjects.

presence of orange discoloured tonsils (X^2=10.374, p=0.001), splenomegaly (X^2=3.923, p=0.04), hepatomegaly (X^2=6.423, p=0.01), corneal opacity (X^2=4.861, p=0.03), anemia (X^2=7.875, p=0.02) and thrombocytopenia (X^2=5.185, p=0.03) (Table 1).

On the other hand, the lipid profile (LDL, HDL, Total Cholesterol, and Triglycerides) of cases with or without CVD was not different (Table 3). Data on cholesterol efflux of primary fibroblasts were reported only in 12 cases (6 with CVD and 6 without), thus we did not consider them in the statistical analysis.

No association was found with CVD and the presence of homozygous or compound heterozygous variants, the patient's gender, and neurological manifestations.

Table 3. Lipid profile of TD patients with or without CV disease.

	Without CVD (n=33)	With CVD (n=25)
Total Cholesterol (mg/dl)	83.5 ± 32.8	85.6 ± 39.8
HDL (mg/dl)	3.4 ± 2.0	3.1 ± 1.9
Triglycerides (mg/ml)	216.2 ± 185.0	206.9 ± 100.6
LDL (mg/dl)	62.0 ± 37.1	66.0 ± 37.1

DISCUSSION

The investigation of TD provides profound insights into the critical role of the *ABCA1* in cholesterol metabolism and cardiovascular health.

ABCA1 protein facilitates the transfer of cholesterol and phospholipids from peripheral cells, such as macrophages, to lipid-poor apolipoprotein A-I (apoA-I)[11]. This step is vital for the formation of nascent HDL particles[12]. In TD, defective or absent *ABCA1* function disrupts this process, leading to the intracellular accumulation of cholesterol and lipids in peripheral tissues[13]. The absence of functional HDL in TD leaves excess cholesterol stranded in the arterial wall, fostering an environment conducive to atherogenesis[14]. Moreover, HDL deficiency eliminates its systemic protective roles, amplifying the risk of cardiovascular complications.

However, even if early studies on TD, patients reported a high incidence of CVD[4-6], when more cases were observed globally, it became evident that the clinical manifestations of TD are highly variable, ranging from neurological symptoms to cardiovascular complications.

In this study, we identified recurrent variants in 45% of TD patients who developed CVD (**Figure 1**). Notably, the p.L548X variant is the most common, found in 20% of CVD patients. Originally described by Rust et al[6] in 1999, this variant was discovered in a German family with premature CVD onset. The variant, a 1-bp deletion in exon 13 (c.1764delG), results in a stop codon that eliminates most of the *ABCA1* protein sequence, including both ATP-binding cassettes.

Further studies comparing fibroblasts from two TD patients – one with the p.L548X variant and another with a p.N935S variant not associated with CVD – revealed significantly lower cholesterol efflux capacity in p.L548X cells. This finding suggests that variants like p.L548X, which severely impair protein function, may be linked to the development of CVD.

Another case study identified a 20% difference in cholesterol efflux capacity between fibroblasts from patients with differing cardiovascular phenotypes and *ABCA1* variants. One subject, who had a severe CVD phenotype, carried a missense variant (p.C1477R), whereas another, without atherosclerosis, harbored a nonsense variant (GG5277,8C) and a *de novo* missense variant (p.T929I). These findings suggest that more severe *ABCA1* variants are likely associated with cardiovascular complications. We hypothesized that recurrent variants in subjects with CVD could disrupt a specific function of *ABCA1*, possibly its interaction with apoA-I or ATPase activity. Since functional domains of *ABCA1* are well-characterized, we analyzed the location of variants on these domains.

Our analysis confirmed that most variants occurred in the large extracellular loops responsible for apoA-I binding, consistent with previous reports on TD patients. However, for the first time, we observed that variants in Transmembrane Binding Domains (TMBs), which regulate substrate transport, were more common in patients with CVD, whereas variants in Nucleotide Binding Domains (NBDs), which function as ATP hydrolases, were more frequent in subjects without CVD.

In addition to genetic findings, we identified specific multi-systemic manifestations associated with CVD, such as orange discolored tonsils, splenomegaly, hepatomegaly, corneal opacity, anemia, and thrombocytopenia. Interestingly, these traits were not linked to the neurological manifestations often seen in TD patients, suggesting two distinct subgroups: one prone to cardiovascular issues with mild neurological symptoms, and another with more severe neurological involvement but without CVD.

Surprisingly, no differences were found in lipid profiles (LDL, HDL, total cholesterol, and triglycerides) or other demographic features, except for age. This discrepancy suggests that the relationship between lipid levels and cardiovascular risk in TD is more complex than previously thought. Factors like environmental influences and the multi-step process of atherosclerosis may obscure the role of HDL levels in CVD risk. More research, particularly cellular models, is needed to clarify this relationship and assess the full risk profile for TD patients.

CONCLUSIONS

Our results indicate that the type and location of *ABCA1* variants play a key role in the molecular mechanisms leading to specific complications in TD. This finding could have significant implications for understanding the pathogenesis of TD and for guiding the genetic evaluation and clinical management of patients.

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