Whole exome sequencing in a family with thromboangiitis obliterans

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Contributions: GM, ADA and FB study concept; PDV and ADA collected clinical data; PDV performed laboratory analysis; BL, NZ, GP and MB, methodology; NZ, GP and MB bioinformatics analysis; BL, NZ and DB data analysis; BL, GM and FB data interpretation and resources; BL, MP, GM and FB writing-review and editing; BL and DB preparation of tables and figure; FB supervision. All the authors approved the final manuscript.

Conflicts of interest: the authors declare no conflict of interest.

Ethical approval: the study, an investigator-initiated institutional review board-approved investigation for improved genetic diagnosis, was conducted according to the guidelines of the Declaration of Helsinki, and approved (June 2015) by the Institutional Review Board of the San Raffaele Hospital.

Informed consent: informed consent was obtained from all family members involved in the study.

Availability of data and material: all relevant data are included in the manuscript. Additional original data will be made available by contacting the corresponding author within the regulations of ethical approval.

Funding: this research was funded by FAR (Fondo di Ateneo per la Ricerca Scientifica) of the University of Ferrara.

Acknowledgments: the authors thank the family for study participation and cooperation.

Editor's note: this manuscript belongs to the issue BTVB 2026;5(3), dedicated to the memory of Armando D'Angelo.

Received: 7 August 2025.
Accepted: 14 January 2026.

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Bleeding, Thrombosis and Vascular Biology 2026; 5:367
doi:10.4081/btvb.2026.367

ABSTRACT

Low-frequency candidates for susceptibility to thromboangiitis obliterans (TAO) were investigated by whole exome sequencing in a family with a young member with migrating thrombophlebitis, artery thrombosis, and ulcerations followed by below-knee amputation. Filtering among 193 candidates for venous thromboembolism (VTE) detected rare heterozygous variants: the *PPIF* (peptidylprolyl-isomerase F) frameshift p.Tyr121fs*0 [minor allele frequency (MAF)= 0.000004], the *F2* (thrombin) synonymous/splicing p.Gly271=, and 5 missense variants. Among these, the novel *PTGIR* (prostaglandin I2 receptor) p.Tyr144Asn would interfere with transmembrane helix interaction, and potentially decrease prostacyclin response. Filtering among 92 genes, suggested by previous genomic and transcriptomic studies in TAO patients, detected a rare combination of *HLA-DQB1* alleles, and two in linkage variants (MAF=0.0003) in titin (*TTN*). Interestingly, *TTN* is also reported among VTE proteome alterations. By exploring the whole exome, the novel *BET1* nonsense p.Glu33Ter change may indirectly influence hemostasis and endothelial integrity. This study pinpoints novel TAO candidate genetic components for further investigation.

Key words: thromboangiitis obliterans, Buerger's disease, whole exome sequencing.

Introduction

Thromboangiitis obliterans (TAO) or Buerger's disease is a rare non-atherosclerotic occlusive disease affecting the small and medium arteries and veins in the upper and lower extremities.[1] The presence of an inflammatory thrombus is the pathological hallmark of TAO. The etiology of TAO remains incompletely understood, with several potential factors implicated, including endothelial dysfunction, immune-mediated mechanisms, hypercoagulable states, and overall inflammatory responses that might contribute to the disease progression.[2]

Genetic components have been suggested to contribute to TAO susceptibility or molecular mechanisms of the disease.[3] The genetic background has been explored through polymorphism analysis in genes involved in immunity, encoding platelet receptors, and regulating vascular tone. However, most association findings, which are quite regional, require replication.[2] Concerning the thrombotic manifestations and altered hemostasis, the role of thrombophilia in TAO is quite controversial.[4]

This study was aimed at investigating, in a family with a young affected member, candidate genetic components of TAO by focusing on low-frequency variants, detected by whole exome sequencing (WES) in genes known to contribute to hemostasis or associated with venous thromboembolism (VTE) and related phenotypes.[5] In addition, genes previously suggested by studies in TAO patients were explored.[3,4,6-8] The progressively declining incidence of TAO in the Caucasian population, coupled with the substantial reduction in the number of pedigrees suitable for genetic investigation, further support this investigation.

Materials and methods

Whole exome sequencing analysis

Genomic DNA was extracted from peripheral blood using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). WES was performed as previously detailed[5] on the NovaSeq platform (Illumina, San Diego, CA, USA) with 150-bp paired-end reads. Reads were mapped against the hg19 human reference sequence. Variants calling was performed by the Complete Genomics Small Variant Caller.

WES analysis was conducted on 193 genes, listed in *Supplementary Table 1* and known to contribute to hemostasis or associated with VTE and related phenotypes. WES analysis was conducted on 92 genes suggested by previous genomic (n=11) and transcriptomic (n=53) studies[3,4,6-8] in TAO patients (*Supplementary Table 1*). WES analysis in the whole exome was focused on premature termination codons unreported in databases, and rare variants homozygous only in the propositus.

Functional prediction

The potential impact of variants on protein function and their pathogenicity were predicted by 14 multi-component bioinformatics tools (AlphaMissense, FATHMM, FATHMM MKL, FATHMM XF Coding, Likelihood Ratio Test, MetaLR, MetaSVM, Mutation Assessor, Mutation Taster, PROVEAN, PhD-SNPg, Polyphen-2-HDIV, Polyphen-2-HVAR and SIFT).[5] Variants features were also investigated by Variant Effect Predictor (VEP, Ensembl resource), and implemented by GeneCards®, database/literature search including ClinVar annotation (NCBI resource), GWAS catalog, quantitative trait loci (QTL) analysis (GTEx Portal) and UniProt resource.

Results and discussion

Patient' clinical and laboratory phenotypes

The patient, referred to as *the case*, developed left femoral-popliteal thrombophlebitis at the age of 18 years followed by migrating superficial thrombophlebitis in both the upper and lower extremities, which lead to ulcerations. At the age of 24 years, after undergoing coagulation tests and receiving diagnosis of a suspected autoimmune disease, the patient began corticosteroid therapy, which provided partial benefit. This was followed by immunosuppressive therapy with cyclosporine and azathioprine, but no improvements were observed. At the age of 27 years, the patient started anticoagulation therapy with Coumadin. Two years later, thrombosis occurred in the femoral-popliteal and anterior tibial arteries, followed by trophic ulcerations on the left leg. The worsening of these ulcerations led to a below knee amputation of the left leg. The patient did not smoke, a TAO risk factor consistently reported.[2,3]

Routine thrombophilia testing excluded the deficiencies of antithrombin, protein C, protein S, APC resistance, and lupus anticoagulant. However, high factor VIII activity values (200%) were detected during heparin prophylaxis. Furthermore, 2 days after temporary suspension of anticoagulation, thrombin generation assays with the addition of soluble thrombomodulin and Fondaparinux showed a two-fold increase in both extrinsic thrombin potential (ETP) and peak parameters. Normal values, observed in the presence of soluble thrombomodulin only, would suggest resistance to this heparin analogue.

Routine genetic assays excluded the presence of factor V Leiden and prothrombin G20210A mutations.

The patient's family (Table 1) consisted of his parents, two sisters, and two brothers, all available for the study, along with two additional brothers, none of them reporting thrombotic episodes.

Whole exome sequencing analysis

WES analysis was conducted on hemostasis-VTE associate genes, on genes suggested by previous genomic and transcriptomic studies in TAO patients, and in the whole exome by focusing on premature termination codons unreported in databases, and rare variants homozygous only in the propositus.

The main genetic findings of WES analysis are reported in Table 1 together with variant inheritance and zygosity status in the family members. The experimental and *in silico* observations, and hypothetical functional interpretation of gene variants are summarized in Table 2.

Venous thromboembolism associated genes

WES analysis of the 193 VTE candidate genes was preliminarily filtered for minor allele frequency (MAF) <0.01. We identified 7 heterozygous variants, including 5 missense mutations, one base deletion leading to premature termination of translation and one synonymous codon (Table 1, listed by frequency).

The rare *PPIF* p.Tyr121fs*0 variant predicts truncation of the peptidylprolyl isomerase F (207aa), also known as cyclophilin D, an ubiquitously expressed protein that is part of the mitochondrial permeability transition pore. The prothrombotic contribution conferred by the potentially loss-of-function truncated cyclophilin D deserves further investigation, in light of its critical role in platelet activation and thrombosis.[9]

Only two missense variants were consistently classified as *deleterious* (Figure 1) by the majority of protein prediction bioinformatics tools: the *PTGIR* p.Tyr144Asn, by 11/14, and *KLK13* p.His109Tyr, by 7/14.

Notably, the novel *PTGIR* variant, unreported in explored databases, affects the receptor for prostacyclin, a G protein-coupled receptor with seven membrane-spanning alpha-helical domains.[10] Secondary structure analysis indicates that p.Tyr144 is the most conserved residue within helix 4, and is involved in hydrogen bond with helix 2 (Figure 1). The substitution of

Table 1. Genetic features of filtered variants.

Gene - SNP	MAF	cDNA	Protein change	Variant inheritance and zygosity in the family						
				I1	I2	II1	II2	II3	II4	II5
VTE associated genes										
PTGIR - no rs	-	c.430T>A	p.Tyr144Asn	h			h	h		
PPIF - rs1855982637	0.000004	c.363delC	p.Tyr121fs*0		h	h		h		
PEAR1 - rs369608309	0.00002	c.2653C>T	p.Arg885Cys	h			h	h	h	
IL13 - rs148077750	0.0001	c.215T>C	p.Leu72Pro	h		h	h	h		
CBS - rs150828989	0.0016	c.1643G>A	p.Arg548Gln	h			h	h	h	h
ZFPM2 - rs187043152	0.0034	c.1632G>A	p.Met544Ile		h	h	h	h	h	h
F2 - rs5899	0.0086	c.813C>T	p.Gly271=		h	h		h	h	
KLK13[#] - rs34089525	0.0221	c.325C>T	p.His109Tyr		h	h		h		h
TAO candidate genes*										
TTN[§] - rs201381085	0.0003	c.68864G>C	p.Gly22955Ala		h	h	h	h		
TTN[§] - rs55886356	0.0056	c.101766G>C	p.Gln33922His		h	h	h	h		
HLA-DQB1 - rs1071637	0.0309	c.266A>G/T	p.Asp89Gly		h_1/h_2	h_2	h_1	h_1	h_2	h_2
HLA-DQB1-DRB5	-	del DQB1-DRB5 143KB		del		del	del	del		
Other candidates										
BET1 - no rs	-	c.97G>T	p.Glu33Ter	h		h		h		h
RASSF9 - no rs	-	c.733C>T	p.Gln245Ter	h		h	h	h	h	h
ARSD° - rs111939179	0.0004	c.992G>A	p.Trp331Ter	He				He	He	He
MIB2 - rs199741261	0.0007	c.153C>A	p.Cys51Ter		h	h		h	h	h
ZNF275° - no rs	-	c.498T>A	p.Asn166Lys		h			He		
FAM240A - rs186252375	0.0022	c.73C>T	p.Arg25Trp	h	h			H	h	

Variants, grouped by venous thromboembolism (VTE) associated genes, thromboangiitis obliterans (TAO) candidate genes and other candidates (see text) are listed according to increasing minor allele frequency (MAF). The gene symbols are reported according to NCBI Gene database (https://www.ncbi.nlm.nih.gov; accessed on May 2025). *ARSD*, Arylsulfatase D, °X-chromosome; *BET1,* Bet1 Golgi Vesicular Membrane Trafficking Protein; *CBS*, cystathionine beta-synthase; *F2*, thrombin; *FAM240A*, family with sequence similarity 240 member A; *HLA-DQB1*, Major Histocompatibility Complex, Class II, DQ Beta 1; *IL13*, interleukin 13; *KLK13*[#], kallikrein related peptidase 13, variant also detected in[5]; *MIB2*, MIB E3 Ubiquitin Protein Ligase 2; *PEAR1*, platelet endothelial aggregation receptor 1; *PPIF*, peptidylprolyl isomerase F; *PTGIR*, prostaglandin I2 receptor; *RASSF9*, Ras Association Domain Family Member 9; *TTN*, titin; *ZNF275*, Zinc Finger Protein 275, °X-chromosome; *ZFPM2*, zinc finger protein, FOG family member 2. *from previous genomic and transcriptomic approaches; [§]in linkage of disequilibrium. The longest transcript (NM_001267550.2) encoding the predicted protein isoform IC (NP_001254479.2) is referred for TTN. Family members are indicated as I1, father; I2, mother; II1 and II2, sisters; II4 and II5 brothers of the propositus (II3). The zygosity status in the family for each single nucleotide polymorphism (SNP) is indicated by h, heterozygous; H, homozygous; He, hemizygous (X-linked); empty rectangles indicate homozygosity for the frequent (WT) alleles. del, heterozygous deletion encompassing the class II HLA genes in the DQB1-DRB5 region (approximately 143 Kb), inferred from multiple SNP zygosity; h_1, heterozygous rs1071637 allele G (Gly); h_2, heterozygous rs1071637 allele T (Gly).

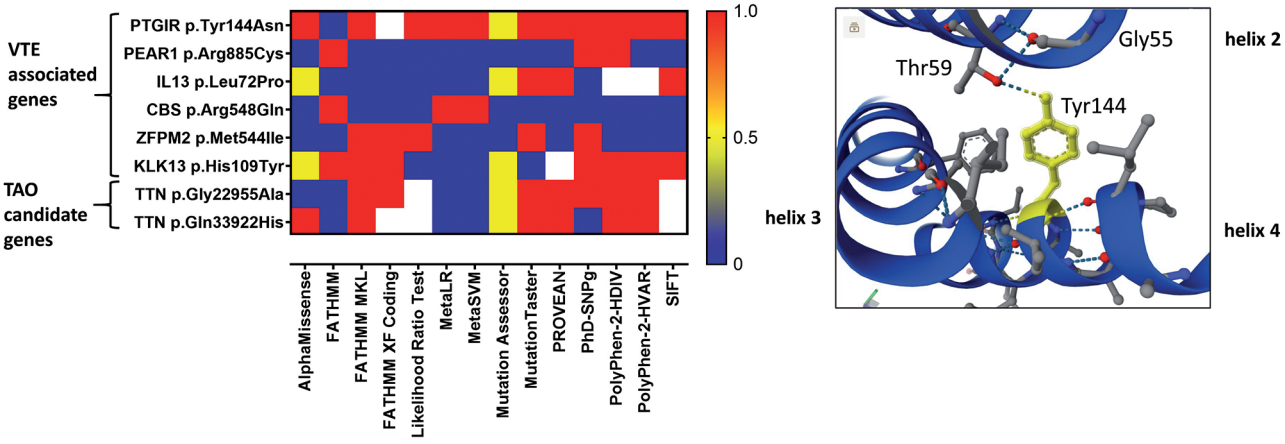

Figure 1. Left Panel. Heatmap representing the degree of deleteriousness of filtered variants. Missense variants are listed in accordance with MAF reported in Table 1. Prediction was conducted by 14 multi-component bioinformatics tools, listed below the heatmap. The probability scores are reported according to the color scale on the right of the panel (0, benign; 0.5 ambiguous/likely pathogenic; 1, pathogenic). No prediction, white boxes. Variants predicted deleterious by several tools (PTGIR and KLK13) as well as variants with ≥3 damaging predictions (red boxes) and additional annotated features (see text for details) were included. Full protein names are reported in the legend to Table 1. Right Panel. Detail of Prostaglandin I2 receptor (*PTGIR*) protein AlphaFold model (https://alphafold.ebi.ac.uk/entry/P43119) showing interaction between membrane helix 4 p.Tyr144 and helix 2 p.Thr59. The p.Tyr144Asn change is predicted to alter (pathogenicity score 0.77) membrane helix interactions.

Table 2. Experimental/*in silico* information and functional interpretation of gene variants.

Gene - SNP	Protein change	Experimental in silico observations/Functional interpretation
VTE associated genes		
PTGIR - no rs	p.Tyr144Asn	Alteration of the hydrogen bond network between membrane helix 2 and 4 /Decreased prostacyclin response
PPIF - rs1855982637	p.Tyr121fs*0	Decreased cyclophilin D function in platelet activation/Role in thrombosis
PEAR1 - rs369608309	p.Arg885Cys	Altered expression of PEAR1/Altered platelet phenotype
IL13 - rs148077750	p.Leu72Pro	Altered structure of cytokine IL13. Elevated IL13 levels in TAO patients/Interference with endothelial function
CBS - rs150828989	p.Arg548Gln	Reduced activity of cystathionine beta-synthase/Reduced vasorelaxation in peripheral vasculature
ZFPM2 - rs187043152	p.Met544Ile	*Locus* associated with VEGF levels/Altered vascularization in TAO
F2 - rs5899	p.Gly271=	Altered splicing of thrombin/Altered thrombin regulation
KLK13[#] - rs34089525	p.His109Tyr	Peptidase domain alteration. Missense change in the irisin interaction region/Inflammation and endothelial dysfunction in TAO
TAO candidate genes		
TTN - rs201381085 *TTN* - rs55886356	p.Gly22955Ala p.Gln33922His	TTN role in vascular smooth muscle cell contraction and arterial stiffness. TTN included in both TAO transcriptome and VTE proteome alterations/Role in vascular alteration in TAO
HLA-DQB1 - rs1071637 *HLA-DQB1-DRB5*	p.Asp89Gly deletion	Very rare combined DQB1 genotype/Altered antigen response in TAO
Other candidates		
BET1 - no rs	p.Glu33Ter	*Locus* association with paraoxonase and TFPI2 levels/Decreased antioxidant defenses and endothelial integrity in TAO

The interpretation sentences are reported in italics. The term *other candidates* refers to whole exome analysis for premature termination codons previously not reported and belonging to pathways of interest for TAO susceptibility. The gene symbols are reported according to NCBI Gene database (https://www.ncbi.nlm.nih.gov; accessed on May 2025). *BET1,* Bet1 Golgi Vesicular Membrane Trafficking Protein; *CBS*, cystathionine beta-synthase; *F2*, thrombin; *HLA-DQB1*, Major Histocompatibility Complex, Class II, DQ Beta 1; *IL13*, interleukin 13; *KLK13*[#], kallikrein related peptidase 13, variant also detected in[5]; *PEAR1*, platelet endothelial aggregation receptor 1; *PPIF*, peptidylprolyl isomerase F; *PTGIR*, prostaglandin I2 receptor; *TTN*, titin; *ZFPM2*, zinc finger protein, FOG family member 2. SNP, single nucleotide polymorphism; TFPI2, tissue factor pathway inhibitor 2; VEGF, vascular-endothelial growth factor; VTE, venous thromboembolism.

asparagine for tyrosine is likely to disrupt the hydrogen bond network connecting the two helical domains, as also predicted for the only known variant at the same position, p.Tyr144His (rs1044504721). The p.Tyr144Asn change could increase propensity for platelet activation, as reported for other *PTGIR* SNPs.[11] This hypothesis aligns with the key roles of prostacyclin and its receptor in vascular smooth muscle relaxation and inhibition of platelet aggregation, as well as with prostanoid therapy in management of TAO.

In *KLK13*, p.His109 is a highly conserved residue in the functional peptidase domain S1. Structural prediction suggests that p.His109 may be located at the interface between KLK13 and Fibronectin Type III Domain-Containing Protein 5 (FNDC5), the precursor of irisin. Interestingly, several evidences suggest a protective role of this myokine, ameliorating inflammation and endothelial dysfunction,[12] processes of noticeable interest for TAO.

We have also detected the *KLK13* p.His109Tyr variant in a family with an unexplained tendency for venous thromboembolism.[5] For these reasons the *KLK13* p.His109Tyr variant is listed in Tables 1 and 2 despite frequency slightly higher than the selected threshold.

Four missense variants, classified as damaging by a lower number (≥3) of bioinformatics tools (Figure 1), might alter gene/protein function, as suggested by structural and experimental evidence. i) The rs369608309, located within an enhancer sequence and causing the missense p.Arg885Cys in the platelet endothelial aggregation receptor 1 (PEAR1), could mediate platelet phenotypes. ii) The recombinant *IL13* p.Leu72Pro variant might increase neuronal vulnerability by potentiating oxidative stress-induced cell death in a human neuroblastoma cell line.[13] Notably, elevated IL-13 levels have been observed in TAO patients,[14] and inflammatory cytokines may contribute to TAO progression by interfering with endothelial function.[8] iii) The p.Arg548Gln variant in cystathionine beta synthase (*CBS*), which mediates vascular tone through hydrogen sulfide production,[15] exhibits in *in vitro* studies reduced enzymatic activity (up to 60% of the wild-type) and diminished capacity to form a multimeric quaternary structure.[16] Noticeably, impaired endothelium-dependent vasorelaxation in peripheral vasculature of TAO patients has been demonstrated (reviewed in Olin),[1] which may support a detrimental role of this variant. iv) The p.Met544Ile change is located around the 5th zinc finger of the FOG family 2 transcription factor (ZFPM2) and might decrease interaction with the transcription regulator GATA4. However, a detailed interaction map is not available and biological effects of this aa change were modest.[17,18] Interestingly, GATA4 is involved in megakaryocyte development and platelet production. Furthermore, the ZFPM2 locus has been associated with circulating levels of vascular-endothelial growth factor (VEGF),[19,20] and VEGF165-mediated neovascularization has been evaluated in TAO gene therapy strategies.[21]

For the rare synonymous thrombin variant *F2* p.Gly271=, located in the mRNA of a key protein for several biological processes in hemostasis, splicing effects were predicted. Whereas the propositus thrombin levels were normal, dysfunctional features potentially conferred by this nucleotide change deserve further mRNA and protein studies.

Thromboangiitis obliterans candidate genes from previous genomic or transcriptomic studies

Based on prior genomic analysis in TAO patients,[3,4,6] we extended our investigation to include 11 genes (*Supplementary Table 1*, TAO candidate genes). Moreover, based on blood transcriptome profiles, WES analysis was extended to 81 genes encoding differentially expressed mRNAs (*Supplementary Table 1*, TAO transcriptome mRNA, Chen *et al*.[7] and Otzan *et al.*[8]). Although no formal overlap exists between transcriptome profiles in the two studies, they include two mitochondrial respiratory chain subunits (NDUFAF4 and NDUFA1). Our focus on genes downregulated in TAO, coupled with the filtering of variants with MAF <0.001, identified (Table 1) the heterozygous p.Gly22595Ala change, in linkage with the p.Gln33922His in the *TTN* gene. Both missense mutations were predicted to be highly deleterious by bioinformatics tools (Figure 1), suggesting their potential role in mediating reduced TTN levels or function. Titin, a giant sarcomere protein, is a key component in the assembly and functioning of striated muscles,[22] and also plays a role in hemostasis and platelet physiology (Reactome pathways). Recent studies have highlighted its involvement in vascular wall stiffness,[23] and its potential association with VTE, as indicated by proteomic analysis in blood.[24]

In this family, we report a rare genetic pattern within the Major Histocompatibility Complex, Class II, *HLA-DQB1*, characterized by a large deletion resulting in a hemizygous condition for the missense variant p.Asp89Gly (Table 1), and a frequent premature termination codon (p.Glu106Ter, not reported in Table 1). The presence of a very rare *DQB1* combined genotype, which mimics homozygosity for the uncommon missense change p.Asp89Gly, aligns with previous studies in TAO, where HLA class II gene polymorphisms, including *DQB1* alleles, were significantly more frequent in the patients (reviewed in Fazeli *et al.*).[2]

Other genetic candidates

To preliminarily explore the whole exome, variants were filtered for i) premature termination codons (PTC) unreported or extremely rare in explored databases and ii) for rare missense SNPs found in the propositus, but not in his siblings, in the homozygous condition or hemizygous state for X-linked nucleotide changes (Table 1).

i) The p.Glu33Ter PTC was found in *BET1*, coding for a SNARE protein involved in the vesicular transport from ER to cis-Golgi membrane. Interestingly, *BET1* has been associated (GWAS catalog) with levels of paraoxonases in serum (PON1 and PON2), components of the antioxidant defenses that were found seriously impaired in Buerger's disease.[25] Moreover, it has been reported (GWAS catalog) the association between *BET1* and levels of tissue factor pathway inhibitor 2 (TFPI2), potentially explained by proximity of these genes (100KB) on chromosome 7. TFPI2 is secreted into the extracellular matrix by endothelial cells, exposed on the plasma membrane and acts as a regulator of angiogenesis and an inhibitor of serine proteases, with a potential role in endothelial integrity and inflammation. The *BET1* p.Glu33Ter nonsense mutation deserves further investigation as an intriguing link both with VTE and TAO.

Among PTCs unreported in databases, the *RASSF9* p.Gln245Ter, paternally inherited in all siblings, affects a protein that may play a role in regulating vesicular trafficking in cells. Among rare PTCs, the hemizygous *ARSD* p.Trp331Ter (MAF=0.0004), detected in all brothers, may affect bone and cartilage matrix sulfatase, and the *MIB2* p.Cys51Ter, maternally inherited in several sibling, may affect an E3 ubiquitin-protein ligase that mediates ubiquitination of Delta receptors in NOTCH signaling. Differently from *BET1, RASSF9, ARSD and MIB2* functional features do not provide evidence to candidate these premature termination codons for TAO susceptibility. ii) In accordance with inheritance of an X-linked recessive disease trait maternally inherited in the propositus only, the hemizygous missense change *ZNF275* p.Asn166Lys was detected. This SNP, previously unreported in databases, would affect a zinc finger protein with an unknown role in transcriptional regulation.

In accordance with inheritance of an autosomal recessive disease trait, the missense variant *FAM240A* p.Arg25Trp (MAF=0.0022) was found to be homozygous in the propositus only. This change is located in a poorly characterized protein and in a low confidence structural region, rendering most of bioinformatics tools ineffective in prediction. The scarce information about *ZNF275* and *FAM240A* is not sufficient to support a relation/link between their genetic variations and TAO.

Limitation of the study

This study is subjected to some genetic and laboratory limitations: i) the presence of only one family member affected by TAO, a disease with uncertain genetic components; ii) the lack of strong genetic evidence. As a matter of fact, the heterozygous conditions for variants in VTE- and TAO-associated genes were present in the propositus and in at least one other unaffected sibling. Although present in the heterozygous condition, combination of multiple gene variants could modulate intermediate phenotypes contributing to TAO; iii) the absence of detailed platelet studies, and of specific plasma assays aimed at investigating intermediate phenotypes potentially stemming from the genetic analysis; iv) although based on *in silico* analysis and literature findings, the interpretation of the variant effects in relation to phenotypes in TAO has to be considered speculative and hypothesis generating.

Conclusions

This study pinpoints, among VTE and TAO candidate genes as well as in the whole exome (Table 2), rare or novel genetic variations that could increase TAO susceptibility. Experimental and *in silico* observations foster further investigation of their potential functional features in TAO.

References

1. Olin JW. Thromboangiitis obliterans (Buerger's disease). N Engl J Med 2000;343:864-9.
2. Fazeli B, Ligi D, Keramat S, et al. Recent Updates and Advances in Winiwarter-Buerger Disease (thromboangiitis obliterans): biomolecular mechanisms, diagnostics and clinical consequences. Diagnostics (Basel) 2021;11:1736.
3. Sun XL, Law BY, de Seabra Rodrigues Dias IR, et al. Pathogenesis of thromboangiitis obliterans: Gene polymorphism and immunoregulation of human vascular endothelial cells. Atherosclerosis 2017;265:258-65.
4. Chen F, Chen ZR, Zhou W. Screening for thrombophilia in patients with thromboangitis obliterans using whole-exome sequencing. Thromb Res 2023;228:117-20.
5. Lunghi B, Ziliotto N, Balestra D, et al. Whole-Exome Sequencing in a family with an unexplained tendency for venous thromboembolism: multicomponent prediction of low-frequency variant deleteriousness and of individual protein interaction. Int J Mol Sci 2023;24:13809.
6. Shi ZF, Fang QB, Limu S, et al. Association between three SNPs and thromboangiitis obliterans in Xinjiang Uyghur population. Genet Test Mol Biomarkers 2016;20:55-62.
7. Chen B, Deng Y, Wang B, et al. Integrated analysis of long non-coding RNA-microRNA-mRNA competing endogenous RNA regulatory networks in thromboangiitis obliterans. Bioengineered 2021;12:12023-37.
8. Öztan G, Bozbuğa N, İşsever H, et al. Comparative analysis of transcriptome profiles in patients with thromboangiitis obliterans. Genes (Basel) 2023;15:19.
9. Jobe SM, Wilson KM, Leo L, et al. Critical role for the mitochondrial permeability transition pore and cyclophilin D in platelet activation and thrombosis. Blood 2008;111: 1257-65.
10. Stitham J, Stojanovic A, Ross LA, et al. Clusters of transmembrane residues are critical for human prostacyclin receptor activation. Biochemistry 2004;43:8974-86.
11. Patrignani P, Di Febbo C, Tacconelli S, et al. Differential association between human prostacyclin receptor polymorphisms and the development of venous thrombosis and intimal hyperplasia: a clinical biomarker study. Pharmacogenet Genomics. 2008;18:611-20.
12. Liu S, Cui F, Ning K, et al. Role of irisin in physiology and pathology. Front. Endocrinol 2022;13:962968.
13. Aguirre CA, Concetta Morale M, Peng Q, et al. Two single nucleotide polymorphisms in IL13 and IL13RA1 from individuals with idiopathic Parkinson's disease increase cellular susceptibility to oxidative stress. Brain Behav Immun 2020;88:920-4.
14. Dellalibera-Joviliano R, Joviliano EE, Silva JS, Evora PR. Activation of cytokines corroborate with development of inflammation and autoimmunity in thromboangiitis obliterans patients. Clin Exp Immunol 2012;170:28-35.
15. Huerta de la Cruz S, Medina-Terol GJ, Tapia-Martínez JA, et al. Hydrogen sulfide as a neuromodulator of the vascular tone. Eur J Pharmacol 2023;940:175455.
16. Urreizti R, Asteggiano C, Cozar M, et al. Functional assays testing pathogenicity of 14 cystathionine-beta synthase mutations. Hum Mutat 2006;27:211.
17. van den Bergen JA, Robevska G, Eggers S, et al. Analysis of variants in GATA4 and FOG2/ZFPM2 demonstrates benign contribution to 46,XY disorders of sex development. Mol Genet Genomic Med 2020;8:e1095.
18. Bashamboo A, Brauner R, Bignon-Topalovic J, et al. Mutations in the FOG2/ZFPM2 gene are associated with anomalies of human testis determination. Hum Mol Genet 2014;23:3657-65.
19. Idei N, Nishioka K, Soga J, et al. Vascular function and circulating progenitor cells in thromboangitis obliterans (Buerger's disease) and atherosclerosis obliterans. Hypertension 2011;57:70-8.
20. Choi SH, Ruggiero D, Sorice R, et al. Six novel loci associated with circulating VEGF levels identified by a meta-analysis of genome-wide association studies. PLoS Genet 2016;12: e1005874.
21. Barć P, Lubieniecki P, Antkiewicz M, et al. Gene therapy of thromboangiitis obliterans with growth factor plasmid (VEGF165) and autologous bone marrow cells. Biomedicines 2024;12:1506.
22. Loescher CM, Hobbach AJ, Linke WA. Titin (TTN): from molecule to modifications, mechanics, and medical significance. Cardiovasc Res 2022;118:2903-18.
23. Zhu C, Bishop T, Gregorich ZR, Guo W. Titin is a new factor regulating arterial stiffness through vascular smooth muscle cell tone in male rats. Physiol Rep 2025;13:e70270.
24. Brækkan SK, Onsaker AL, Nøst TH, et al. The plasma proteome and risk of future venous thromboembolism-results from the HUNT study. Thromb Haemost 2025;125:574-84.
25. Arslan C, Altan H, Beşirli K, et al. The role of oxidative stress and antioxidant defenses in Buerger disease and atherosclerotic peripheral arterial occlusive disease. Ann Vasc Surg 2010;24:455-60.

Online supplementary material:

Supplementary Table S1. List of candidate gene grouped by disease [venous thromboembolism (VTE) / thromboangiitis obliterans (TAO) or by transcriptome studies (Chen B / Oztan G)].