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Investigation of von Willebrand factor polymorphisms in congenital Thrombotic Thrombocytopenic Purpura

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INTRODUCTION

Von Willebrand factor (VWF) is a multimeric glycoprotein with a central role in primary haemostasis.

Under high shear stress, VWF is released as ultra-large and highly adhesive multimers that form long strings to facilitate platelets adhesion to the endothelium and aggregates formation. This prothrombotic activity is controlled by ADAMTS13 cleavage in the A2 domain (Y1605-M1606 bond) of the VWF multimers.

Many polymorphisms in VWF gene have been reported and investigated for their role and association with thrombogenicity and cardiovascular diseases.

AIM

This retrospective observational study wanted to investigate the presence of polymorphic variants in the coding region of the functional domains of VWF in congenital Thrombotic Thrombocytopenic Purpura (cTTP).

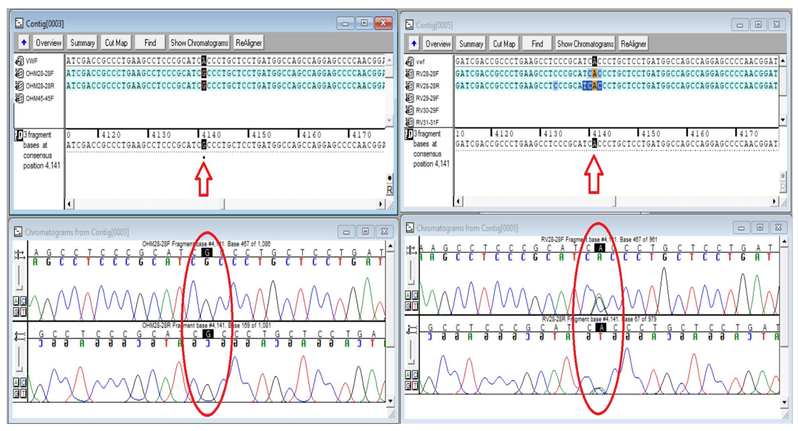
METHOD

63 cTTP patients from the UK TTP registry were analysed with Sanger Sequencing. Genomic DNA was extracted from EDTA whole blood samples.

VWF exons from 28 to 32, and exon 45, were amplified by polymerase chain reaction (PCR) with corresponding introns primers.

PCR products were sequenced by GENEWIZ and sequences data were analysed bidirectionally using Sequencher 5.4.6 (Gene Codes Corporation). Genomic

database Ensembl was used to identify known VWF polymorphisms.



***Example of DNA sequences analysis with Sequencher**. The pictures show how the rs216311 variant can appear in different patients: the first one has the homozygous variant while the second is heterozygous for the polymorphism.*

RESULTS

The cTTP patients were included in the study: 75% (n=47) female and 25% (n=16) male, median age 35 years (range: 2-74).

The **A1**, **A2**, **A3** and **C4** domains, corresponding to the binding sites for GPIbα, ADAMTS13, collagen and GPIIb/IIIa on VWF, were investigated.

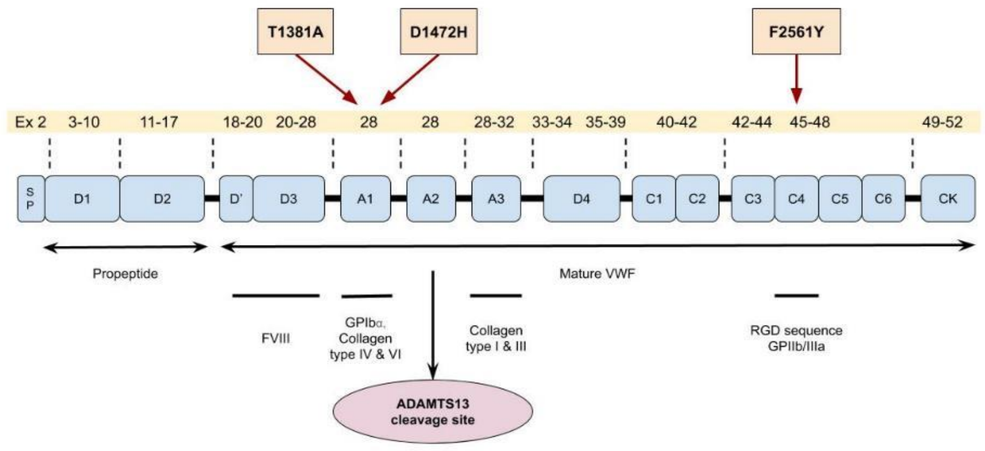
VWF polymorphisms were identified in **47.6%** (n=30) cTTP patients.

DNA sequencing revealed 3 missense variants:

- p.T1381A (rs216311)
- p.D1472H (rs1800383)
- p.F2561Y (rs35335161)

The first two variants were in exon 28, located in the A1 domain: the first one (rs216311) was located in the middle of the A1 loop, while the second one (rs1800383) was just after the C-terminal disulphide bond on the A1 loop.

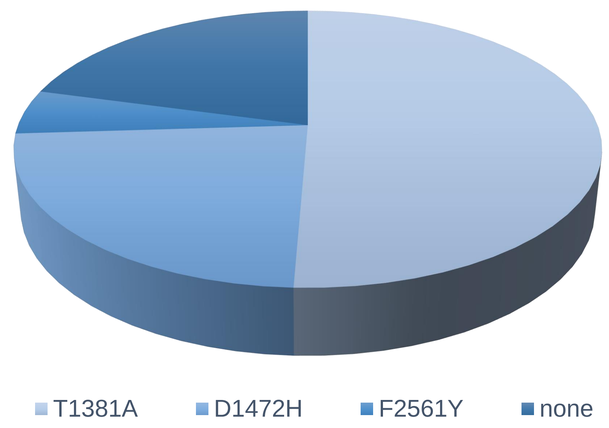
The third one was found in exon 45, corresponding to C4 domain, where the RGD sequence is located.



***Schematic representation of VWF domain architecture and correspondent exons**.*
The location of each investigated VWF variants are indicated by red arrow to the corresponding exon and domain (top). VWF spans~178 kilobases, containing 52 exons. The VWF precursor consists of a signal peptide (SP), propeptide and mature subunit. The pro-VWF is organized into repeats of homologous structural domains (A, C and D). VWF binding sites for factor VIII, platelet glycoprotein (GP) Iba, collagen, platelet GPIIb/IIIa and ADAMTS13 cleavage sites are shown.

16% of patients (n=10) **T/T1381** homozygotes and 46% (n=29) **A/T1381** heterozygotes were identified.
D1472H heterozygous variants was detected in 28.6% (n=18) of patients.
F2561Y heterozygous variants was present in 6% (n=4) of patients.

Incidence of VWF SNPs in our cTTP population



Onset period	n° (% total)	Female, %	Ethnicity n° (%)
Childhood (<14 y)	11 (17%)	36%	White: 5 (45%), Asian: 4 (36%), African: 2 (18%)
Pregnancy	16 (25%)	100%	White: 15 (94%), Asian: 1 (6%)
Adulthood	26 (41%)	77%	White: 18 (69%), Asian: 6 (23%), African: 1 (4%), Mixed: 1 (4%)
Late onset (> 50 y)	10 (16%)	70%	White: 10 (100%)

The composition of our study population, from the UK TTP Registry

CONCLUSIONS

Although all the variants identified in this observational study have been defined to be “likely benign” by PolyPhen-2, they may have a prognostic meaning in our patients cohort.

Homozygous T/T1381 variant has been demonstrated to have a higher affinity for the platelet receptor GPIbα. Similarly, F2561Y has been investigated as a gain-of-function variant which increases the force sensitivity of VWF interaction with platelets. The aminoacidic substitution in D1472H variant seemed to require an increased concentration of ADAMTS13 for the cleavage of VWF.

In conclusion, VWF variants were more common than anticipated in cTTP cases and may alter the prothrombotic risk and phenotypic diversity in cTTP patients.

REFERENCES

1. **Schneppenheim R**, Hellermann N, Brehm MA, Klemm U, Obser T, Huck V, et al. The von Willebrand factor Tyr2561 allele is a gain-of-function variant and a risk factor for early myocardial infarction. *Blood. 2019;133(4):356–65*.

2. **Szánto T**, Schlammadinger Á, Staelens S, De Meyer SF, Freson K, Pareyn I, et al. The A/T1381 polymorphism in the A1-domain of von Willebrand factor influences the affinity of von Willebrand factor for platelet glycoprotein Ibα. *Thromb Haemost. 2007;98(1):178–85*.

3. **Pruss CM**, Notley CRP, Hegadorn CA, O’Brien LA, Lillicrap D. ADAMTS13 cleavage efficiency is altered by mutagenic and, to a lesser extent, polymorphic sequence changes in the A1 and A2 domains of von Willebrand factor. *Br J Haematol. 2008;143(4):552–8*.

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