

CO-DOMINANT MIXED PHENOTYPE VON WILLEBRAND DISEASE CAUSED BY A NOVEL CYSTEINE VARIANT THAT RESULTS IN BOTH QUALITATIVE AND QUANTITATIVE VON WILLEBRAND FACTOR DEFECTS

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Introduction/Background

Von Willebrand factor (VWF) is an extremely cysteine rich molecule, with cysteine residues comprising 8.2% of the total amino acid content, as compared with an average of 2.3% in other human proteins. Cysteine residues cluster mainly in the VWF N- and C- termini where they are involved in extensive intra- and inter-molecular disulfide bonding. Preservation of these disulfide bonds is essential for maintaining VWF conformation, multimer structure and thus haemostatic function. Indeed, loss of cysteine residues has widely been reported as causative of von Willebrand disease (VWD) and has frequently been associated with both type 1 and type 2 VWD. Herein we report a consanguineous family with a novel cysteine variant (c.3251G>A, p.Cys1084Tyr) that maps to the C8-3 subunit of the VWF D3 domain, and is associated with both qualitative and quantitative VWF deficiencies resulting in mixed phenotype VWD.

Materials & Methods

Bleeding phenotype was assessed using the ISTH BAT. Relevant hemostatic values were measured using standard laboratory assays. Expression studies were carried out in HEK293T cells. Imaging studies were carried out in HEK293 cells.

Results

The index case (IC) was diagnosed with VWD following episodes of epistaxis requiring intervention. Family phenotypic values are outlined in table 1. In 3 family members, heterozygosity for the p.(Cys1084Tyr) variant was associated with a qualitative VWF deficiency. Normal VWF:Ag levels but significantly reduced VWF:GPIbM and VWF:CB, as well as loss of high molecular weight multimers were observed, consistent with a diagnosis of type 2A VWD. Interestingly however, homozygosity for this variant as observed in the IC and her sibling, was further associated with significantly reduced FVIII-binding and an additional quantitative defect. Plasma VWF:Ag levels were significantly reduced (<0.40IU/mL) and subsequent analysis of DDAVP response kinetics revealed markedly reduced post-DDAVP survival of VWF:Ag, VWF:GPIbM and FVIII:C. Quantitative *in vitro* expression studies revealed impaired synthesis/secretion of p.(Cys1084Tyr) for both the heterozygous and homozygous states (58.4±5.2% and 25.2±7.0% of wild-type VWF respectively, p<0.0001) and subsequent imaging

studies also demonstrated qualitative differences in pseudo-Weibel Palade body (pseudo-WPB) formation. Analysis of the crystal structure of the D'D3 assembly revealed the close proximity of the C1084-C1060 disulfide to C1099, the cysteine involved in inter-subunit disulfide formation. Loss of C1084 with subsequent generation of a novel free cysteine at C1060 likely interferes with D'D3 structural conformation and multimerization, leading to both the quantitative and qualitative defects observed.

Conclusion

The novel p.(Cys1084Tyr) variant causes codominant inheritance of a complex, mixed phenotype VWD associated with reduced VWF:Ag levels due to accelerated VWF clearance and impaired VWF synthesis, packaging and secretion, as well as deficiencies in VWF:GPIbM and FVIII-binding.