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## **The homozygous variant p.Gln1311\* in exon 28 of *VWF* is associated with the development of alloantibodies in 3 unrelated patients with type 3 VWD**

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Affiliations collapse

### **Affiliations**

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Type 3 von Willebrand disease (VWD3), the rarest and most severe form of the disease, is inherited as an autosomal recessive trait. It is characterized by a near-total absence of von Willebrand factor (VWF) and very low levels (1–5%) of FVIII.<sup>1</sup> Replacement therapy with VWF concentrates is the mainstay of treatment but there is an approximately 5%–10% risk of developing alloantibodies against VWF, rendering the treatment ineffective or even dangerous due to allergic reactions.<sup>2</sup> The majority of VWD3 patients who develop anti-VWF antibodies have a partial or large homozygous deletion of the *VWF* gene although it has also been described in some patients with homozygous nonsense or frameshift variants.<sup>3</sup>

Type 3 von Willebrand disease has been diagnosed in 107 patients (99 families) of the 3954 patients included in the database of the French Reference Center for VWD. Among them, 6 patients developed an anti-VWF antibody. Two of them have a homozygous complete *VWF* deletion and another has a heterozygous nonsense variant p.Arg324\* on one allele and an unknown variant on the second allele. Finally, the 3 remaining patients (Table 1) were found to be homozygous for the same nonsense variant p.Gln1311\* (c.3931C>T) in exon 28. This variant can be the result of a gene conversion with the pseudogene *VWFP1*,<sup>4</sup> but no other additional neighbouring variants derived from the pseudogene has been identified in our patients to prove this mechanism. No other patient homozygous for this variant was identified in the database. A prophylaxis regimen has been initiated for the 3 patients early after their

diagnosis of VWD3 because of severe bleeding symptoms (ISTH-BAT score: 14–24) (Table 1). Inhibitor detection was performed with 2 techniques: on one hand, with a mixing study (anti-VWF:Rco) where residual VWF activity was measured after incubation of the patient plasma with a normal plasma, and on the other hand with an enzyme-linked immunosorbent assay (ELISA) using recombinant VWF. The first patient (P1) is a 19-year-old girl (score = 18) on long-term prophylaxis since the age of 6 due to knee haemarthrosis with an intensification of the treatment since adolescence because of menorrhagia. Her alloantibody was detected in 2019, after 12 years of prophylaxis 2 or 3 times a week, due to a poor therapeutic efficacy. The second patient (P2) is a 12-year-old boy. He has the highest haemorrhagic score of the 3 patients (score = 24), with notably severe and life-threatening epistaxis. The alloantibody was detected early, on a routine blood test at the 6th day of exposure. He also has a mild haemophilia A (c.2113+466\_2113+473del in intron 13 of *F8*) leading to an undetectable FVIII level (FVIII:C<1%) in combination with VWD3. He developed an additional high-titre anti-FVIII alloantibody and for this reason was switched to emicizumab. Both these patients have a history of consanguinity in their family, leading to the *VWF* homozygous variant heredity. The third patient (P3) is a 9-year-old girl (score = 14) who started prophylaxis approximately at the age of 15 months. The alloantibody was detected at the age of 8 years, after 7 years of prophylaxis 2 or 3 times a week, at the occasion of a preoperative screening. The mother was found to be heterozygous for the variant but the father was missing, with apparently no history of consanguinity. The sequencing of P3 *VWF* gene showed only homozygous polymorphisms on a large region around the p.Gln1311\* variant, which initially led us to assume a hemizygoty but the presence of a large deletion was excluded after performing Multiplex Ligation-dependent Probe Amplification assay. Our second hypothesis was the presence of a uniparental disomy (UPD). We performed a SNP array with a CytoScan® HD (Thermo Fischer, Waltham, MA, USA) chip to detect a copy-neutral loss of heterozygosity corresponding to imbalance between maternal and paternal genetic material inheritance. In the case of UPD, the two chromosomes of a pair, or a part of them, are exclusively of maternal or paternal origin after duplication, replacing material coming from the second parent. Thus, UPD allows the expression of deleterious variants usually silent which are present here in a homozygous state, leading to clinical expression of recessive diseases. The results of the CytoScan® HD for chromosome 12, obtained with the software ChAS Chromosome Analysis Suite version 2.1, are shown in Figure 1 for patient P3. There is a normal number of copy (CN =2) on the total length of the chromosome 12, meaning that 2 alleles are present but with an imbalanced allelic expression on around 8.6 Mb, on the genomic interval chr12:4,744,500–13,346,450. This fragment includes

the *VWF* gene (chr12:5,948,874–6,124,770) but also 194 other ones. Thus, P3 expresses only one parental allele in a homozygous state, presumably the maternal allele carrying the same variant p.Gln1311\*.

Visualization of the analysis of chromosome 12 on the CytoScan<sup>®</sup> HD chip for patient P3. The chromosome 12 is represented in the bottom of the figure. The *VWF* gene is located in its short arm (red arrow). The black arrow represents the 8.6 Mb fragment involved in uniparental disomy: arr[hg19] 12p13.32p13.1 (4,744,500–13,346,450) hmz.

The p.Gln1311\* variant has been described several times in VWD3, especially in Spain<sup>5</sup> and Portugal.<sup>6</sup> This variant was originally reported in Spanish patients of Gypsy origin with a probable shared ancestry,<sup>7</sup> and this could apply to two of our patients (P1 and P3). It has also been described in patients from India, Iran and occasionally Caucasian patients<sup>4, 8-10</sup> which is not unexpected as the original Gypsy population is known to have migrated from north-west India to Europe.

However, until now this variant had not been specifically identified as a high inhibitor risk variant. Only two cases of inhibitors have already been reported in homozygous patients. One is a 6-year-old child from Indian origin who developed a high-titre inhibitor detected after a poor response to treatment.<sup>4</sup> The second is a 21-year-old woman from Portugal who showed an ineffective response after replacement therapy secondary to the development of an inhibitor detected by an anti-VWF ELISA.<sup>6</sup> About 30 patients homozygous for p.Gln1311\* have been reported in the literature without mention of the presence of an inhibitor.<sup>5, 7-10</sup> This can be due to a lack of inhibitor detection or the fact that some patients were rarely exposed to replacement therapy. Nevertheless the finding that all 3 patients in our cohort homozygous for this p.Gln1311\* variant developed an anti-VWF antibody strongly suggests that this variant may be at high risk of inhibitor. However, the mechanism leading to a higher immunogenicity for this variant is not yet understood. A homozygous nonsense variant as p.Gln1311\*, if escaping from nonsense mediated decay, is leading to a truncated protein and we can hypothesize that patient who only synthesizes truncated and dysfunctional VWF have a higher risk to develop antibodies after treatment. In addition, the fact that this variant might result from a gene conversion may also contribute to alloantibody development. When present at a homozygous state in a patient, this should encourage to closely monitoring the therapeutic efficacy of the replacement therapy and, if any doubt, to investigate the presence of an anti-VWF antibody by the most sensitive techniques.

The prophylaxis treatment for these patients who developed an anti-VWF antibody has to be discussed as life-threatening anaphylactic reactions when re-exposed to VWF may occur, due to

immune complex formation and complement activation.<sup>2</sup> However none of the 3 reported patients developed allergic reactions before or after alloantibody detection.

In conclusion, we characterized the homozygous p.Gln1311\* variant as a VWD3 variant associated with a high risk of alloimmunization. This information must be considered for future patients in the discussion of prophylaxis instauration.

### **CONFLICT OF INTEREST**

A.H. reports fees from Sobi, Roche, LFB, CSL Behring, Octapharma, Novonordisk, Bayer and Takeda. C.F. reports grants or personal fees from LFB, Bayer, Novo Nordisk, Pfizer, Shire-Takeda, Sobi and Roche. A.R. reports consultant fees from BioMarin, LFB, Octapharma, Roche and Sobi, grants from CSL Behring and Roche. S.S. reports grants from Carmat, CorWave and Stago, grants and personal fees from LFB-Biotechnologies, Roche, Sobi and Siemens Healthineers, personal fees from HEMA Biologics. J.G. reports personal fees from LFB-Biotechnologies, Novo Nordisk, Shire-Takeda and Roche. The remaining authors declare no competing financial interests.

### **AUTHORS CONTRIBUTION**

F.L. and JG wrote the manuscript; F.L., C.Z., E.J., P.B. and N.D. performed and analysed the data; A.H., C.B.A. and C.F. collected clinical data; and all the authors edited the manuscript.

### **REFERENCES**

- 1Leebeek FWG, Eikenboom JCJ. Von Willebrand's disease. *N Engl J Med*. 2016; **375**(21): 2067-2080.
- 2James PD, Lillicrap D, Mannucci PM. Alloantibodies in von Willebrand disease. *Blood*. 2013; **122**(5): 636-640.
- 3Baronciani L, Cozzi G, Canciani MT, et al. Molecular defects in type 3 von Willebrand disease: updated results from 40 multiethnic patients. *Blood Cells Mol Dis*. 2003; **30**(3): 264-270.
- 4Surdhar GK, Enayat MS, Lawson S, Williams MD, Hill FGH. Homozygous gene conversion in von Willebrand factor gene as a cause of type 3 von Willebrand disease and predisposition to inhibitor development. *Blood*. 2001; **98**(1): 248-250.
- 5Borràs N, Batlle J, Pérez-Rodríguez A, et al. Molecular and clinical profile of von Willebrand disease in Spain (PCM-EVW-ES): comprehensive genetic analysis by next-generation sequencing of 480 patients. *Haematologica*. 2017; **102**(12): 2005-2014.
- 6Fidalgo T, Salvado R, Corrales I, et al. Genotype-phenotype correlation in a cohort of Portuguese patients comprising the entire spectrum of VWD types: impact of NGS. *Thromb Haemost*. 2016; **116**(07): 17-31.

- 7Casaña P, Martínez F, Haya S, Lorenzo JI, Espinós C, Aznar JA. Q1311X: a novel nonsense mutation of putative ancient origin in the von Willebrand factor gene. *Br J Haematol*. 2000; **111**(2): 552-555.
- 8Sutherland MS, Keeney S, Bolton-Maggs PHB, Hay CRM, Will A, Cumming AM. The mutation spectrum associated with type 3 von Willebrand disease in a cohort of patients from the North West of England. *Haemophilia*. 2009; **15**(5): 1048-1057.
- 9Gupta PK, Adamtziki E, Budde U, et al. Gene conversions are a common cause of von Willebrand disease. *Br J Haematol*. 2005; **130**(5): 752-758.
- 10Elayaperumal S, Fouzia NA, Biswas A, et al. Type-3 von Willebrand disease in India—Clinical spectrum and molecular profile. *Haemophilia*. 2018; **24**(6): 930-940.