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RESEARCH LETTER

Variant p.Tyr1584Cys: a frequent von Willebrand factor variant in search of von Willebrand disease

The first report of the von Willebrand factor (VWF) variant p.Tyr1584Cys (c.4751A>G, p.Y1584C) dates back 2 decades [1], but its pathogenic nature is still unclear. Over the course of these 2 decades, p.Tyr1584Cys has been seeking its position to be classified among disease-causing *VWF* variants. p.Tyr1584Cys is the most frequently occurring variant in patients with type 1 von Willebrand disease (VWD), is located in the VWF A2 domain, and apparently does not share a common etiology with other type 1 VWD variants [2–4]. Previous studies on a limited number of cases showed that this variant is usually associated with a mild reduction in VWF levels (~40 International Units [IU]/dL) with incomplete penetrance and a variable bleeding tendency [5–7]. Type 1 VWD appears to occur particularly when p.Tyr1584Cys is associated with blood group O. A recent study by Christopherson et al. [8] investigated a large group of subjects with the p.Tyr1584Cys variant (n = 58). The study population was included from 2 cohort studies: the Zimmerman Program and the Canadian type 1 VWD, and p.Tyr1584Cys showed a prevalence of 6.2% and 7.3% in these cohorts, respectively [8]. The variant has an overall minor allele frequency of 0.003442 in the Genome Aggregation Database. We previously showed that it is among the most recurrent VWD-associated variants in the Non-Finnish European population [9]. In order to determine the role of this frequently reported VWF variant, it is necessary to gather data from multiple centers on large populations.

In our center, 18 subjects (male/female, 8/10) were identified to carry the p.Tyr1584Cys variant, and all were heterozygous except 2 cases who were compound heterozygous (p.Arg854Gln/p.Tyr1584Cys and p.Tyr1584Cys/p.Arg1597Gln). We compared the results of our cohort (n = 16) with 50 local healthy controls (ABO-adjusted) in order to better understand the variant pathophysiology and addressed the 2 cases with more than 1 variant separately. VWD panel and genetic testing were performed as previously reported [10,11]. According to the VWF levels, subjects could be classified as type 1 VWD (<30 IU/dL; n = 4; 25%), low VWF (30-50 IU/dL; n = 10; 63%), or normal (>50 IU/dL; n = 2; 12%). The cohort had a median factor (F)VIII procoagulant activity (FVIII:C) of 70 IU/dL (range, 33-99 IU/dL), VWF antigen (VWF:Ag) of 46 IU/dL (range, 22-118 IU/dL), platelet-dependent VWF activity using the VWF:glycoprotein Ib-binding assay (VWF:GPIbR) of 38 IU/dL (range, 15-84 IU/dL), and VWF collagen binding assay (VWF:CB) of 39 IU/dL (range, 20-76 IU/dL). On the whole, the variant was associated with a quantitative VWF deficiency with preserved ratios of VWF:GPIbR/VWF:Ag (0.77; range, 0.63-1.08) and VWF:CB/VWF:Ag (0.87; range, 0.64-1.28) in all 16 cases. In the study of Christopherson et al. [8], 60% of subjects with p.Tyr1584Cys had VWF:Ag ≤ 50 IU/dL, similar to our cohort where 56% had VWF:Ag ≤ 50 IU/dL, with VWF:GPIbR and VWF:CB ≤ 50 IU/dL being found in 81% (13 of 16) of our cohort. The majority of the cohort (11/16; 69%) had blood group O, at variance with the Italian general population (42%). This is in line with the study by Christopherson et al. [8], who reported that 56.9% of cases with p.Tyr1584Cys had blood group O, in contrast with the frequency of blood group O in the Canadian and US (46% and 44%, respectively) general populations.

The International Society on Thrombosis and Haemostasis Bleeding Assessment Tool (ISTH-BAT) was available in 15 of 16 cases of our cohort with a median of 3 (range, 0-12). Using age and gender normal ranges, 5 of 15 cases (33%) had abnormal bleeding. Of note, at least 1 bleeding symptom (ie, ISTH-BAT ≥ 1) was seen in 14 of our 15 cases (93%). Of these 5 cases with abnormal ISTH-BAT, 3 were females and 2 were males. Similar results were reported by Christopherson et al. [8]. They found that in the Zimmerman cases with p.Tyr1584Cys, 36% had an abnormal ISTH-BAT score, with no significant difference in bleeding scores between p.Tyr1584Cys group O and p.Tyr1584Cys nongroup O.

To determine the underlying mechanisms of the p.Tyr1584Cys variant, we used as markers the VWF propeptide (pp) antigen (VWFpp) and FVIII:C/VWF:Ag ratio for VWF synthesis/secretion and the VWFpp/VWF:Ag ratio for the VWF clearance. Compared with the healthy controls, lower VWFpp [12] and higher FVIII:C/VWF:Ag [13] ratio are indications of defective VWF synthesis/secretion and a higher VWFpp/VWF:Ag ratio of enhanced VWF clearance [14]. Our cohort showed an overall decreased VWF synthesis/secretion with a median FVIII:C/VWF:Ag ratio of 1.44, significantly higher than the 50 healthy controls (ratio of 0.92; P < .0001; Figure 1). VWFpp, the other marker of VWF synthesis/secretion, was also significantly lower than in controls (median of 72 vs 91 IU/dL; P = .0007).

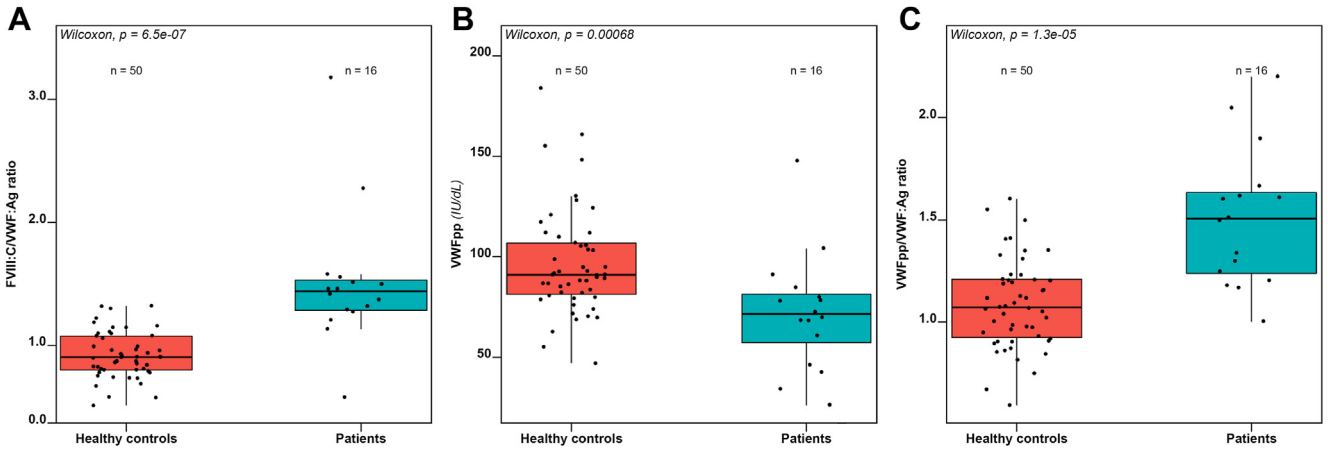

FIGURE 1 Underlying mechanisms of the variant p.Tyr1584Cys. Patients with p.Tyr1584Cys showed a higher median of factor (F)VIII procoagulant activity/von Willebrand factor (VWF) antigen (FVIII:C/VWF:Ag) ratio (A) and a lower median of VWF propeptide (pp) antigen (VWFpp) (B) in comparison with controls, suggesting defective VWF synthesis/secretion. In addition, the median VWFpp/VWF:Ag ratio was higher in patients than in controls, indicating an enhanced clearance of VWF (C).

Our patients had a median VWFpp/VWF:Ag ratio of 1.5 (range, 1-2.2), significantly higher than in healthy controls (median ratio of 1.0; $P < .0001$), suggesting an enhanced VWF clearance (Figure 1). An enhanced clearance was identified in 6 out of 16 patients (37%) using a cutoff of >1.6 for VWFpp/VWF:Ag ratio [11,15]. The overall clearance rate is similar to our previous investigations in both type 1 VWD (34%) [11] and low VWF (33%) [12]. Altogether, these findings indicate that the variant p.Tyr1584Cys is associated with a

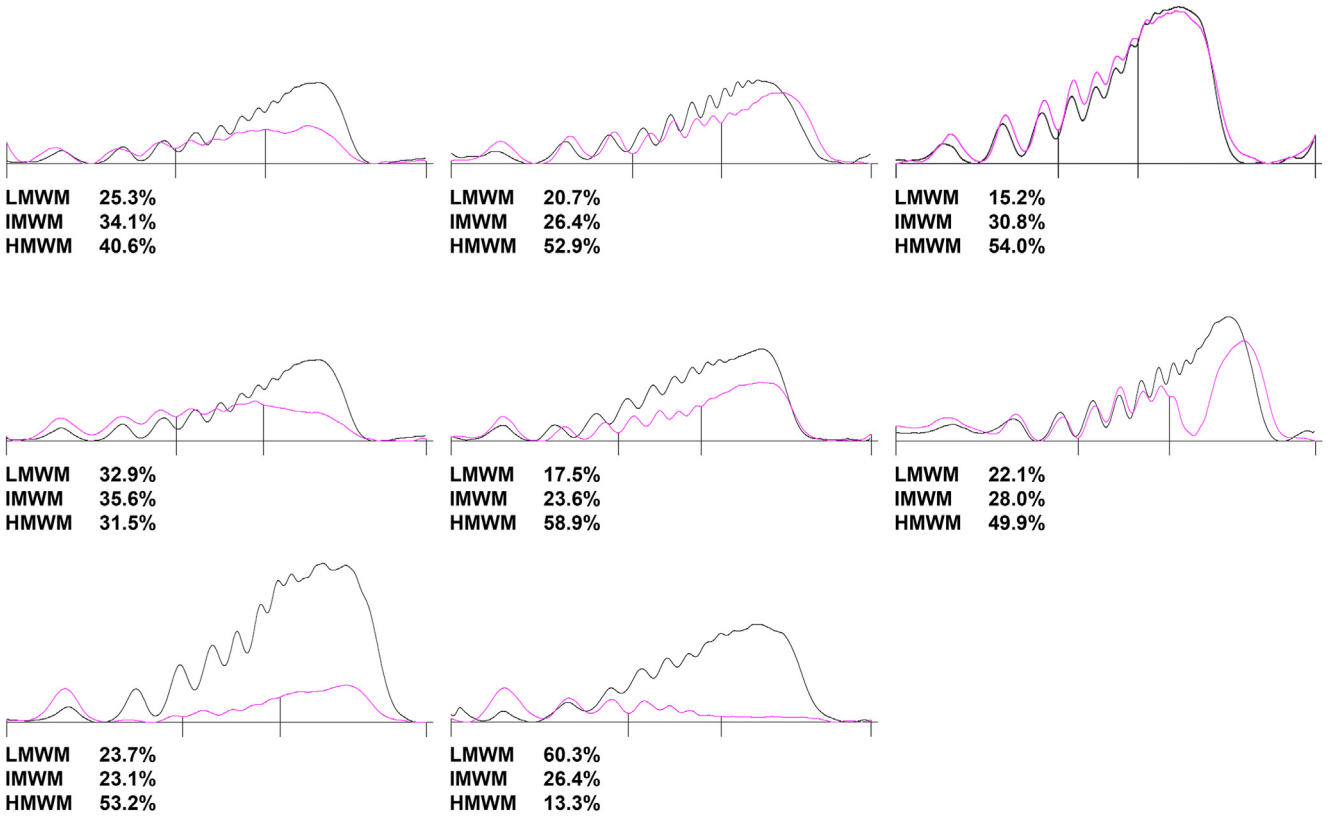

FIGURE 2 The multimer pattern (densitometric analysis) for patients with the variant p.Tyr1584Cys. Densitometric representation of peaks from left to right, with peaks 1 to 3 being low molecular weight multimers (LMWM), peaks 4 to 7 being intermediate molecular weight multimers (IMWM), and all other peaks representing high molecular weight multimers (HMWM). Overall, cases with p.Tyr1584Cys showed a lower ratio of HMWM/LMWM compared with that of pooled plasma (2.17 vs 3.93), suggesting a slight quantitative reduction of HMWM. The last multimer densitometry is for a patient with type 2A von Willebrand disease phenotype who carries p.Tyr1584Cys/p.Arg1597Gln. Gray, pool of normal samples; pink, tested samples.

quantitative reduction in plasma VWF because of combined synthesis/secretion and survival defects.

Multimer analysis was available for 8 of our cases using a semi-automatic Hydrasys 2 scan (Sebia), 7 cases with p.Tyr1584Cys alone and 1 with a type 2A phenotype due to p.Tyr1584Cys/p.Arg1597Gln (Figure 2). This multimer analysis shows the details of the multimer proportion: low (L), intermediate (I), and high (H) molecular weight multimers (MWMs). We used a ratio of HMWM/LMWM among the 7 cases with p.Tyr1584Cys and compared the result with the same ratio obtained from the normal pooled plasma on the same gel used to evaluate these patients. Accordingly, patients showed a lower ratio of HMWM/LMWM compared with the pooled plasma (2.17 vs 3.93), suggesting a slightly quantitatively reduced HMWM VWF. However, the HMWMs were still the highest proportion of multimers (Figure 2). The variant p.Tyr1584Cys showed mild impairments in secretion and increased cleavage by ADAMTS-13 in animal models [4]. In the majority of their cohort with available multimer analysis, Christopherson et al. [8] found normal multimer profiles.

Two of our cases carried a second variant. One case with p.Arg854Gln/p.Tyr1584Cys had a FVIII:C of 50 IU/dL, VWF:Ag of 56 IU/dL, and VWF:GPIbR of 55 IU/dL. These borderline results suggest a slight quantitative reduction of VWF due to p.Tyr1584Cys and of FVIII:C compared with VWF:Ag as a consequence of p.Arg854Gln. The case showed an enhanced VWF clearance with a VWFpp/VWF:Ag ratio of 2.5. Another interesting case was a type 2A VWD carrying p.Tyr1584Cys/p.Arg1597Gln with 30 IU/dL FVIII:C, 20 IU/dL VWF:Ag, and 6 IU/dL VWF:GPIbR. In this case, the effect of p.Tyr1584Cys in quantitative reduction of VWF:Ag (20 IU/dL) was highlighted when we observed that the median VWF:Ag in 8 type 2A patients with p.Arg1597Gln only in our center was 56 IU/dL. The VWFpp/VWF:Ag ratio was 2.7 in this case.

To sum up, our study reports that almost all cases carrying the variant p.Tyr1584Cys had VWF levels below 50 IU/dL. At least 1 bleeding symptom was observed in 93% of the cases with this variant and 33% had abnormal bleeding. Based on the recent VWD guidelines of the American Society of Hematology (ASH), ISTH, the National Hemophilia Foundation, and the World Federation of Hemophilia [16], among our 16 cases with p.Tyr1584Cys alone, 50% are classified as type 1 VWD. The laboratory phenotype and clinical presentations of our cases with this variant appear to be mostly blood group dependent, although 5 of 16 cases (31%) with p.Tyr1584Cys were non-O blood group and had reduced VWF levels. According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines, p.Tyr1584Cys was classified as Likely Pathogenic [8], and our results support this classification.

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AUTHOR CONTRIBUTIONS

O.S. designed the study. O.S. collected and analyzed data and wrote the manuscript. P.C. and G.C. performed the laboratory tests. A.C., S.M.S., and F.P. were involved in the clinical evaluation and L.B. in the laboratory diagnosis of these patients. L.B. and F.P. critically revised the manuscript. All authors have approved the final manuscript.

RELATIONSHIP DISCLOSURE

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