Published in final edited form as:

Ann Hematol. 2014 March ; 93(3): 505–506. doi:10.1007/s00277-013-1817-y.

p.P2063S: a neutral VWF variant masquerading as a mutation

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Dear Editor,

Kasatkar and colleagues recently reported that the previously described von Willebrand factor (VWF) variant c.6187C>T (p.P2063S) appears to be a common cause of type 3 von Willebrand disease (VWD3) in the Indian population [1]. In support of their conclusion, p.P2063S was the only VWF variant observed in 11 Indian VWD3 index cases (IC), tracked with disease phenotype and was predicted to be deleterious via *in silico* protein prediction analysis. In order to ensure accurate molecular characterisation of VWF variants, it is essential to consider all available information. Regarding p.P2063S, there are several lines of evidence that contradict the conclusion drawn.

p.P2063S is also observed in healthy controls with reported population frequencies between 0.6–2.5% in African-Americans / Caucasians [2–4] and (as reported by Kasatkar and colleagues [1]) 3% in Indians. Given a frequency of 3% in the general population, Hardy-Weinberg equilibrium predicts homozygosity for this variant in approximately 1/1100 individuals. This would suggest a high population frequency of VWD3 if this variant were causative. It is also notable that p.P2063S has not been reported as causal in previous studies of VWD3 in Indians [5–7].

The majority of missense mutations reported in VWD3 patients occur in the VWF propeptide and C-terminus where they are reported to affect protein folding and multimerisation resulting in intracellular retention of the protein [2,7,8]. Kasatkar and colleagues argue that diagnosis of VWD3 relies on defective release or synthesis of VWF [1], however given that p.P2063S has no effect on VWF expression *in vitro* [2] it seems unlikely that this variant would affect VWF *in vivo*. This is supported by observations of p.P2063S in VWD patients inherited together with other VWF variants that more readily explain their VWD phenotype (Table 1), most notably in a Turkish VWD3 IC [8].

In previous Indian VWD3 studies [5–7], molecular analysis failed to fully account for the genetic cause in 8/54 IC (15%). It is therefore likely that the actual disease causing mutation has yet to be identified by Kasatkar and colleagues, who imply that DNA scanning methods were used to identify mutations in their patient cohort [1]. DNA scanning methods are not 100% sensitive to all sequence variations so may fail to detect the ‘true’ mutation, for example chemical cleavage mismatch analysis identified a homozygous p.P2063S variant in a Turkish VWD3 IC but failed to identify the actual p.Q1734* causative mutation (Table 1) [8]. Alternatively, the causative mutation may have been overlooked if, for example, it were

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Conflict of interest

The authors state that there is no conflict of interest.

a synonymous variant that disrupts normal splicing or protein folding, or a deep intronic variant causing disruption of splicing outside the *VWF* regions analysed [9].

In summary, review of the available information supports the conclusion that p.P2063S is a common neutral *VWF* polymorphic variant. Further studies (for example using mRNA as the template investigated) may identify the underlying cause of VWD in this interesting patient group.

Acknowledgments

The authors are supported by the National Institutes of Health program project grant HL081588 Zimmerman Program for the Molecular and Clinical Biology of VWD.

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Table 1

Co-inheritance of p.P2063S and actual VWD causing variants

VWD type	Co-inherited variant	Allelic inheritance	Analysis method(s)	Reference
1	c.4135C>T (p.R1379C)	p.[R1379C(;);P2063S]	Seq	Johansson <i>et al.</i> [3]
1	c.6859C>T (p.R2287W)	p.[P2063S;R2287W]	Seq	Eikenboom <i>et al.</i> [2]
2A	c.605G>C (p.R202P)	p.[R202P];[P2063S]	Unknown	Schneppenheim <i>et al.</i> [10]
3	c.5200C>T (p.Q1734*)	p.[Q1734*;P2063S]	CCMA, MLPA, Seq	Hampshire <i>et al.</i> [8]

CCMA, chemical cleavage mismatch analysis; MLPA, multiplex ligation-dependent probe amplification; Seq, direct DNA sequence analysis; Unknown, exact analysis method(s) not reported.