Article:
Duval, C., Ali, M., Chaudhry, W.W. et al. (3 more authors) (2016) Factor XIII A-Subunit V34L Variant Affects Thrombus Cross-Linking in a Murine Model of Thrombosis. Arteriosclerosis, Thrombosis, and Vascular Biology, 36 (2). pp. 308-316. ISSN 1079-5642

https://doi.org/10.1161/ATVBAHA.115.306695

SUPPLEMENTAL MATERIAL

Factor XIII A-subunit V34L variant affects thrombus cross-linking rather than size in a murine model of thrombosis

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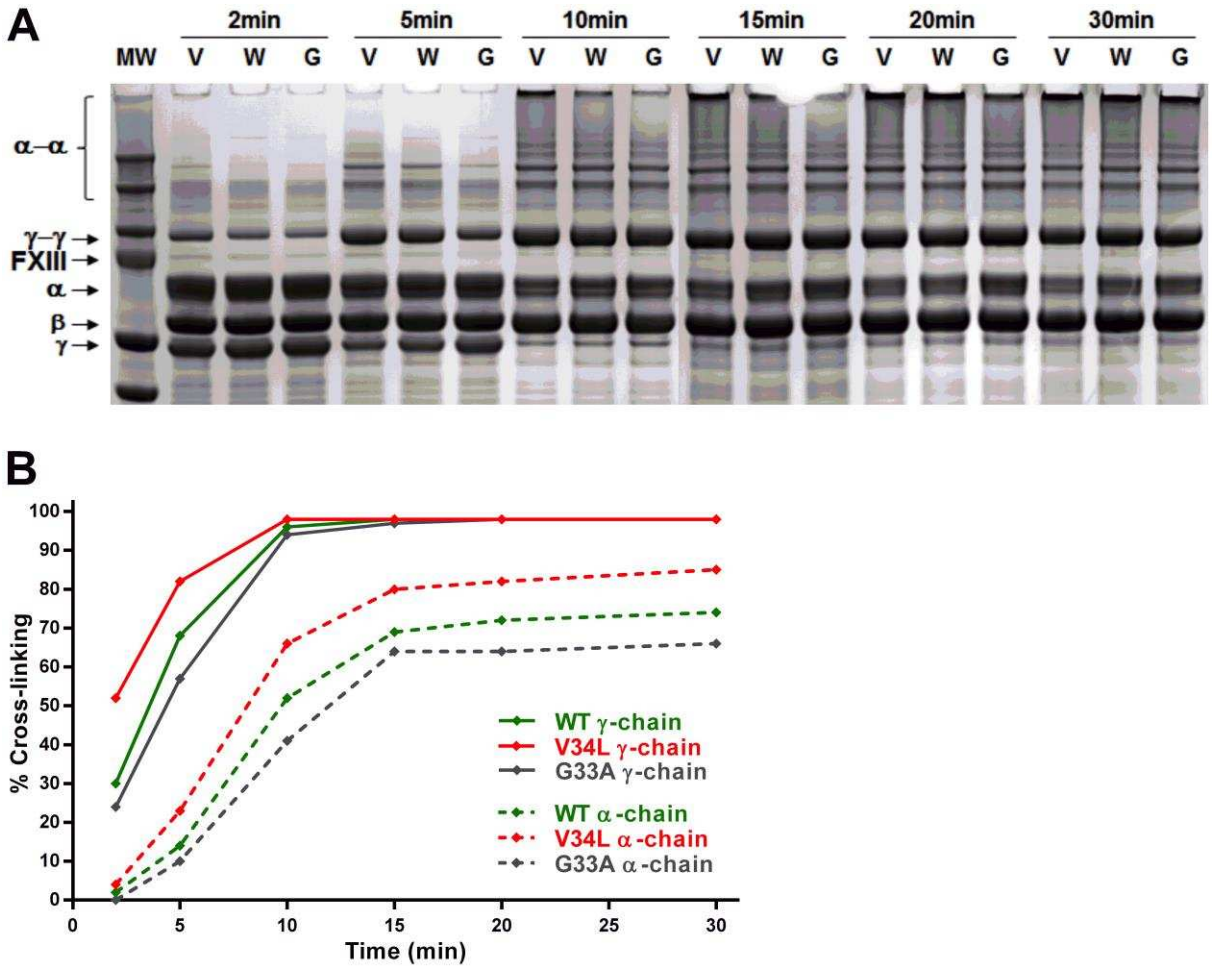

Figure I: Effects of rhFXIII-A_2 variants on formation of fibrin α- and γ-chain cross-links. Fibrin (2mg/ml) α- and γ-chain cross-linking by 15μg/ml rhFXIII-A_2 V34L (V), WT (W) and G33A (G) was observed over time by SDS-PAGE (A), in the presence of 0.5U/ml thrombin and 10mM $CaCl_2$. Band densitometry was performed in order to quantify the percentage of α- and γ-chain cross-linking, both expressed relative to the uncross-linked β-chain band intensities to correct for any loading differences (B). At 2 and 5 minutes, γ-chain cross-linking was increased for V34L and decreased for G33A, compared to WT. From 10 minutes, all the γ-chains were cross-linked, and the α-chain cross-linking increased over time. Cross-linking of the α-chain was also increased for V34L and decreased for G33A, compared to WT. Panel A shows a composite of 2 SDS-PAGE gels (lanes 1-10 and 11-19). n=1.

Table I: Site-directed mutagenesis primers for rhFXIII-A_2 variants. Mutated bases are highlighted.

Primer	Forward primer	Reverse primer
T28A	GGAAGATGACCTGCCC**G**CAGTGGAGCTTCAGGG	CCCTGAAGCTCCACTG**C**GGGCAGGTCATCTTCC
V29A	GCAGCGGAAGATGACCTGCCCACAG**C**GGAGCTTCAG	GGCACCACGCCCTGAAGCTCC**G**CTGTGGGCAGGTC
E30A	CCTGCCCACAGTGG**C**GCTTCAGGGCGTGG	CCACGCCCTGAAGC**G**CCACTGTGGGCAGG
L31A	CCTGCCCACAGTGGAG**GC**TCAGGGCGTGGTGC	GCACCACGCCCTGA**GC**CTCCACTGTGGGCAGG
Q32A	CCCACAGTGGAGCTT**GC**GGGCGTGGTGC	GCACCACGCCC**GC**AAGCTCCACTGTGGG
G33A	GTGGAGCTTCAGG**C**CGTGGTGCCCCGGGGCGTCAAC	GTTGACGCCCCGGGGCACCACG**G**CCTGAAGCTCCAC
V34A	GGAGCTTCAGGGCG**C**GGTGCCCCGG	CCGGGGCACC**G**CGCCCTGAACCTCC
V34L	GGAGCTTCAGGGC**C**TGGTGCCCCGG	CCGGGGCACCA**G**GCCCTGAAGCTCC
V34M	GGAGCTTCAGGGC**A**TGGTGCCCCGG	CCGGGGCACCA**T**GCCCTGAAGCTCC
V35A	GAGCTTCAGGGCGTGG**C**GCCCCGGGGC	GCCCCGGGGC**G**CCACGCCCTGAAGCTC
P36A*	CTTCAGGGCGTGGTG**G**CCCGGGGCGTCAACC	GGTTGACGCCCCGGG**C**CACCACGCCCTGAAG
R37A	CGTGGTGCCC**GC**GGGCGTCAACCTGC	GCAGGTTGACGCCC**GC**GGGCACCACG
G38A	CGTGGTGCCCCGGG**C**CGTCAACCTGC	GCAGGTTGACG**G**CCCGGGGCACCACG
V39A	CGTGGTGCCCCGGGGCG**C**CAACCTGC	GCAGGTTG**G**CGCCCCGGGGCACCACG
N40A	CGGGGCGTC**GC**CCTGCAAGAGTTTCTTAATGTC	GACATTAAGAAACTCTTGCAGG**GC**GACGCCCCG
L41A	GGGCGTCAAC**GC**GCAAGAGTTTCTTAATGTC	GACATTAAGAAACTCTTGC**GC**GTTGACGCCC

** P36A variant was successfully mutated but transformation step failed.*