Co-localization analysis of pathological *FXIIIB* subunit mutants confirm an indirect but varied effect on secretion

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Introduction & Aim

Inherited defects in the *F13A* and *F13B* genes of coagulation Factor XIII transglutaminase may result in a bleeding predisposition. The symptoms are milder in heterozygous deficiency that is more frequent in the population. Recently an investigation[1/2] of 7 FXIII-B subunit missense variants revealed a varied impact on secretion. Further investigation of these 7 variants was done to identify if organelle-specific intracellular accumulation is the reason for the reduced secretion. Using confocal microscopy and co-localization analysis (Fig. 1) we demonstrate that a direct effect on secretion is observed only for the *Cys5Arg* missense mutation.

Methods

Mutations were introduced into the Factor XIII B wild type cloned pEZ-MO1 expression vector and transfected into *HEK293T* cells. The Factor XIII B protein was stained with an anti-mouse IgG labelled Alexa488 antibody and both Golgi and Endoplasmic Reticulum (ER) were immunostained with anti-rabbit coupled Alexa594 Antibody for Confocal analysis. Calculation of the degree of co-localization was done with Image J version 1.43m software visualization and analysis software. MD (molecular dynamic) simulation was performed on wild type and variant modelled sushi domain in order to analyse the effect of the mutation on the structure (not shown here).

Results

Analysis of the co-localization with the Trans-Golgi showed accumulation of the *Cys5Arg* variant in ER. Mutations *Cys316Phe* and *Pro428Ser* showed reduced levels of co-localization with Golgi as well as ER. Mutations *Leu116Phe* and *Val217Ile* showed marginal decrease in co-localization in both Golgi and ER. Mutations *Ile81Asn* and *Val401Glu* showed similar levels of co-localization to the wild type in the ER and Golgi (Fig.1&2).

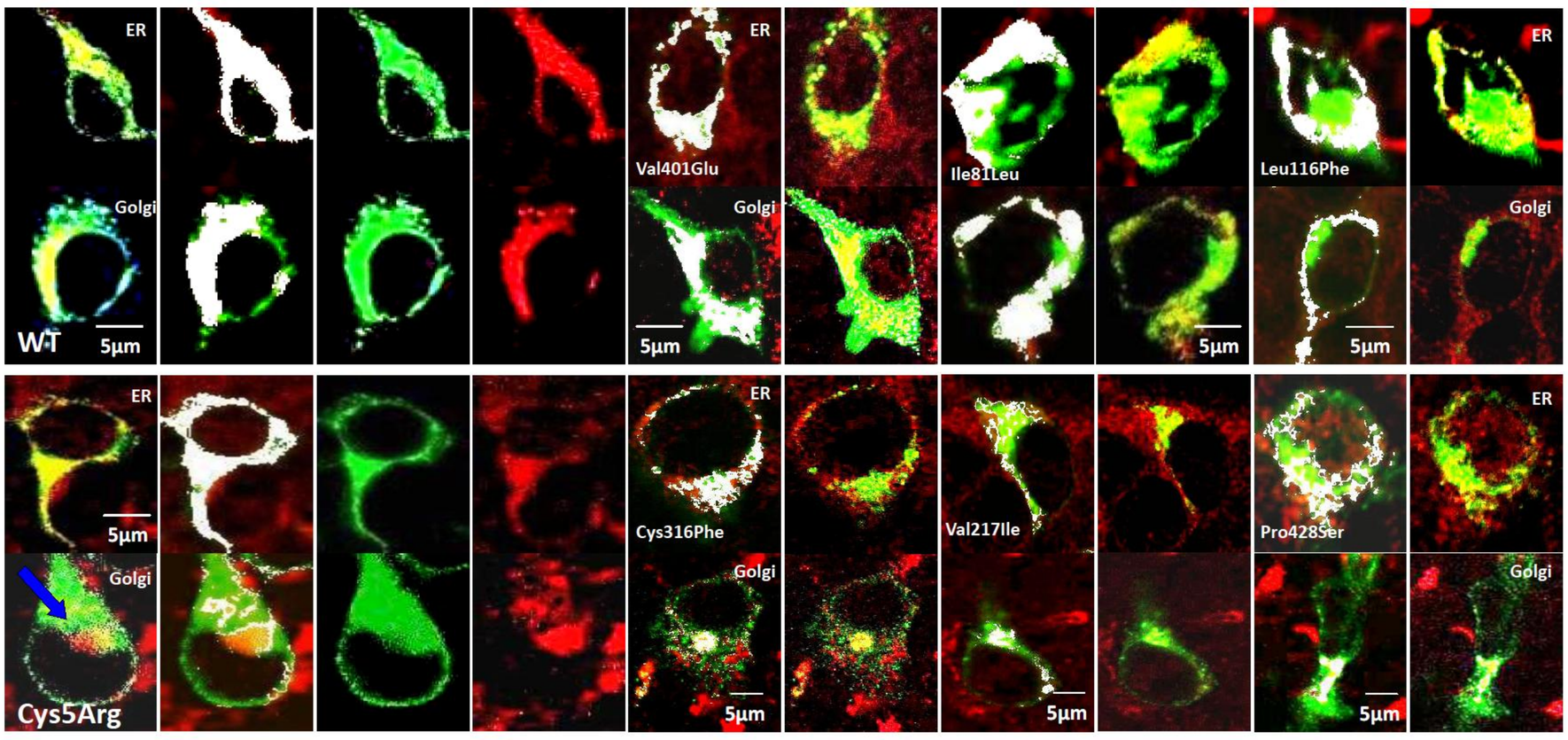


Figure 1: This figure shows the comparative co-localization status for the wild type and 7 variants. Green color represents the FXIIIB protein and Red the organelle (Endoplasmic Reticulum and Golgi body). The co-localized regions are shown in yellow (depending on the extent of co-localization) or in white using the co-localization highlighter in Image J visualization software. Only for Wildtyp and Cys5Arg ER and Golgi staining alone are shown. For the other 6 variants only the co.localized images are shown.

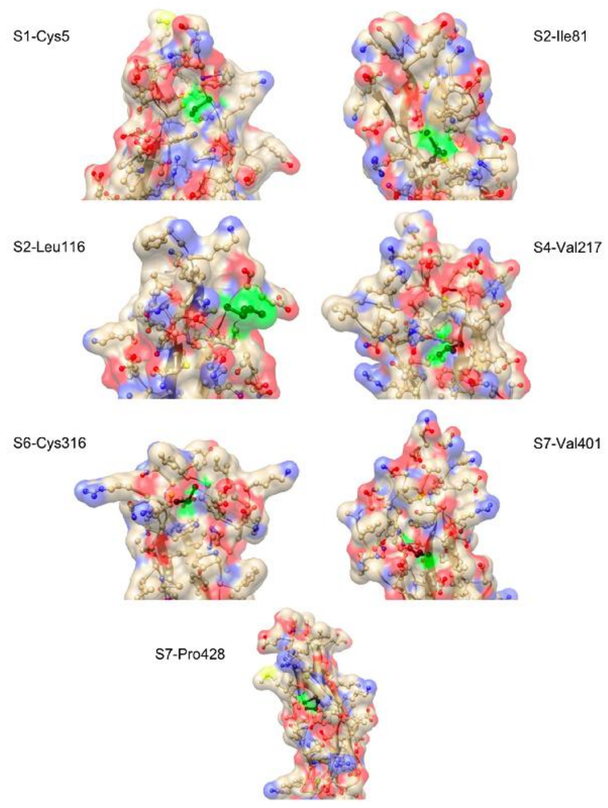
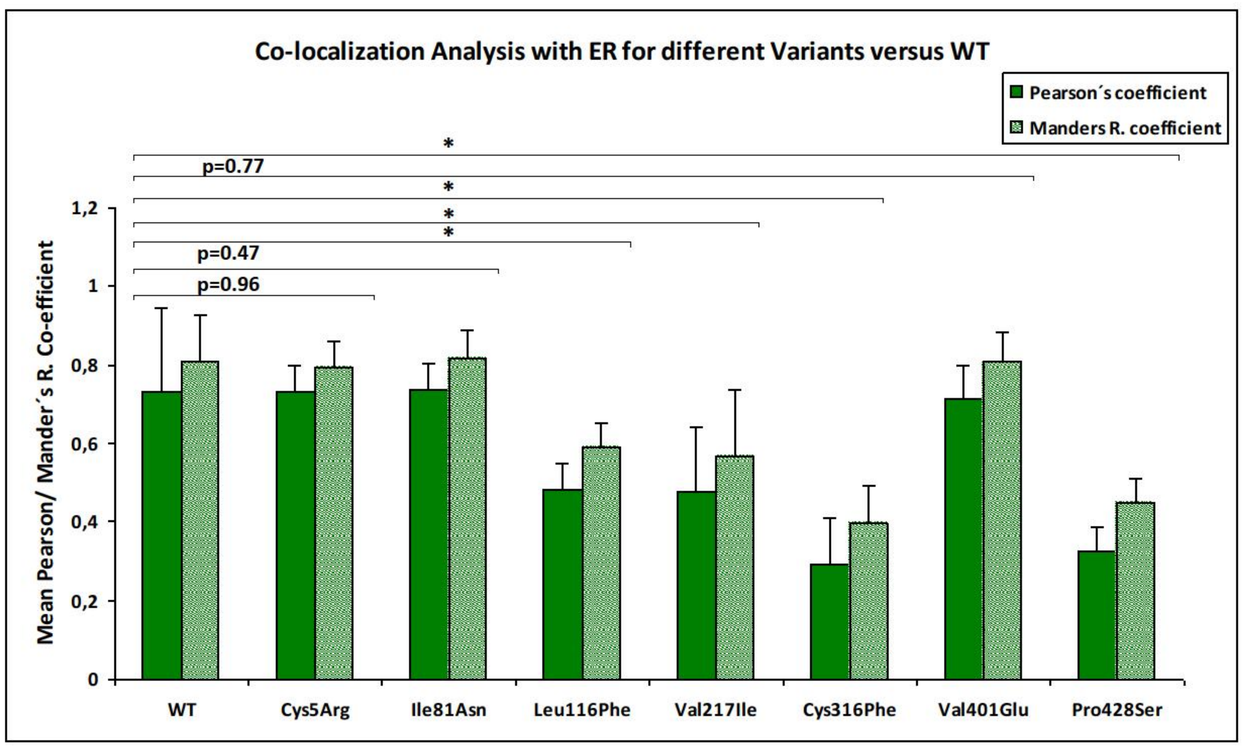


Figure 2: Shows the particular residue for each mutation on the FXIIIB modelled Sushi Domains.



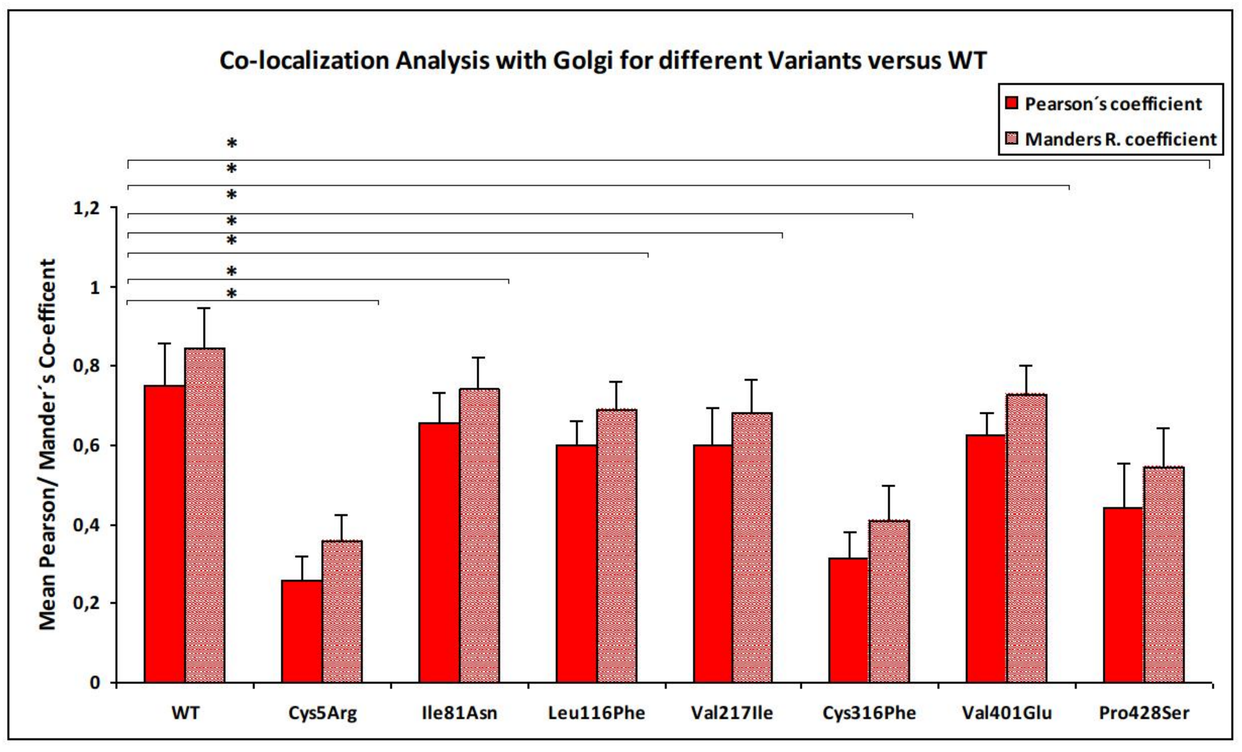


Figure 3: This graph shows the comparative Degree of co-localization for wild type and the 7 mutamts represented by mean Pearson and Mander´s R. co-efficient. Calculations were done on n =10 regions of interest (ROI). The bars represent the mean values and the error bars represent standard deviation. P-Value is only given for the Pearson´s coefficient. Black shows the degree of Co-localization with ER and Blue shows the degree of Co-localization with Golgi.

Discussion & Conclusion

Out of 7 missense mutants only one variant *Cys5Arg* confirms a direct impact on secretion. For the *Cys5Arg*, the affected cysteine residue is a buried residue in the Sushi Domain 1. The mutated *Arg* residue exposes hydrophobic surfaces as a result of induced misfolding which is recognized by the quality control system in the ER and removed gradually which leads to low levels of the remaining mutated protein in the Golgi. Therefore, we observe a lower degree of co-localization in the Golgi. Our antigen measurements for the intra- and extracellular expression support this hypothesis[1]. Mutations *Cys316Phe*, *Pro428Ser*,*Leu116Phe* and *Val217Ile* all show a signigicant or at least marginal decrease in co-localization with ER and Golgi when compared with the wild type. We assume that the impact on secretion observed for these variants is the indirect characteristic effect of each variant on the rate of biosynthesis and folding of the protein.

It seems that the diversity in the impact on secretion is a reflection of the differential impact on the degree and rate of folding of the mutant variants. The varied nature of folding/folding rates influences the rate of biosynthesis as well as clearance of the mutant variants.

[1] Biswas and Thomas et al. *In Vitro* Secretion Deficits are Common Among Human Coagulation Factor XIII Subunit B Missense Mutants: Correlations with Patient Phenotypes and Molecular Models. Human Mutations. 2013 Nov;34(11):1490-500

[2] Ivaskevicius V. et al. Mutations affecting disulphide bonds contribute to a fairly common prevalence of F13B gene defects: results of a genetic study in 14 families with factor XIII B deficiency. Haemophilia. 2010 Jul;1(16(4)):675-82