The D2194G mutation is responsible for increased levels of FV1 in carriers of the factor V R2 haplotype

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Dear Sir,

Factor V (FV) exists in plasma in two isoforms, FV1 and FV2. FV1 has a slightly higher molecular weight than FV2 due to partial glycosylation at N2181 in the C2 domain of the FV light chain (1). It was further shown that FV1 and FV2, beside the different glycosylation at N2181, have two N-linked glycosylations in common, at N1675 and at N1982 in the A3 and C1 domain, respectively (1). FV1 and FV2 have different characteristics with respect to their procoagulant activity, inactivation by APC and anticoagulant function in the protein C pathway. Consequently, FV1 is more thrombogenic than FV2 (2).

The FV R2 haplotype (FV-R2) is a common genetic variation, which has been associated with decreased plasma FV antigen levels, mild APC-resistance and an increased FV1: FV2 ratio (3). Furthermore, several missense mutations in the FV gene co-segregate and appear to contribute to the in vivo phenotype. To date, only few studies have characterised the plasma phenotypes associated with FV-R2 in detail. Hoekema et al. reported that FV-R2 carriers have increased FV1: FV2 ratios and suggested that the D2194G mutation plays a key role for the observation (3).

Here we report the effect of the D2194G mutation on the FV1: FV2 ratio by using in vitro expression studies to confirm the previous in vivo plasma-based data and, moreover, directly correlate glycosylation to mutation at D2194.

Recombinant wild-type FV and five mutant FV molecules were expressed in COS-1 cells as previously described (4). Primary translated FV molecules were radiolabelled, immunoprecipitated from cell lysates, cleaved by thrombin and subjected to SDS-PAGE after which densitometric analyses were performed.

Figure 1A shows the light chains of the recombinant FV molecules from a typical experiment. The amount of FVα1 was clearly increased when the recombinant FV molecule contained the D2194G mutation, with a relative increase in the FVα1: FVα2 ratio by over three-fold. In contrast, three other mutations, linked to FV-R2, did not affect the FVα1: FVα2 ratio.

We have previously reported that D2194 is crucial for the proper conformation of the FVα light chain, and more specifically for the C-terminal C2 domain in which D2194 is present (5). Thus, we hypothesise that D2194G mutation leads to altered folding kinetics of the FVα light chain, which might affect the glycosylation efficiency at N2181. Furthermore, the D2194G mutation has been reported to result in ER retention of mutant FV molecules (4).
Since protein folding and glycosylation occur during protein transport through the ER and Golgi and are two mutually dependent processes (6), we speculate that prolonged ER retention facilitates FV1 isoform formation, i.e. when a FV molecule resides in the ER longer, this will result in a more efficient glycosylation at N2181. To check this hypothesis, we added Brefeldin A, a compound known to block protein transport from the endoplasmatic reticulum (ER) to the Golgi apparatus, to the culture medium of wild-type human FV-expressing COS-1 cells. As a consequence, we observed a relative increase of the FVa1 isoform in the presence of Brefeldin A (Fig. 1B). Thus, our results are in agreement with the hypothesis that the ER retention caused by D2194G mutation results in an increase in glycosylation efficiency at N2181. In the case of FV-R2 is has been established that an altered FV1: FV2 ratio contributes to the proposed procoagulant phenotype (8). Also other coagulation proteins besides FV have been reported to express glycosylation variants with diverse functions. In the case of, e.g. antithrombin, FX, FVII, PAI-1 and FIX (9) different functional glycovariants have been described indicating the clinical relevance of the glycosylation state of plasma coagulation proteins. Increased ER retention should be considered as a causal factor contributing to heterogeneity in glycoproteins.

References

Corrigendum
In the original article by Shomron et al. “A splice variant of ADAMTS13 is expressed in human hepatic stellate cells and cancerous tissues” (Thromb Haemost 2010; 104: 531–535) one co-author, Alexandra Dobkin (e-mail: alexandradobkin@hotmail.com, affiliation: Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA) was inadvertently not listed. The authors apologise for this oversight.