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Tissue-factor induced allosteric enhancement of factor VIIa activity by stabilization of segment 215–219 and taming of W215 flexibility

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Background: The complex of coagulation factor VIIa (FVIIa) and tissue factor (TF) initiates blood coagulation upon vascular injury. Previous work has shown that TF binding to FVIIa generates new exosites for macromolecular substrates and induces allosteric changes in and around the FVIIa active-site, leading to enhanced FVIIa catalytic efficiency. Structural studies have added detailed molecular insights into the TF binding epitope, but have not elucidated the extensive and long-range structural effects in FVIIa.
Aims: To understand the molecular basis of TF-mediated allosteric regulation of FVIIa activity.
Methods: In the current study we have produced 3 FVIIa variants in which the 170-loop has been replaced with the corresponding loop from trypsin. The resulting variants have been investigated using a combined approach of functional and biophysical characterization, x-ray crystallography and molecular dynamics (MD) simulations.
Results: The functional characterization and x-ray crystal structures of these FVIIa variants suggest that stabilization of the segment 215–219 leads to maturation of the primary specificity pocket and enhanced catalytic efficiency, independent of protease domain N-terminus insertion. MD simulations support the experimental results and vouch for the direct involvement of W215 in TF-induced allosteric changes in the FVIIa protease domain. Our results are further corroborated by novel tryptophan quenching studies and solvent accessible surface area calculations.
Conclusion: We propose that in the absence of TF, FVIIa exists in a conformation where segment 215–219 is highly flexible, capable of occupying either a collapsed or an open conformation, functioning as an activity regulating mechanism. In addition to other changes, the binding of TF to FVIIa stabilizes segment 215–219 in an open conformation leading to enhanced functional properties of FVIIa. Taken together, our results shed new insights into molecular mechanism of TF-induced allosteric enhancement in FVIIa activity.
Disclosure of Interest: A. Sorensen Employee of: Novo Nordisk A/S, J. Madsen: None declared, A. Svensson Employee of: Novo Nordisk A/S, H. Stennicke Employee of: Novo Nordisk A/S, H. Østergaard Employee of: Novo Nordisk A/S, M. Overgaard: None declared, O. Olsen Employee of: Novo Nordisk A/S, P. Gandhi Employee of: Novo Nordisk A/S.

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Relationships of circulating coagulation factor VIIa (FVIIa) with common single nucleotide polymorphisms and risk of incident ischemic stroke: the Cardiovascular Health Study

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Background: A small but variable fraction (~1%) of coagulation factor VII circulates as an active protease (FVIIa) in human plasma. Data for FVIIc, (influenced by both FVIIa and total FVII), as a cardiovascular disease (CVD) risk factor has been inconsistent. To date, no studies have evaluated the genetic and epidemiologic associations of FVIIa.
Aims: Evaluate the associations of FVIIa with common single nucleotide polymorphisms (SNPs) and incident CVD events in a prospective population-based cohort of older adults.
Methods: FVIIa was measured by commercial clot-rate assay (Stago) in citrated plasma of ~3500 men and women ≥ 65-years from the Cardiovascular Health Study. Associations of FVIIa with ~50K SNPs were evaluated using the IBCv2 genotyping array. Cox proportional hazards models were used to calculate associations with incident CVD outcomes (median follow-up 14.5 years).
Results: FVIIa was correlated with FVIIc (Pearson $r^2 = 0.44$; $P < 0.0001$). Mean FVIIa was higher in women than men [57.9 vs. 45.9 mU mL^{-1}] ($P < 0.0001$) and in European-Americans (EAs) than African-Americans ($P < 0.05$). In EAs, 15 SNPs located in the *F7*/*F10* locus (chromosome 13) and 7 SNPs located in the *PROCR* locus (chromosome 20) were significantly associated with FVIIa. The top *F7* SNP, rs6046 ($P < 4.9 \times 10^{-24}$), was associated with 23.8 mU mL^{-1} lower FVIIa per minor allele; the top *PROCR* SNP, rs867186 ($P < 1.7 \times 10^{-12}$), was associated with 7.5 mU mL^{-1} higher FVIIa per minor allele. After adjustment for CVD-risk factors, FVIIa was associated with incident ischemic stroke ($P = 0.03$). Those in the highest FVIIa tertile [≥ 61 mU mL^{-1}] had a 29% increased risk vs. those in the lowest tertile [< 42 mU mL^{-1}]. There were no significant associations of FVIIa with incident MI, CHD, or CVD-related mortality.
Conclusion: These results support the importance of the *F7*/*F10* and *PROCR* loci on variation in circulating FVIIa, and suggest FVIIa may be a risk factor for ischemic stroke in older adults.
Disclosure of Interest: None declared.

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Epidermal tissue factor in wound healing

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Background: Healing of skin wounds relies on several biological processes, including hemostasis, inflammation, cell proliferation and tissue remodeling. It has been hypothesized that tissue factor (TF), an initiator of coagulation and regulator of inflammation, plays a role in the re-epithelialization of skin wounds. To investigate this function, wound healing was studied in Keratin-14 Cre+ TF$^{fl/fl}$ mice, which do not produce TF in their keratinocytes.
Aims: To confirm that a lack of epidermal tissue factor influenced wound healing and to explore the mechanisms underlying this defect.
Methods: Full thickness dermal wounds were placed on the backs of K14 Cre+ TF$^{fl/fl}$ mice and Cre- littermate controls using a 3-mm biopsy punch. The wound areas and number of healed mice were observed daily. Wound samples were collected during the course of healing for histological analyses and primary keratinocyte cultures were used to study signaling.
Results: Cre+ mice exhibited deletion of TF in the epidermis and the leading edge of healing wounds but maintained TF expression around blood vessels. Cre+ mice had larger wounds and slower healing times than Cre- mice and were more prone to bleeding.
Conclusion: Epidermal tissue factor is required for normal wound healing and influences bleeding, wound size and healing time. Further studies will investigate the cellular players underlying this phenotype.
Disclosure of Interest: None declared.