

The disruption of the platform would likely interfere with binding to high-molecular-weight kininogen,¹⁰ a protein that forms a complex with fXI in plasma. This, and the absence of fXI coagulant activity,¹ raise questions about the function of such a protein, if it was expressed.

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Acknowledgment: This work was supported by grants HL58837 and HL81326 from the National Heart, Lung, and Blood Institute.

Contribution: D.G. wrote the initial draft of the letter; M.-f.S. and Q.C. prepared fXI and performed Western blots; A.M. characterized IgG O1A6 and

14E11; A.G. and E.I.T. generated and characterized IgG O1A6 and 14E11; and J.E. modeled fXI apple domains. All authors contributed to writing the final version of the letter.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Response

Further thoughts on the "phantom" Δ 6/7 FXI isoform

We are pleased that our work raised the interest of the scientific community. Indeed, the main aim of the paper was to characterize the pattern of alternative splicing of the factor XI (F11) gene and to study its role in the regulation of F11 expression by the coupling of nonproductive splicing and nonsense-mediated mRNA decay.

In their letter, Gailani and coworkers focused their efforts in providing evidence against the existence of a factor XI (FXI) protein isoform in plasma originating from the skipping of exons 6 and 7 and therefore lacking the C-terminal half of the A2 domain and the N-terminal half of the A3 domain (FXI- $\Delta 6/7$). The rationale of their strategy was to show that the FXI- $\Delta 6/7$ is not recognized by monoclonal antibodies against A2 and A3 domains, whereas these same antibodies readily react against an approximately 105-kDa band immunoprecipitable from both human plasma and media conditioned by cells expressing full-length recombinant FXI. However, Gailani and colleagues' position is based on the assumption that the 105-kDa band corresponds to the hypothetical $\Delta 6/7$ band we found in human plasma. This is unlikely to be true for the following reasons: (1) The 105-kDa band was easily detectable by Coomassie staining loading just 2 to 4 µg of FXI. Conversely, we could detect the putative $\Delta 6/7$ FXI isoform by Coomassie-stained gels only loading at least 10 µg of commercial FXI, or by Western blotting after FXI immunoprecipitation from 1.5 mL of human plasma. (2) At variance with Gailani et al, we cannot see a 105-kDa band (or any band of a similar molecular weight) when expressing

recombinant full-length FXI in eukaryotic cells. The same result was found in other papers in the literature.^{1,2} Moreover, also in the data presented by Gailani et al, this band is undetectable in lysates of FXI-expressing HEK293 cells (compare panels C and E of Figure 1 in Gailani et al). (3) When immunoprecipitating FXI from human plasma with a mouse monoclonal antibody, we detected a band (under nonreducing conditions) migrating below FXI that was shown, by mass-spectrometry analysis, to correspond to contaminating mouse IgGs (data not shown in Asselta et al³). (4) The matching between the 105-kDa species and the putative $\Delta 6/7$ FXI isoform is further hampered when considering that more than one band is detectable in nonreducing SDS-PAGE of plasma FXI purified by affinity chromatography with goat anti-human FXI polyclonal antibody (our unpublished data). Mass spectrometry of these additional excisable bands demonstrated that they correspond to contaminating proteins (ie, ceruloplasmin, complement factor H, haptoglobin, and serum albumin).

In conclusion, we agree that our data do not unequivocally demonstrate the existence of a circulating FXI isoform originating from the $\Delta 6/7$ FXI transcript (as thoughtfully stated in our original paper³). However, we feel that the results presented by Gailani and colleagues are still not conclusive. A future possibility could be to purify enough FXI from human plasma to be able to analyze fast-migrating FXIspecific bands by mass spectrometry after digestion with a protease (such as Glu-C) generating $\Delta 6/7$ -specific peptides.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

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