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POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Increased Activated Protein C Response Rates to Thrombin Formation in Asymptomatic Factor V Leiden Carriers Are Driven By the Endothelium

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Background

The thrombophilic factor V Leiden (FVL) mutation shows a highly variable clinical expressivity. We have shown in vivo that asymptomatic FVL carriers respond to extrinsic coagulation activation with higher activated protein C (APC) formation rates than those with a history of venous thromboembolism (VTE). Recently we have established an ex vivo model for personalized assessment of the protein C (PC) pathway combining endothelial colony forming cells (ECFCs) and autologous plasma. Aim of this study was to investigate potential modulating factors of the APC response in the endothelium unraveling the enigma of the variable expressivity of the FVL phenotype.

Methods

ECFCs were isolated from FVL carriers with (FVL VTE+) or without (FVL VTE-) a history of unprovoked VTE and healthy controls (non-FVL) (n=7 each). Quality control of cultured cells included cobblestone morphology as examined by light microscopy, positive staining for CD31, CD309, CD201, and CD141, CD34, and negative staining for CD45 analyzed by flow cytometry. We either added autologous or pooled normal defibrinated plasma to the confluent cell culture and inititated thrombin formation by addition of tissue factor (1 pmol/L) and $CaCl_2$ (16.6 mmol/L) (**Fig. 1A**). Thrombin and APC formation were measured over time using oligonucleotide-based enzyme capture assays (OECAs) and the ratio between the area under the curve (AUC) of APC formation and the AUC of thrombin formation was calculated as measure for APC response to thrombin. Moreover, we quantified thrombomodulin (TM) and endothelial protein C receptor (EPCR) expression in cell-based enzyme-linked immunosorbent assays (cell-ELISAs) and measured activation of purified PC on ECFCs in the APC-OECA.

Results

ECFC morphology in light microscopy and characteristic surface marker expression in flow cytometry did not differ between non-FVL, FVL VTE-, and FVL VTE+ subgroups. In the ECFC-based ex vivo model, the APC response in plasma on autologous cells was significantly higher in asymptomatic FVL VTE- carriers compared to non-FVL carriers ($P = .0072$) and compared to the FVL VTE+ subgroup ($P = .0292$) whereas it did not differ significantly between non-FVL and FVL VTE+ carriers. EPCR expression on ECFCs measured by cell-ELISA was higher in non-FVL carriers compared to FVL VTE+ subjects ($P = .0374$) but did not differ significantly when comparing non-FVL versus FVL VTE-, and FVL VTE+ versus FVL VTE-. Similarly, we showed that TM expression as well as activation of purified PC on ECFCs did not differ between cohorts. However, when the APC response was assessed on FVL VTE- and FVL VTE+ ECFCs using non-FVL plasma, the difference in the APC response between asymptomatic FVL carriers and FVL carriers with a history of VTE persisted ($P = .0111$) (**Fig. 1B**).

Conclusion

Consistent with previous in vivo experiments, APC response rates to thrombin formation were increased on ECFCs obtained from asymptomatic FVL carriers compared to those from FVL carriers with a history of VTE in autologous plasma. Although TM and EPCR expression did not differ between both groups, we showed that the endothelial APC response in asymptomatic FVL carriers remained increased when using plasma from healthy controls. Our observations suggest that the increased APC response to thrombin in asymptomatic FVL carriers is driven by the endothelium. Further studies are warranted to elucidate the underlying mechanism for a better understanding of endogenous protective factors in FVL carriers. These findings may pave the way towards personalized, precision medicine approaches for managing the thrombotic risk in FVL and other disorders related to the PC pathway.

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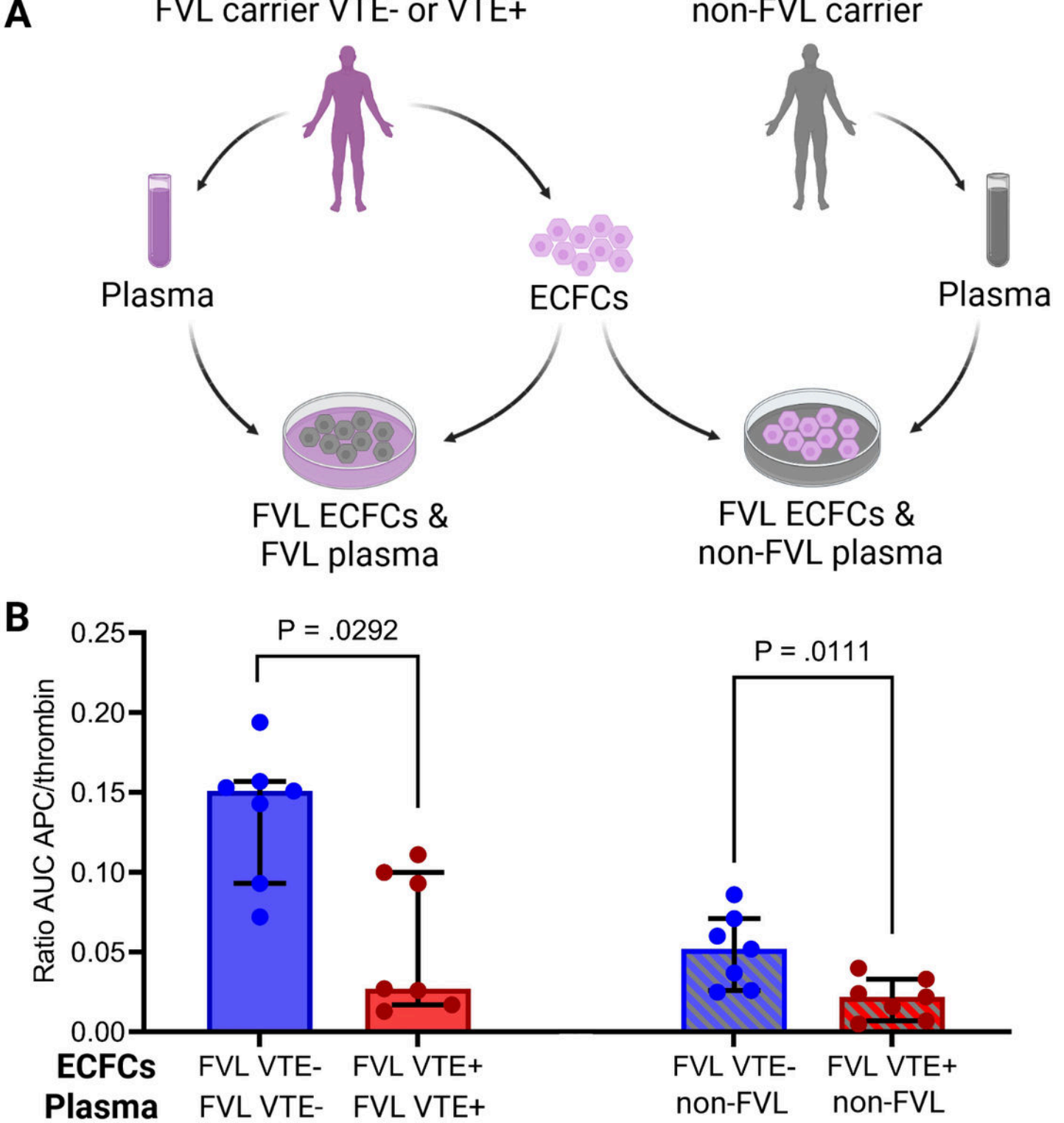

Figure 1