Abstract 137: Identification Of A Noncoding Genetic Variant In The Tissue Factor Locus That Reverses Lethal Thrombosis In Mice

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Abstract

Background: Thrombosis is initiated by tissue factor (TF, gene name *F3*) binding to coagulationFVII, with tissue factor pathway inhibitor (TFPI) inhibiting this complex. Alterations in TF orTFPI expression significantly affect thrombosis. Reducing TFPI expression by 50% (*Tfpi*+/-) inmice results in a perinatal lethal phenotype on the Factor V Leiden homozygous($F5^{L/L}$)prothrombotic background. We used the $F5^{L/L}$*Tfpi*+/- lethal phenotype to conduct a dominantsensitized whole genome ENU mutagenesis screen to suppress the $F5^{L/L}$*Tfpi*+/- lethality. Weidentified a Modifier of Factor 5 Leiden 6 (MF5L6) line with 72% penetrance and 85 $F5^{L/L}$*Tfpi*+/- offspring. A significant linkage peak (LOD=4.35),explaining half the suppressing effect andcontaining *F3* (Chromosome 3) was identified.

Goals/Hypothesis: To identify the genomic variant controlling F3 expression in the MF5L6 line.

Methods: To quantify *F3* expression in the surviving mice from MF5L6, quantitative PCR onliver, lung, and heart tissues from MF5L6 was performed. We used Sanger DNA and highthroughput sequencing to identify candidate TF regulatory variants in the F3 locus. Theprothrombin time assay was used to test the effects of reduced TF expression on in vitro bloodcoagulation.

Results: Two distinct expression profiles in the lung and liver of the MF5L6 mice wereobserved, those that had a 50% reduction in *F3* mRNA and those that did not. Heart tissuesexhibited one expression profile, suggesting that the variant regulates F3 expressiontissue-specifically. Sanger sequencing of the *F3* coding region revealed no coding mutations inMF5L6 mice. Whole genome sequencing identified two novel candidate variants (in unknownF3 regulatory elements) in the 200 kilobase upstream region of *F3*. The 50% reduction in *F3*resulted in significant changes in coagulation by the prothrombin assay ($n=18, p<0.0009$).

Conclusion: We identified novel candidate variants for regulating *F3* gene expression and aredetermining their mechanism of action. Investigation of these variants will provide new insightsinto the regulation of *F3* and enable us to identify the variant(s) responsible for the remainder ofthe thrombosis suppressing effect in MF5L6. Our findings provide new insights into the geneticregulation of thrombosis.