

On the role of genetic variation and epigenetics in hemostatic gene regulation

Akademisk avhandling

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Avhandlingen baseras på följande delarbeten:

- I. Olsson M, Hultman K, Dunoyer-Geindre S, Curtis MA, Faull RLM, Kruihof EKO, Jern C. Epigenetic regulation of tissue-type plasminogen activator in human brain tissue and brain-derived cells. *Gene Regulation and Systems Biology* 2016;10:9-13.
- II. Olsson Lindvall M, Hansson L, Klasson S, Davila Lopez M, Jern C, Stanne TM. Hemostatic genes exhibit a high degree of allele-specific regulation in liver. *Thrombosis and Haemostasis* 2019;119:1072-1083
- III. Olsson Lindvall M, Davila Lopez M, Klasson S, Hansson L, Nilsson S, Stanne TM**, Jern C**. A comprehensive sequencing-based analysis of allelic methylation patterns in hemostatic genes in human liver. *Thrombosis and Haemostasis* 2020; *Epub ahead of print*.
- IV. Olsson Lindvall M*, Angerfors A*, Andersson B, Nilsson S, Davila Lopez M, Hansson L, Stanne TM**, Jern C**. Comparison of DNA methylation profiles of hemostatic genes between liver tissue and peripheral blood within individuals. *In manuscript*.

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**SAHLGRENKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN**



On the role of genetic variation and epigenetics in hemostatic gene regulation

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Many genetic variants have been identified to associate with circulating levels of hemostatic proteins and with thrombotic or hemorrhagic disorders. However, the underlying molecular mechanisms remain largely unknown.

The overall aim of this thesis was to study how genetic variation and epigenetic mechanisms influence the regulation of hemostatic gene expression. The specific aims were to investigate epigenetic mechanisms regulating tissue-type plasminogen activator (t-PA) gene expression in the human brain (Paper I); to identify *cis*-acting variants involved in hemostatic gene regulation in liver (Papers II and III); and to investigate whether DNA methylation patterns in hemostatic genes in blood can reliably predict those in liver (Paper IV).

In Paper I, human astrocytes and neurons were treated with histone deacetylase (HDAC) inhibitors. Protein and mRNA levels of t-PA were measured using ELISA and real-time qPCR, respectively. Histone modifications were assayed with chromatin immunoprecipitation, and DNA methylation analysis of the t-PA promoter was performed by bisulfite sequencing.

In Papers II-IV, liver tissue and blood samples were collected from patients undergoing liver surgery and targeted DNA-, RNA- and methylation sequencing was performed for 35 hemostatic genes with predominant expression in the liver. These data were used in Papers II and III to performed allele-specific analyses of mRNA expression (ASE) and DNA methylation (ASM) in liver. In Paper IV, the extent to which blood can be used as a surrogate for DNA methylation of hemostatic genes in the liver was investigated.

In Paper I, cell treatments with HDAC inhibitors resulted in an increase in t-PA mRNA and protein expression, and in a significant increase in histone H3 acetylation. DNA methylation analysis revealed that the t-PA promoter was hypomethylated in neurons, astrocytes, and in post-mortem brain tissue, which indicates active transcription. In Paper II, ASE was identified in 60% of the hemostatic genes studied and 14 novel genotype-expression associations were discovered. In Paper III, a detailed DNA methylation map of the targeted hemostatic genes in liver was created, and novel associations between SNPs and DNA methylation were identified. The analyses performed in Paper IV showed that the correlation of hemostatic gene methylation between liver and blood was generally low. However, about 3% of the investigated CpGs had methylation levels that were significantly correlated between the two tissues, and for these, blood may potentially be used as a surrogate tissue to detect liver methylation.

Taken together, these findings highlight the importance of integrating genetic, epigenetic, and expression analyses in the relevant tissue, and demonstrate that this approach can contribute to new insights into the biological processes affecting hemostasis and thrombosis.

Keywords:

Hemostasis, tissue-type plasminogen activator, genetics, epigenetics, DNA methylation