Transcriptional regulation of the human immunodeficiency virus type 1 enhancer binding protein 1 (HIVEP1)

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A. Salomon1,2, B. Schmitz2, M. Herrmann3, A. Rötrige1, E. Brand3, P.E. Morange4, F. Cambien5, L. Tiri5, D. Trégouët5, S.-M. Brand1,2

1Leibniz-Institute for Arteriosclerosis Research, Münster; 2Medical Faculty of the Westfalian Wilhelms-University of Münster, Department of Molecular Genetics of Cardiovascular Disease, University of Münster, Münster; 3University Hospital Münster, Internal Medicine D, Münster; 4INSERM, UMR_S 626, Marseille; 5INSERM, UMR_S 525, Paris

Objective: As a ZAS-family member, HIVEP1 binds specific DNA sequences, including NF-κB and other proinflammatory consensus sequences. We recently identified rs169713, positioned 90 kb upstream of the HIVEP1 gene, to be replicatively associated with venous thrombosis (AJHG 2010) in a multistage study following GWAs.

Methods: Serial HIVEP1 promoter deletion constructs were cloned into a pGL3-Basic vector, and a 332 bp potential enhancer fragment, harbouring rs169713C/T, into the pGL3-Promoter vector. Reporter gene assays were performed by transient transfection and overexpression of transcription factors was executed by cotransfection assays in vascular endothelial cells EA.hy926 and THP1 monocytes. Bandshift assays were performed with EA.hy926 and THP1 nuclear extracts.

Results: In EA.hy926 cells, endogenous HIVEP1 expression was increased by proinflammatory cytokines TNFα, IL1β and IL4. The construct harbouring rs169713T showed significantly higher transcriptional activity over the empty pGL3-Promoter vector and the one including rs169713C (both P<0.001); similar results were revealed in THP1 cells. Reporter gene assays demonstrated a regulatory effect on HIVEP1 expression for intron 1. Cotransfection of SP1 and EGR1 led to an increase in transcriptional activity, while this increase by overexpression of WT1 was only observed for constructs comprising part of intron 1. By bandshift assays combined with specific antibody detection, we identified binding of SP1 to intron 1.

Conclusion: The rs169713 site harbours potential activational capacity for HIVEP1 gene transcription. Basal HIVEP1 expression is regulated by SP1, a potential interaction partner of EGR1 and WT1, combined in a module under basal and/or inflammatory conditions. These factors could be part of a coactivator complex, which communicates with the putative enhancer element 90 kb upstream of HIVEP1. Unknown HIVEP1 target genes and signal transduction pathways will be identified by knockdown via si-RNA and Illumina BeadChip analysis validated by HIVEP1 overexpression.