Common Genetic Variation in Relation to Brachial Vascular Dimensions and Flow-Mediated Vasodilation

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Running Title: FMD GWAS in community cohorts

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Brachial artery dimensions and reactivity as assessed by flow-mediated dilation (FMD) using ultrasound have been associated with cardiovascular disease.[1] Systematic analysis of common genetic variation and brachial artery reactivity may help explain the hereditary component of vascular function.[2] Because of the sensitive nature of the data collected for this study, requests to access the dataset from qualified researchers trained in human subject confidentiality protocols may be sent to Prof. Dr. med. Renate B. Schnabel at Department of General and Interventional Cardiology, University Heart Center Hamburg-Eppendorf, Germany. We performed an inverse variance weighting in a fixed effects meta-analysis of six genome-wide association studies on brachial artery diameter, maximum brachial artery diameter adjusted for baseline diameter, and FMD totaling 17,151 community-based individuals of European ancestry (Cardiovascular Health Study, Framingham Heart Study, Gutenberg Health Study (GHS), Prospective Investigation of the Vasculature in Uppsala Seniors, Study of Health in Pomerania (SHIP), and Young Finns Study). For replication we genotyped up to 9,555 independent individuals of the GHS using functionally tested standard 5´ nuclease assays on a 7900HT Fast Real-Time PCR system (Applied Biosystems). We selected eight SNPs (6 for brachial artery diameter, 2 for maximum brachial artery diameter adjusted for baseline diameter, 0 for FMD) with genome-wide significance or nominally significant regions with several supporting signals in the region and pathophysiological plausibility for replication by *de novo* genotyping in an independent GHS sample. Furthermore, we performed a stage 2 meta-analysis including all individuals. All studies were approved by the local ethics committees.

The mean age of the study participants was 55 years and approximately 50% were women. We report two novel loci for baseline brachial artery diameter that replicated (**A**); none of the SNPs chosen for replication with close to genome-wide significance in stage 1 meta-analysis for brachial artery diameter adjusted for baseline replicated (**B**), and no SNPs reached near genome-wide significance for the FMD phenotype in the discovery phase. The first one is a single nucleotide polymorphism (SNP) rs924140 on chromosome 7 (minor allele frequency [MAF] 0.43) in the insulin-like growth factor binding protein 3 (*IGFBP3*) gene, effect size beta per mm 0.033±0.006, *P* discovery=1.34x10^{-8}. IGFBP3 protein concentrations measured in 1485 individuals of the SHIP-1 sample by automated chemiluminescence immunoassays (Siemens Immulite 2500; Siemens Healthcare Medical Diagnostics) were related with brachial artery diameter (beta per ng/mL 0.000039, *P*=0.003) in multivariable-adjusted analysis. Further, rs1926034 on chromosome 10 (MAF 0.38) in the arsenite methyltransferase (*AS3MT*)-*CNNM2* locus was identified, beta per mm 0.037±0.006, *P* discovery=2.06x10^{-9} in a genomic region related to blood pressure traits. The SNP was associated with AS3MT mRNA in monocytes of 1367 individuals of the GHS, *P*=8.79x10^{-36} for which we examined the replicated SNPs in relation to whole genome transcriptome data of circulating monocytes with RNA hybridized to Illumina HT-12 v3 BeadChips (Illumina Inc,). Boxplots of expression intensity revealed a gradual decrease with a higher number of T alleles in monocytes and arterial tissues from different locations drawn from the GTEx Portal (gtexportal.org) for post mortem tissue (**C**). Further *trans* associations were observed for expression of B-cell receptor-associated protein 29 (*BCAP29*), chromosome 7 and the zinc finger protein 644 (*ZNF644*) in monocytes in the GHS. No monocyte gene expression signal was observed for the *IGFBP3* gene locus.

IGFBP3 serves in the regulation of insulin-like growth factor I (IGF-I) concentrations and bioavailability. The circulating protein has been related to intima-media thickness and incident ischemic heart disease.[3] The genetic locus identified in relation to baseline brachial artery diameter in our study has been related to circulating IGFBP3 protein concentrations.[4] Thus, our results are in context with the literature indicating a possible pathophysiological pathway from genetically determined IGFBP3 protein concentrations through vascular remodeling to cardiovascular disease and mortality. Whether modulation of such a speculative pathway may improve cardiovascular health needs to be evaluated.

The *AS3MT-CNNM2* locus belongs to a gene-rich genomic region related to blood pressure phenotypes. The *AS3MT-CNNM2* locus has been associated with coronary artery disease,[5] and gene expression consistently across arterial tissue of different vascular beds. Long-term impact of elevated blood pressure may result in changes of the vascular wall that increase brachial artery dimensions and are correlated with coronary artery dysfunction and vulnerability to coronary artery disease.

Few genetic associations for FMD have been reported to date, most of which have not been replicated in independent samples including initial genome-wide association study results. This observation is not unexpected since the heritability of FMD is low even in twin studies indicating that the genetic component at the population level may not be strong. The low variability explained by genetics may suggest an important role of environment factors contributing to FMD variability. The clinical value of FMD may thus remain the ability to quantify vascular reactivity in the current state.

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Disclosures

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Figure legend

Summary study data

A. Association results of baseline brachial artery diameter in the discovery (stage 1 meta-analysis), replication and combined stage 2 meta-analysis. NA stands for not applicable. Effect size is given for mm increase. SNPs which reached genome-wide significance in stage 1 meta-analysis are printed in bold.

B. Top association results of maximum brachial artery diameter adjusted for baseline diameter in the discovery (stage 1 meta-analysis), replication and combined stage 2 meta-analysis. Effect size is given for change of baseline diameter in mm.

C. Boxplots of expression intensity for AS3MT mRNA in relation to rs2297786 and rs1926034 genotypes in monocytes (Gutenberg Health Study) and different arterial vessels (GTEx portal).

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