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

Authors
Marcus Dörr, MD, FHS\textsuperscript{1,2*}; Naomi M. Hamburg, MD, MSc\textsuperscript{3*}; Christian Müller, PhD\textsuperscript{4,5*}; Nicholas L. Smith, PhD\textsuperscript{6,7,8}; Stefan Gustafsson, PhD\textsuperscript{9}; Terho Lehtimäki, MD, PhD\textsuperscript{10}; Alexander Teumer, PhD\textsuperscript{11}; Tanja Zeller, PhD FHS\textsuperscript{4,5}; Xiaohui Li, MD, MSc\textsuperscript{12}; Lars Lind, MD, PhD\textsuperscript{9}; Olli T. Raitakari, MD, PhD\textsuperscript{13,14}; Uwe Völker, PhD\textsuperscript{2,15}; Stefan Blankenberg, MD, PhD\textsuperscript{5}; Barbara McKnight, PhD\textsuperscript{16}; Andrew P. Morris, PhD\textsuperscript{17}; Mika Kähönen, MD, PhD\textsuperscript{18,19}; Rozenn N. Lemaitre, PhD\textsuperscript{20}; Philip S. Wild, MD MSc\textsuperscript{21,22,23,24*}; Matthias Nauck, MD\textsuperscript{2,25*}; Henry Völzke, MD\textsuperscript{2,11}; Thomas Münzel, MD\textsuperscript{23,24,26}; Gary F. Mitchell, MD\textsuperscript{27}; Bruce M Psaty, MD, PhD\textsuperscript{6,7}; Cecilia M. Lindgren, PhD\textsuperscript{28,29,30}; Martin G. Larson, ScD\textsuperscript{31,32}; Stephan B. Felix, MD\textsuperscript{1,2*}; Erik Ingelsson, MD, PhD\textsuperscript{3,33*}; Leo-Pekka Lyytikäinen, MD\textsuperscript{34*}; David Herrington, MD, MHS\textsuperscript{34*}; Emelia J. Benjamin MD, ScM\textsuperscript{31,35}; Renate B. Schnabel, MD, MSc \textsuperscript{4,5*}

Affiliations
1 Department of Internal Medicine B, University Medicine Greifswald, Greifswald, Germany;
2 DZHK (German Centre for Cardiovascular Research), partner site Greifswald, Germany;
3 Department of Medicine, Sections of Cardiology and Vascular Medicine, Boston University School of Medicine, Boston, MA, USA;
4 Department of General and Interventional Cardiology, University Heart Center Hamburg-Eppendorf, Germany;
5 DZHK (German Center for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Germany;
6 Cardiovascular Health Research Unit, Department of Medicine, Epidemiology, and Health Services, University of Washington, Seattle, WA, USA;
7 Kaiser Permanente Washington Health Research Institute, Seattle, WA, USA;
8 Seattle Epidemiologic Research and Information Center, Department of Veteran Affairs Office of Research and Development, Seattle WA, USA;
9 Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden;
10 Department of Clinical Chemistry, Finnlab Laboratories, and Finnish Cardiovascular Research Center - Tampere, Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland
11 Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany;
12 Institute for Translational Genomics and Population Sciences, Department of Pediatrics, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center;
13 Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland;
14 Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Finland;
15 Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany;
16 Cardiovascular Health Research Unit, Department of Biostatistics, University of Washington, Seattle, WA, USA;
17 Department of Biostatistics, University of Liverpool, Liverpool, UK;
18 Department of Clinical Physiology, Tampere University Hospital, Tampere, Finland;
19 Department of Clinical Physiology, Finnish Cardiovascular Research Center - Tampere, Faculty of Medicine and Life Sciences, University of Tampere, Tampere 33014, Finland;
20 Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, USA;
21 Preventive Cardiology and Preventive Medicine, Center for Cardiology, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany;
22 Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany;
23 DZHK (German Center for Cardiovascular Research), partner site Rhine-Main, Mainz, Germany;  
24 Center for Translational Vascular Biology (CTVB), University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany;  
25 Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany;  
26 Center for Cardiology, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany;  
27 Cardiovascular Engineering Inc., Norwood, MA, USA;  
28 Li Ka Shing Centre for Health Information and Discovery, The Big Data Institute, University of Oxford, Oxford, UK;  
29 Wellcome Trust Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, UK;  
30 Broad Institute of the Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA.  
31 Boston University and the NHLBI's Framingham Heart Study, Boston, MA, USA;  
32 Department of Biostatistics, Boston University School of Public Health, Boston, MA, USA;  
33 Department of Medicine, Division of Cardiovascular Medicine Stanford University School of Medicine and Diabetes Research Center Stanford, CA 94305, USA;  
34 Section on Cardiovascular Medicine, Wake Forest University School of Medicine, Winston Salem, NC, USA;  
35 Boston University Schools of Medicine and Public Health, Boston, MA, USA.

*indicates equal contribution

**Running Title:** FMD GWAS in community cohorts

**Addresses for Correspondence:**  
Renate B. Schnabel, MD, MSc  
University Heart Center  
Department of General and Interventional Cardiology  
Martinistr. 52  
Building O50  
20246 Hamburg, Germany  
Phone: +49-1522-2816064  
Fax: +49-40-7410-55310  
E-mail: r.schnabel@uke.de

Marcus Dörr, MD  
University Medicine Greifswald  
Department of Internal Medicine B  
Ferdinand Sauerbruch-Str.  
17475 Greifswald, Germany  
Phone: +49-3834-8680510  
Fax: +49-3834-8680502  
E-mail: mdoerr@uni-greifswald.de

**Key words:** flow-mediated dilation, genome-wide association, insulin-like growth factor binding protein 3, epidemiology
Brachial artery dimensions and reactivity as assessed by flow-mediated dilation (FMD) using ultrasound have been associated with cardiovascular disease. Systematic analysis of common genetic variation and brachial artery reactivity may help explain the hereditary component of vascular function. 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, $P_{\text{discovery}}=1.34\times10^{-8}$. 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, $P_{\text{discovery}}=2.06\times10^{-9}$ in a genomic region related to blood pressure traits. The SNP was associated with AS3MT mRNA in monocytes of 1367 individuals of the GHS, $P=8.79\times10^{-36}$ 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 \textit{AS3MT-CNNM2} locus belongs to a gene-rich genomic region related to blood pressure phenotypes. The \textit{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.
Sources of Funding

Cardiovascular Health Study: This CHS research was supported by National Heart, Lung, and Blood Institute (NHLBI) contracts HHSN268201200036C, HHSN26820080007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, R01HL120393, and R01HL130114 with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences (CTSI) grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center.

Dr. Herrington has received multiple NHLBI and NIH grants related to vascular function research.

Framingham Heart Study was funded by National Heart, Lung, and Blood Institute HHSN268201500001I; N01-HC 25195; 1R01HL60040; 1RO1HL70100; 1R01HL128914; 2R01 HL092577.

Gutenberg Health Study: The Gutenberg Health Study is funded through the government of Rhineland-Palatinate („Stiftung Rheinland-Pfalz für Innovation“, contract AZ 961-386261/733), the research programs “Wissen schafft Zukunft” and “Center for Translational Vascular Biology (CTVB)” of the Johannes Gutenberg-University of Mainz, and its contract with Boehringer Ingelheim and PHILIPS Medical Systems, including an unrestricted grant for
the Gutenberg Health Study. Philipp S. Wild is funded by the Federal Ministry of Education and Research (BMBF 01EO1503) and he is PI of the German Center for Cardiovascular Research (DZHK). This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No 648131). This work was performed in the context of the Junior Research Alliance symAtrial project funded by the German Ministry of Research and Education (BMBF 01ZX1408A) e:Med – Systems Medicine program (RBS, TZ). RBS is supported by Deutsche Forschungsgemeinschaft (German Research Foundation) Emmy Noether Program SCHN 1149/3-1.

The Study of Health In Pomerania: SHIP is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (grants no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs as well as the Social Ministry of the Federal State of Mecklenburg-West Pomerania, and the network ‘Greifswald Approach to Individualized Medicine (GANI_MED)’ funded by the Federal Ministry of Education and Research (grant 03IS2061A). Genome-wide data have been supported by the Federal Ministry of Education and Research (grant no. 03ZIK012) and a joint grant from Siemens Healthcare, Erlangen, Germany and the Federal State of Mecklenburg- West Pomerania. The University of Greifswald is a member of the ‘Center of Knowledge Interchange’ program of the Siemens AG and the Caché Campus program of the InterSystems GmbH.

The Young Finns Study has been financially supported by the Academy of Finland: grants 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and
Disclosures

Dr. Ingelsson serves as a scientific advisor for Precision Wellness, Inc and Olink. Proteomics for work unrelated to the present project. Dr. Psaty serves on the DSMB of a clinical trial funded by Zoll LifeCor and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. Dr. Lindgren is supported by the Li Ka Shing Foundation; WT-SSI/John Fell funds and by the National Institute for Health Research (NIHR) Biomedical Research Centre, Oxford; by Widenlife and NIH (5P50HD028138-27).

None for all remaining authors.
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).
References