Impact of carotid atherosclerosis loci on cardiovascular events

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1. Introduction

Coronary artery disease (CAD) and stroke are leading causes of death worldwide [1], influenced by common genetic factors. Subclinical atherosclerosis, a thickening of the artery wall caused by the deposition of cholesterol material, often precedes these events [2,3]. Carotid intima-media thickness (cIMT) and plaque, measures of subclinical atherosclerosis, have been shown to predict subsequent cardiovascular events [4–6]. In this sense, genetic association studies may identify susceptibility genes and pathways involved in the initiation and early phases of these diseases.

Recently, genome-wide association studies (GWAS) identified 6 single-nucleotide polymorphisms (SNPs) [7,8] associated to cIMT or plaque. The extent to which these loci relate to other vascular diseases and subsequent cardiovascular events is unclear. Therefore, we aimed to demonstrate the external validity of these findings by testing whether these SNPs relate to cIMT, plaque, ankle-brachial index (ABI), ischaemic stroke (IS), abdominal aortic aneurysm (AAA), peripheral artery disease (PAD), and CAD in the SMART (Second Manifestations of ARTerial disease) cohort. We also modeled the 3 cIMT-related SNPs as a multilocus genetic risk score (GRS) and tested for association with CAD, IS, AAA, PAD, ABI, and cIMT itself, as well as with recurrent vascular events.

Methods and results: We tested six SNPs (rs4888378, rs11781551, rs445925, rs6601530, rs17398575 and rs1878406) for association with subclinical atherosclerotic measures (cIMT, plaque presence and ankle-brachial index), as well as ischemic stroke, abdominal aortic aneurysm, peripheral or coronary artery disease (CAD) in the Second Manifestations of ARTerial disease (SMART) cohort. Four SNPs were associated with cIMT and two with plaque (p < 0.05). One SNP was also significantly associated to CAD (rs1878406, OR = 1.24, 95% CI = 1.08–1.42, p = 2 × 10⁻²). A genetic risk score (GRS) based on the cIMT-related SNPs was associated to increased risk of cIMT itself (p = 1 × 10⁻³), but not to other secondary outcomes or vascular events during follow-up (p = 0.86).

Conclusions: In addition to replicating previously published associations for cIMT, we confirmed a nominally significant effect between the GRS and cIMT.
2. Methods

2.1. Study populations and phenotyping

We used data from the SMART cohort, consisting of patients from the University Medical Center Utrecht (UMCU), the Netherlands, included on the basis of manifest atherosclerotic vascular disease or the treatment of atherosclerotic risk factors [9,10] (Supplemental Table S1). From the 8210 patients included in the study, 3743 had CAD, 640 had AAA, 1726 had PAD, and 1764 had IS, with overlap among traits. Patients free of cardiovascular disease who had one or more risk factors for cardiovascular disease and were included in the SMART follow-up phase, served as a control group (n = 1981). All patients provided informed consent, and the Medical Ethics Committee of the UMCU approved the study.

2.2. SNP selection, genotyping and quality control

Based on a GWAS meta-analysis for cIMT and plaque [7], we selected 3 cIMT-associated SNPs (rs11781551, rs445925, and rs6601530), and 2 plaque-associated SNPs (rs17398575 and rs1878406). A second study associated rs4888378 with cIMT [8] and was added to this analysis, resulting in a total of 4 cIMT-associated and 2 plaque-associated SNPs. Community standard quality control (QC) [11] was applied in all 6 SNPs. We excluded rs445925 from further analysis as it was out of Hardy–Weinberg Equilibrium (p = 4.14 × 10^-9).

Wet-lab genotyping was carried out at KBiosciences (Hertfordshire, UK, www.kbioscience.co.uk) whose personnel were blinded to patient status, using the proprietary KASP PCR technique and TaqMan. Genotype calling was done using an automated system, with results checked manually using SNPviewer software.

2.3. Statistical analysis

Single SNP and GRS analyses were performed using linear and logistic regression models where appropriate, adjusting for sex and age. We considered the presence of plaque when cIMT > 1.1 mm, following the same criteria as in Bis et al. [7].

For each individual in our cohort we constructed an unweighted GRS using PASW Statistics 21 (SPSS, Inc., 2012, Chicago, IL, USA, www.spss.com). The GRS was calculated as the sum of the number of risk alleles at the 3 cIMT-related SNPs (rs11781551, rs6601530, and rs4888378).

We used Cox proportional hazards model to analyze the association between GRS and clinical events during follow-up, considering age, sex and inclusion criteria as covariates. To estimate quantitative effect sizes of the GRS on subsequent disease risk, we divided individuals into quartiles according to the GRS distribution and computed hazard ratios (HR) between the quartiles, with the first quartile as reference.

Since all 5 SNPs that passed QC were previously associated with cIMT or plaque at genome-wide significance (p < 5 × 10^-8), we considered a p < 0.05 threshold (for the same risk allele in the same direction previously reported) as significant for these two phenotypes. Correcting for multiple testing, the Bonferroni threshold defined for the other five tested vascular beds (IS, AAA, CAD, PAD, and ABI) was p < 2 × 10^-3.

3. Results and discussion

In this study SMART comprised a total of 8210 individuals (6229 cases and 1981 controls), aged 17–83 years, whose majority (67.5%) is male.

The single-SNP analysis for association with cIMT and plaque in SMART (Table 1) resulted in 4 SNPs associated with cIMT and 2 with plaque (p < 0.05), all showing a concordant direction of effect with the originally reported associations (binomial p = 0.03). This independent replication supports the role of these loci as genetic determinants of cIMT and plaque.

In order to test the relation between these loci and other vascular beds, we tested their association with IS, AAA, CAD, PAD, and ABI (Table 2). One variant (rs1878406) was significantly associated with CAD (odds ratio [OR] = 1.24, 95% confidence interval [CI] = 1.08–1.42, p = 2 × 10^-3). This SNP is located 8.5 kb from EDNRA, a gene related to endothelial dysfunction. The endothelin receptor is a target to reduce blood pressure, given the vasoconstrictor role of endothelins in blood pressure elevation and vascular hypertrophy [12]. Interestingly, genetic variations in this gene have also been associated with atherosclerosis in hypertensive patients [13] and with ambulatory blood pressure [14]. Convincingly, previous reports also linked the same locus to CAD [7,15]. This supports the hypothesis that EDNRA might affect atherosclerosis causing changes in blood pressure and thereby increasing the risk on CAD. SNP rs6601530 showed a nominally significant association with CAD (p = 0.04) and ABI (p = 0.006). This SNP locates in an intron of PINX1, encoding Pin2-interacting protein 1, a telomerase inhibitor [16] that relates to chromosomal segregation in mitosis [17] and has been implicated in cancer [18,19]. A recent study [20] found that SNP rs7840785, also located in the intron of PINX1, was significantly associated with right carotid IMT (p = 0.0003) in a non-European population. The same study also conducted a gene-based analysis in which PINX1 was significantly associated with right carotid IMT. Even after removing the significant SNP rs7840785, PINX1 was still significantly associated in the overall sample (p = 1 × 10^-7). However, after correcting for multiple testing, we did not find significant associations with IS, AAA, PAD, or ABI.

We further tested the combined effects of the cIMT-related SNPs in a multilocus GRS for association with disease risk (Supplemental Table S2). We confirmed the association between the GRS and cIMT (p = 0.04) [7]. We did not find a significant association between the

### Table 1

<table>
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<th>Locus</th>
<th>SNP</th>
<th>Chr</th>
<th>Alleles</th>
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<th>HWE</th>
<th>OR (95%CI)</th>
<th>p</th>
<th>EAF</th>
<th>OR (95%CI)</th>
<th>P (cIMT)</th>
<th>P (plaque)</th>
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<tr>
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<td>T/C</td>
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<td>0.5535</td>
<td>1.22 (1.15–1.29)</td>
<td>6.90 × 10^-12</td>
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<td>1.18 (1.12–1.23)</td>
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<td>1.17 (1.04–1.30)</td>
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<tr>
<td>Locus</td>
<td>SNP</td>
<td>Chr</td>
<td>Alleles</td>
<td>EAF</td>
<td>β (95%CI)</td>
<td>P</td>
<td>EAF</td>
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</table>

SNP: single-nucleotide polymorphism. Alleles: effect and non-effect alleles. EAF: effect allele frequency. Results listed in bold are nominally significant (p < 0.05).
risk score and IS, AAA, CAD, PAD, or ABI. This can be due to the small effect the combined SNPs confer on the susceptibility of disease, or cardiovascular traits.

We also analyzed the association between the GRS and vascular events following up using Cox proportional hazard models (HR = 0.977, 95% CI 0.93–1.07, survival curves in Supplemental Figure S1). We found no increased risk of vascular events during follow-up when comparing individuals in the highest GRS quartile with those in the lowest quartile (p = 0.86). We may have had limited power due to the modest number of secondary events to find such an association, if it exists.

The results showed in this study confirm the original findings [7,8], adding to the evidence of the effect these loci have on cIMT or plaque presence. The GRS based on cIMT-related SNPs did not show a significant effect on other vascular beds, or secondary vascular events, suggesting against pleiotropy. An explanation for this finding could be that risk factors and their underlying genetic background may impact differently on each vascular bed in the atherogenic process. In conclusion, our findings provide support for previously claimed SNP associations for cIMT and plaque, specifically highlighting the role of rs1878406 in both atherosclerosis and CAD. This should motivate further research focused on the underlying mechanisms involved.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.10.017.

References