Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018

Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery

Janne Pott[1,2], Ralph Burkhardt[2,3], Frank Beutner[2,4], Katrin Horn[1], Andrej Teren[2,4], Holger Kirsten[1,2], Lesca M. Holdt[2,5], Gerhard Schuler[4], Daniel Teupser[2,5], Markus Loeffler[1,2], Joachim Thiery[2,3], Markus Scholz[1,2]

[1]Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany

[2]LIFE Research Center for Civilization Diseases, University of Leipzig, Leipzig, Germany

[3]Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital, Leipzig, Germany

[4]Heart Center Leipzig, Leipzig, Germany

[5]Institute for Laboratory Medicine, Ludwig-Maximilians University, Munich, Germany

Contact Information:

MS: markus.scholz@imise.uni-leipzig.de

Keywords: atherosclerosis, carotid artery plaque, genome-wide association analysis

Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018

ABSTRACT

Background and aims: Carotid artery plaque is an established marker of subclinical atherosclerosis and common patho-mechanisms with coronary artery disease (CAD) are hypothesized. We aimed to identify genetic variants associated with carotid plaque and examine the potential shared genetic basis with CAD.

Methods: After investigating the reliability of plaque detection, we performed a genome-wide meta-association study in two independent cohorts (LIFE-Adult, n=4,037 and LIFE-Heart, n=3,152) for carotid plaque score (PS), defined as the sum of the plaque load of common carotid artery and carotid bulb. Further, we analyzed whether previously reported CAD and stroke loci were also associated with PS.

Results: We identified two loci with genome-wide significance for PS. One locus is the known CAD-locus at chromosome 9p21 (lead SNP rs9644862, $p=8.73 \times 10^{-12}$). We also describe a novel locus at on chromosome 10q24 within the *SFXN2* gene as the most probable candidate (lead SNP rs2902548, $p=1.97 \times 10^{-8}$). In addition, 17 out of 58 known CAD loci and six of 17 known stroke loci were associated with PS at a nominal level of significance.

Conclusion: We showed that PS is a reliable trait to analyze genetics of atherosclerosis. Two new loci of genome-wide significant association with PS were found. The observed non-random overlap of CAD and PS associations strengthens the hypothesis of a shared genetic basis for these atherosclerotic manifestations.

Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018

Introduction

Coronary artery disease (CAD) is a complex disease determined by numerous environmental and genetic factors [1]. Over the last several years, genome-wide association analyses have led to the identification of several genetic loci associated with CAD and myocardial infarction (MI). The most recent meta-analysis of Nikpay et al. [2] included about 185,000 cases and controls adding further evidence and resulting in a total of 58 loci considered as well-established. However, these studies typically comprise a highly heterogeneous mixture of assessments and information to determine disease status, e.g. anamnestic data, clinical records, coronary angiography or acute myocardial infarction. Therefore, to gain a better understanding of the underlying genetic patho-mechanisms, it appears worthwhile to study whether these loci are also related to other manifestations of atherosclerosis.

Carotid intima-media thickness (cIMT) and carotid artery plaque are promising assessment for this purpose. Intima-media thickness was reported to be predictive for cardiovascular events [3, 4]. However, the importance of cIMT in comparison to established risk scores such as the Framingham Risk Score was challenged by others [5]. Studies have also shown an association of carotid parameters with prevalent CAD with the predictive value of carotid plaque outperforming that of intima-media thickness [6, 7]. In a large cohort of patients with suspected CAD receiving coronary angiography (LIFE-Heart), we have recently also found a strong correlation of plaque status in the carotid artery and lesions in coronary vessels with an odds ratio (OR) >3.7 [8]. Again, carotid plaque showed a considerably stronger association with CAD than intima-media thickness proposing carotid plaque as a non-invasively assessable marker of coronary lesions. Based on these findings, we hypothesized that there may be a shared genetic basis for carotid artery plaques and CAD.

Here, we performed a large genome-wide association analysis of carotid plaques in two independent cohorts collected in the LIFE Research Center of Civilization diseases. While previous studies focused on plaque prevalence [9, 10] or plaque size [11], we used the number of carotid plaques as primary end point.

In a secondary analysis, we investigated whether there is an enrichment of plaque associations for CAD risk loci to detect possible genetic similarities and differences of these atherosclerotic phenotypes.

In analogy, we analyzed loci associated with ischemic stroke (IS) as found by Dichgans et al. [12] and Pulit et al. [13]. Both authors defined subtypes of stroke using the Trial of Org 10 172 in Acute Stroke Treatment (TOAST) classification system [14]. One subtype is large artery stroke (LAS), which requires a stenosis of greater than 50% of the extracranial *internal carotid artery* (ICA). We analyzed this sub-phenotype separately since plaque assessment was available in proximal ICA in our cohorts.

Materials and methods

Cohort description

LIFE-Adult

LIFE-Adult is a population-based cohort of 10,000 adult inhabitants of the city of Leipzig, Germany. Participants are well characterized regarding life-style and environmental risk factors and clinical and subclinical signs of diseases such as cardiovascular diseases, type 2 diabetes or cognitive impairment. Detailed description of the cohort can be found elsewhere [15]. CAD status in LIFE-Adult was determined by the following anamnestic criteria: MI, stent implementation during a coronary angiography or diagnosed CAD.

LIFE-Heart

LIFE-Heart is a cohort of patients with suspected and confirmed stable coronary artery disease or myocardial infarction as first manifestation of CAD. All patients underwent coronary angiography and vascular phenotyping at the Heart Center of the University of Leipzig. Details of the study can be found elsewhere [16]. A total of about 7,000 patients were recruited so far. CAD status is defined by the presence of at least one coronary lesion with more than 50% luminal reduction.

Both, LIFE-Adult and LIFE-Heart, meet the ethical standards of the Declaration of Helsinki. They have been approved by the Ethics Committee of the Medical Faculty of the University Leipzig, Germany (LIFE-Adult: Reg. No 263-2009-14122009, LIFE-Heart: Reg. No 276-2005). LIFE-Heart is registered at ClinicalTrials.gov (NCT00497887). Written informed consent including agreement with genetic analyses was obtained from all participants.

A description of basic parameters of LIFE-Adult in comparison with those of LIFE-Adult is shown in table 1.

Carotid ultra-sound and plaque assessment

For both cohorts, carotid ultrasound was performed using the same standard operating procedures. Subjects with cervical spine disorder, wounds at the scanning area and patients with acute myocardial infarction in LIFE-Heart were excluded from carotid ultrasound.

Eligible patients were scanned at both sides of four anatomical regions: *common carotid artery* (CCA), *carotid bulb* (Bulb), proximal parts of *internal carotid artery* (ICA) and *external carotid artery* (ECA) respectively. High-resolution B-mode ultrasound images of carotid vessels were acquired using the GE Vivid ultrasound platform with a 12.0-MHz linear-array transducer (GE-Healthcare). For the assessments, subjects were in supine position.

Carotid artery plaque was defined according to the American Society of Echocardiography Intima-Media Thickness Task Force [17]. In detail, a lesion was counted as plaque if echogenic thickening of intimal reflection that extends into the arterial lumen at least 0.5 mm or 50 % of the surrounding intima-media thickness or thickness of intima and media >1.5 mm. Plaque presence was documented as 'present' or 'absent' or 'missing' if the quality of the image was insufficient. Details can be found elsewhere [8].

Prior to LIFE recruitment, we performed a feasibility study of N=47 volunteers receiving repeated plaque readings by six different investigators. We observed a high intra- (κ=0.93 for the overall agreement) and inter-rater reliability (κ=0.88 for the overall agreement with the consented plaque assessment, data not shown). Based on the single plaque assessments, we defined the plaque score (PS) as follows. PS is the sum of the plaque assessments at CCA and Bulb on both sides. Thus PS takes values from 0 to 4. Single missing values are counted as zeros for this purpose. If there is more than one missing value, the score is set to missing.

A possible alternative to this phenotype definition is a more comprehensive evaluation of all four scanned carotid areas on both side (PS8, with values in between 0 and 8). Plaque scores PS and PS8 were highly correlated (r=0.91 for both LIFE-Heart and LIFE-Adult). However, as ICA and ECA were more difficult to scan, there is a substantially higher percentage of missingness for PS8 compared to PS. Therefore, we decided to use PS as our primary endpoint. However, for consistency we verified our main results using this alternative plaque phenotype. For the purpose of comparisons with established stroke loci, we analyzed the four anatomical regions separately considering scores of corresponding plaque burden which take values 0, 1 and 2.

Carotid ultra-sound was available for 9,858 participants of LIFE-Adult and 3,501 patients of LIFE-Heart. Distributions of PS in both cohorts are presented in table 1.

Genotyping

LIFE-Adult

A total of 5,101 randomly selected individuals were genotyped using the genome-wide SNP array *Affymetrix Axiom CEU1*. Genotype calling was performed following the best practice steps recommended by Affymetrix [18]. The software "*Affymetrix Power Tools*" (APT, version 1.17.0) was used with the latest library "*Axiom GenomeWide CEU 1 Array Plate, Analysis Files, release 6*".

We filtered 73 samples failing the dishQC criteria (dishQC ≥ 0.82) and sample call rate criteria (SCR ≥ 97%) in the initial calling round. Genotypes of 587,352 SNPs were determined in the final calling round. Sex-mismatches were filtered. Additionally, the intensities of gonosomal SNPs were plotted to check for further abnormalities as proposed by Laurie et al. [19]. Cryptic relatedness was assessed according to Wang [20]. Duplicates were removed keeping the sample with the higher quality. Principal component analysis (PCA) as suggested by Patterson et al. [21] was performed using PLINK [22, 23] (version 1.9). Ethnical outliers (>6 SD of first ten PCs) were removed, which resulted in a final set of 4,985 unrelated high quality samples for autosomal analyses and 4,978 for gonosomal analyses.

Autosomal SNPs were filtered for call rate (CR < 97%), the cluster plot quality criteria as suggested by Affymetrix (i.e. Fisher's Linear Discriminant, heterozygous strength offset, homozygote ratio offset), *p* value of exact test for Hardy-Weinberg equilibrium (HWE<10^{-6}), monomorphic SNPs and *p* value of plate association (<10^{-7}, i.e. test for dependency of allele frequency to plate). A total of 532,875 SNPs fulfilled all quality criteria.

For X-chromosomal SNPs, filter criteria proposed by Konig et al. [24] were applied (*p* value of exact test for HWE for women <10^{-4}, CR<98%, and minor allele frequency (MAF)<1% after setting all heterozygous calls in male samples on missing). This resulted in 13,554 X-chromosomal SNPs eligible for analysis.

Finally, all 4,985 samples were imputed, using 1000 Genomes Phase 1, Version 3 [25] (2012) as reference, SHAPEIT [26] (version v2.r790) for phasing, and IMPUTE2 [27] (version 2.3.2) for imputation. Imputation for X-chromosomal SNPs was performed using the same reference panel and software, but with the subset samples eligible for X-chromosomal analysis as mentioned above.

LIFE-Heart

LIFE-Heart samples were genotyped with either *Affymetrix Axiom CEU1* or *Affymetrix Axiom CADLIFE*. The latter is an array containing Axiom CEU as genome-wide backbone and an additional custom content of about 62,500 SNPs from CAD loci. Genotype calling relied on *Affymetrix Power Tools* (APT, version 1.17.0 for Axiom-CADLIFE, version 1.16.1 for Axiom-CEU) with their latest libraries (Axiom CADLIFE1, release 3 respectively Axiom GenomeWide CEU 1 Array Plate, Analysis Files, release 6). Genotype calling and SNP filtering was performed separately for the two array products. For sample filtering, high quality SNPs in the intersection of both arrays were used. The same steps of sample and SNP filtering were performed as in LIFE-Adult. In summary, 5,700 samples and 504,593 SNPs fulfilled all quality criteria for autosomal analyses. For X-chromosomal analyses, additional 12 samples were removed and a total of 12,715 SNPs fulfilled the specified quality criteria.

Imputation was performed using the same reference, software and software settings as in LIFE-Adult. The intersection of SNPs of *Affymetrix Axiom CEU1* and *Affymetrix Axiom CADLIFE* was used for this purpose.

Statistical Analysis

PS was available for 4,037 successfully genotyped samples of LIFE-Adult and 3,152 samples of LIFE-Heart sub cohort of patients initially admitted for suspected CAD. Separate genome-wide analysis was

performed for each cohort first. The analysis was executed with PLINK [22, 23] (version 1.9) using the additive frequentist model and expected genotype counts, adjusting for sex and age (LIFE-Heart: beta=0.54 for males and beta=0.03/year for age; LIFE-Adult: beta=0.44 for males and beta=0.03/year for age; all betas significant with $p<0.001$). X-chromosomal SNPs were analyzed assuming total X inactivation. PS was treated as continuous since the difference of test statistics compared to those of proportional odds logistic regression appeared to be small (correlation of test statistics r>0.99 for both cohorts).

SNPs not in the intersection of the studies, with MAF<1% or info-score<0.3 in one of the studies were initially filtered, leaving 9,846,747 million SNPs for further analysis. A fixed effect model (FEM) meta-analysis was then performed for all remaining SNPs using the package *meta* of R. A genome-wide significance threshold of 5.0×10^{-8} was applied. The study has a power of 80% respectively 90% to detect variants explaining 0.5% respectively 0.6% of variance.

To determine independent variants, we applied a priority pruning using linkage disequilibrium data from 1000 Genomes Phase 1, Version 3 (2012), restricted to individuals with European ancestry. Variants which are in linkage disequilibrium (LD) with an association of higher significance were removed if $r^2 \geq 0.5$.

We performed a comprehensive annotation of our top-SNPs using a number of bioinformatic resources: We reported known genome-wide association study (GWAS) hits, using the *GWAS catalogue* [28], if in linkage disequilibrium ($r^2>0.2$) with one of our top-hits. Analogously, we add expression quantitative trait loci (eQTL) data as explained in Kirsten et al. [29]. Pathway enrichment was performed for each SNP by including physically nearby genes, all within 50 kb distance and up to four within 250 kb distance, and eQTLs in LD ($r^2>0.2$) with the variant. Pathways were retrieved from KEGG, GO, DOSE [30], and reactome. We also calculated deleteriousness scores according to Kircher et al. [31].

In addition, we tried to replicate five SNPs which were reported to be associated with carotid plaque prevalence (Bis et al. [9]) with our score. To analyze a possible shared genetic background of carotid plaques and CAD, we analyzed 58 loci for which associations with CAD were described in the literature [2]. Of the 58 top-SNPs of these loci, ten were directly genotyped in both of our cohorts. All other SNPs were successfully imputed. To analyze enrichment of significant associations with concordant direction of effects, we compared this percentage with 2.5% expected by chance.

We also analyzed SNPs in LD ($r^2>0.5$) with the reported CAD SNPs and determined the SNP with strongest plaque association per locus. This results in a higher number of CAD loci showing nominal significance for plaque. To compare this number with the one expected by chance, we performed permutation analysis. 10,000 permutations of phenotypes (PS, sex, age) and genotypes were analyzed. An empirical *p* value was calculated assuming a Poisson distribution of the number of significant loci. Additionally, we calculated a genetic risk score (GRS) of CAD using the beta estimates of Nikpay et al. [2] in order to analyze its association with our trait.

Similarly, we analyzed loci associated with cIMT [9, 32], ischemic stroke (IS) [12, 13] and large artery stroke (LAS) [12].

Results

Genome-wide significant hits for plaque score

Meta-analysis of 9,576,485 SNPs in the LIFE-Adult and LIFE-Heart studies showed no signs of inflation ($\lambda = 1.004$). QQ-Plot and Manhattan plot can be found in figure 1. We identified two loci at chromosome

9p21 and 10q24 with genome-wide significant hits ($p<5x10^{-8}$) in our study (Table 2, further results are summarized in supplementary table 1).

The strongest association with PS was observed for SNP rs9644862, located at 9p21 in the sequence of *CDKN2B-AS1* (also known as *ANRIL*), i.e. the best established common locus of CAD association. This SNP showed robust associations with similar effect sizes in both cohorts (LIFE-Heart: beta=0.174, $p=3.36x10^{-7}$, LIFE-Adult: beta=0.111, $p=1.87x10^{-6}$, FEM: beta=0.131, $p=8.73x10^{-12}$). No interactions with sex were observed (LIFE-Heart: $p=0.53$, LIFE-Adult: $p=0.47$). Our lead SNP rs9644862 is in linkage disequilibrium ($r^2=0.75$) with rs2891168, which was reported as top-SNP in the most recent GWAS of CAD [2] (see also supplementary table 2). For our top-SNP no direct eQTLs were observed. But it is in strong LD (r2=0.75) with rs4977574 which is an expression quantitative trait nucleotide (eQTN) of *CDKN2B* [33]. Other, partly stronger eQTLs but in weaker LD with the top-hit are presented in supplementary table 3.

After pruning with $r^2=0.5$, another SNP at the 9p21 locus (rs7853090) also showed genome-wide significance. This SNP is in weak LD with the top-SNP ($r^2=0.28$). A regional association plot of the locus is shown in figure 2A.

Besides the 9p21 locus, we identified a second locus at 10q24 reaching genome-wide significance (lead SNP rs2902548; FEM: beta=-0.141, $p=1.97x10^{-8}$, Table 2). The lead SNP is located in the intron of *SFXN2*. Other nearby genes are *ARL3* (13 kb), *WPL1P* (16 kb) and *TRIM8* (69 kb, figure 2B). SNP rs2902548 is an eQTN for several cis-regulated genes including those mentioned above (supplementary table 3). The strongest eQTL ($p=8.6x10^{-26}$, The GTEx Consortium [34]) was observed for *SFXN2* in esophagus tissue, and a weaker one in left ventricular heart tissue ($p=6.1x10^{-10}$, The GTEx Consortium [34]). There is also an eQTL-SNP in LD with rs2902548 at *ARL3* in tissue of the artery aorta ($p=1.5x10^{-5}$, $r^2=0.56$, The GTEx Consortium [34]). Restricted to blood tissue, the strongest eQTL was found for *SFXN2*, *C10orf32*, and *ARL3* ($p=3.6x10^{-15}$, $p=1.8x10^{-11}$, and $p=4.2x10^{-10}$, respectively, Westra et al. [35]). SNP rs2902548 is also in weak LD ($r^2<0.3$) with several SNPs previously reported for cardiovascular risk factors such as BMI and hypertension (supplementary table 2). Further, it is in weak LD ($r^2=0.20$) with SNP rs12413409 which has been associated with CAD [12, 33, 36]. But conversely, the latter was only weakly associated with PS ($p=0.01$).

Suggestive plaque score loci identified in the Meta-analysis

A total of five SNPs showed suggestive association ($p<1x10^{-6}$) with PS but failed genome-wide significance. While two of them correspond to the 9p21 locus and one to 10q24, two additional new loci were found: At chromosome 6q13, the lead SNP rs141249844 is located in the intron of *KCNQ5* and at chromosome 3p24 the lead SNP rs1349287 is located in close proximity to *KCNH8*. Statistics of all loci are summarized in table 2. Interestingly, both genes belong to the calcium channel family.

Verification of top and suggestive SNPs with PS8

We verified our results with an alternative plaque endpoint, namely PS8. The 9p21 locus remained genome-wide significant for PS8 (beta=0.193, $p=7.34x10^{-10}$). The second hit on 10q24 reached suggestive evidence for PS8 (beta=-0.211, $p=2.63x10^{-7}$). For the suggestive loci at 6q13 and 3p24 no suggestive significance was found for the phenotypic alternatives ($p=5.35x10^{-5}$ and $p=3.08x10^{-4}$, respectively, see also supplementary table 4). Interestingly, higher absolute betas were observed for PS8 which however did not resulted in better *p* values due to the smaller sample size available for PS8.

Replication of SNPs associated with plaque prevalence

A recent meta-GWAS for carotis plaque prevalence (Bis et al. [9]) reported two SNPs with genome-wide significance and three SNPs with suggestive *p* values. We tested, whether these 5 SNPs were also associated with carotid plaque score in our study.

We found evidence for replication in the LIFE studies (nominal significance and same direction of effect) for four of the five SNPs, including the two SNPs with genome-wide significance in Bis et al. [9] (table 3 and supplementary table 4 and 5). Only SNP rs17045031 / *LRIG1* at chromosome 3p14 failed replication. The same results were observed if analyzing plaque prevalence instead of PS (not shown).

Analysis of known CAD loci for association with plaque score

To investigate if the development of atherosclerotic plaques in the carotid and coronary arteries may share a common genetic background, we analyzed whether the 58 lead SNPs at known CAD-loci were also associated with PS (see table 3 and supplementary table 4 and 5). CAD association statistics were retrieved from Nikpay et al. [2], the most recent meta-GWAS on CAD.

The CAD lead SNP at the chromosome 9p21 locus, rs2891168, also reached genome-wide significance with PS (p=2.31x10^{-8}). This SNP is in high LD (r^2=0.75) with the PS lead SNP rs9644862 of our study. In addition, seven other CAD lead SNPs reached nominal significance for PS and of those, five SNPs showed concordant direction of effects (table 3). Thus, concordant SNPs with nominal significance are enriched in this analysis (OR=4.6, p=3.2x10^{-3}).

Additionally, the literature OR of the CAD SNPs are correlated with the beta estimates (r=0.4, p=0.0017, after transforming the OR as suggested by Chinn [37], see also supplementary figure 1). However, the beta estimates for PS were on average smaller than those for CAD (t-test of difference, after recoding for effect allele, p=1.5x10^{-7}). Restricted to the eight nominal significant SNPs, correlation increased (r=0.7, p=0.049), and the difference became insignificant (p=0.68). Using PS8 instead did not improve the results.

We next included sets of proxy SNPs that are in high LD (r^2>0.5) with the lead SNP of a CAD locus in our analysis. Of note, this included only 57 SNPs, as two CAD SNPs were tagged by the same proxy SNP. The number of CAD loci that were also significantly associated with PS (p<0.05) increased to 16, suggesting that these CAD loci might also be associated with carotid plaque. To verify this result, we performed a permutation test with 10,000 randomly combined sets of genotypes and phenotypes. Assuming the data to be Poisson distributed, the empirical p value is 1.03x10^{-8}, which indicates a strong enrichment. We also considered the combined effect of all CAD SNPs by calculating a GRS. The GRS showed a strong association with PS (beta=0.264, p=1.99x10^{-6}).

Analysis of known cIMT, IS, and LAS loci for association with plaque score

For four SNPs, association with cIMT [9] was reported. One of them showed nominal significance with PS, and the direction of effects are the same. The locus of this SNP is *APOC1*, which is also reported to be a CAD and plaque prevalence locus (with suggestive evidence) [2, 9].

We found no significant association with the eight SNPs of the most recently published stroke GWAS [13]. Dichgans et al. [12] reported 17 SNPs in association with IS or LAS. Three of them showed nominal significance with our PS trait, namely rs1333047 at 9p21 (reported for LAS), rs7937106 at 11q22 (reported for LAS), and rs4792143 at 17p12 (reported for IS). For both LAS SNPs the effect with PS had the same direction as reported. However, the SNP reported for IS has opposite direction of effect in

our data. Using best proxy SNPs in high LD (r^2>0.5) there were six loci significantly associated with PS (p<0.05). Permutation test was performed and resulted in an empirical p-value of $2.44x10^{-4}$, indicating a non-random overlap of stroke loci and loci associated with carotid plaque score.

In addition, we calculated the genetic risk score for stroke using the published ORs of Dichgans et al. [12] and tested the association between this GRS and PS, PS8 and plaque burden at each of the four anatomical regions investigated (see methods and supplementary table 6). There was no significant association between PS and GRS using all 17 stroke SNPs (beta=0.0658, p=0.20). Restricting the GRS on the eleven LAS SNPs, the association becomes significant (beta=0.114, p=0.039). We repeated the analysis with PS8, which resulted in significant association for all 17 SNPs (beta=0.225, p=$6.8x10^{-3}$) as well as LAS SNPs only (beta=0.296, p=$9.4x10^{-4}$). Interestingly, this association is mainly triggered by plaque in ICA.

Comparison of LIFE-Heart and LIFE-Adult

Prevalence of carotid plaque is higher in LIFE-Heart. Therefore, we compared the risk allele frequencies (RAF) of our genome-wide significant and suggestive PS hits between LIFE-Heart and LIFE-Adult. The same was done for the RAF of the 58 CAD SNPs and the corresponding GRS. Additionally, we compared CAD cases and controls (noCAD) of LIFE-Heart with respect to these frequencies. Frequencies are shown in supplementary table 7.

For the PS hits, three of them had higher RAF in LIFE-Heart compared to LIFE-Adult (see also table 2). This also applies for the comparison of CAD and noCAD.

For the 58 CAD SNPs we found no enrichment of risk variants in LIFE-Heart, as only 35 SNPs had higher RAF (binomial test, p=0.15). In contrast, comparing CAD and noCAD in LIFE-Heart we observed 39 SNPs with higher RAF in cases (binomial test, p=0.012). Results are displayed at supplementary figure 2.

Similarly, we compared the GRS between our cohorts and between cases and controls of LIFE-Heart. As expected, subjects of LIFE-Adult have smaller risk scores than those of LIFE-Heart (t test, p=0.005). Controls of LIFE-Heart have smaller GRS than cases (t test, p=0.001). Of note, scores in LIFE-Adult and LIFE-Heart are significantly associated with PS (LIFE-Adult: beta=0.22, p=$8.7x10^{-4}$, LIFE-Heart: beta=0.36, p=$3.7x10^{-4}$).

Discussion

Carotid artery plaque was proposed as a marker to support prediction of prevalent and incident coronary events [6–8]. However, assessing plaque status in sufficiently large cohorts is demanding and only one meta-GWAS was published for this trait so far [9]. Moreover, there is no generally accepted gold standard of carotid plaque assessment, resulting in large heterogeneity of the trait between studies. Here, we performed a genetic meta-analysis of two large epidemiologic studies using the same operating procedures of plaque assessment, thereby guaranteeing a high degree of comparability and high intra- and inter-rater reliability as shown by our feasibility study. We also propose to consider the number of affected segments as a more refined trait. We have previously shown [8] that a plaque score considering the number of affected segments is a better predictor for obstructive CAD than plaque prevalence alone [9]. For our analyses, we relied on a plaque score of the segments CCA and bulb. A score including also ICA and ECA was also considered. Although effect sizes appeared to be stronger compared to PS, the higher percentage of missingness resulted in inferior power. Nevertheless, our study is the first GWAS exploiting plaque score as primary phenotype and resulted in the identification

of two loci with genome-wide significance not yet identified with plaque prevalence in genome-wide studies.

The strongest association was observed for SNPs at the 9p21 locus, the most widely replicated locus of CAD. Our lead SNP is in high LD with the lead SNP of this locus. The underlying patho-mechanism of this locus is subject of intense research and evidence was collected that the non-coding RNAs of *CDKN2B-AS1* expressed at this locus play a key role [38–40]. The locus is known to be associated with several other vascular phenotypes and cardiac events [41, 42].

The situation for the second strongest locus at chromosome 10 is less obvious. Our top-hit is in an intron of *SFXN2* and the expression of this gene is strongly controlled by the SNP in several tissues. Thus, *SFXN2* is the most plausible candidate here. However, our lead SNP is in low LD with the CAD lead SNP (rs12413409) [33]. While the latter shows only marginal association with PS (p=0.01), proxy SNPs considered by Nikpay et al. [2] (rs11191416) and us (rs284841) showed some association (p=5.17x10^{-3} and p=1.71x10^{-4}, for rs11191416 and rs284841, respectively). The SNP rs284841 is an intron modifier in *WBP1L*. The same applies for rs9663711 showing suggestive significance in our data. Therefore, *WBP1L* is another plausible candidate gene for this locus.

Both top-SNPs of our genome-wide significant loci are not described in the study of Bis et al. [9] Conversely, we aimed at replicating their loci in our data. It turned out that both genome-wide significant SNPs reported in Bis et al. showed at least nominal significance with the same direction of effect in our data. We further found supportive evidence, i.e. same direction of effect, for the other three SNPs reported as suggestive. Two of them showed nominal significance. This analysis shows that further studies and meta-analyses are required to underpin associations with carotid plaque phenotypes.

Another interesting question is to which extend there is a common genetic origin of different atherosclerotic manifestations. Although the correlation of carotid plaque and cardiovascular lesion is well established [6–8], it is not clear how this translates into common genetic patho-mechanisms. Therefore, we present association statistics of reported top-SNPs of CAD loci with our phenotype, which takes the number of plaques into account. Although only one of the SNPs showed genome-wide significance in our data, we observed an enrichment of concordant effects with at least nominal significance. Effect sizes of CAD and PS are positively correlated. This strongly supports our hypotheses of common genetic mechanisms underlying the development of atherosclerosis in carotid and coronary vessels. In line with this, the strongest CAD locus 9p21 also showed strongest association with our plaque score. The GRS of CAD loci was also associated with PS.

In analogy to the analysis of CAD loci, we investigated the association of cIMT and stroke loci with our phenotype. Regarding cIMT, one of the four reported loci was also associated with PS (*APOC1*). Regarding stroke, six of the 17 loci were associated which was significantly more than expected by random. Interestingly, the genetic risk score of stroke variants showed stronger association with PS8 than PS which was mostly triggered by a strong association of LAS loci with ICA. This is in well agreement of the pathophysiology of LAS, i.e. plaques in ICA are required for the definition of LAS [14].

In conclusion, we propose carotid plaque score as a valuable trait of genetic association analysis. By performing the first GWAS of this phenotype we identified two loci of genetic association. While the 9p21 locus is robustly associated with carotid artery plaque score, our hit at chromosome 10 requires further replication in independent cohorts as well as identification of the causal gene. Using our plaque score, we could replicate four hits associated with carotid plaque prevalence. Comparisons of plaque score and CAD associations strongly suggest that there is a larger overlap of genetic causes of the two atherosclerotic manifestations. Likewise, we observed an overlap with genetic loci associated with

stroke. This overlap becomes stronger for LAS loci analyzed for genetic associations with plaque scores comprising ICA. Larger studies and meta-analyses are required to confine this overlap and possible differences in genetic patho-mechanisms.

Conflict of interest

The authors declare that they have no competing interests.

Financial support

LIFE-Heart and LIFE-Adult are funded by the Leipzig Research Center for Civilization Diseases (LIFE). LIFE is an organizational unit affiliated to the Medical Faculty of the University of Leipzig. LIFE is funded by means of the European Union, by the European Regional Development Fund (ERDF) and by funds of the Free State of Saxony within the framework of the excellence initiative (project numbers 713-241202, 14505/2470, 14575/2470).

Author contributions

JP: data analysis, manuscript writing, RB: genotyping, contribution to discussion, FB, AT: carotid plaque phenotyping, KH: data analysis, HK: data analysis, GS: study design of LIFE-Heart, LMH: genotyping, DT: study design of LIFE-Heart, genotyping, ML: design and conduction of LIFE-Adult, JT: design and conduction of LIFE-Heart, MS: study design, manuscript writing. All authors read and approved the final manuscript.

Acknowledgments

We like to thank the participants of LIFE-Adult and the patients of LIFE-Heart very much for spending their time and blood samples. We thank Kerstin Wirkner very much for running the LIFE study center. We thank Sylvia Henger very much for data quality control. We thank Kay Olischer and Annegret Unger very much for technical assistance.

References

[1] Kessler, T., Erdmann, J., Schunkert, H., Genetics of coronary artery disease and myocardial infarction--2013. *Current cardiology reports* 2013, *15*, 368, DOI: 10.1007/s11886-013-0368-0.

[2] Nikpay, M., Goel, A., Won, H.-H., Hall, L. M. *et al.*, A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nature genetics* 2015, *47*, 1121–1130, DOI: 10.1038/ng.3396.

[3] Lorenz, M. W., Markus, H. S., Bots, M. L., Rosvall, M. *et al.*, Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007, *115*, 459–467, DOI: 10.1161/CIRCULATIONAHA.106.628875.

[4] Bots, M. L., Sutton-Tyrrell, K., Lessons from the past and promises for the future for carotid intima-media thickness. *Journal of the American College of Cardiology* 2012, *60*, 1599–1604, DOI: 10.1016/j.jacc.2011.12.061.

[5] den Ruijter, H. M., Peters, S. A. E., Anderson, T. J., Britton, A. R. *et al.*, Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis. *JAMA* 2012, *308*, 796–803, DOI: 10.1001/jama.2012.9630.

[6] Sillesen, H., Muntendam, P., Adourian, A., Entrekin, R. *et al.*, Carotid plaque burden as a measure of subclinical atherosclerosis: comparison with other tests for subclinical arterial disease in the High Risk Plaque BioImage study. *JACC. Cardiovascular imaging* 2012, *5*, 681–689, DOI: 10.1016/j.jcmg.2012.03.013.

[7] Inaba, Y., Chen, J. A., Bergmann, S. R., Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. *Atherosclerosis* 2012, *220*, 128–133, DOI: 10.1016/j.atherosclerosis.2011.06.044.
[8] Weissgerber, A., Scholz, M., Teren, A., Sandri, M. *et al.*, The value of noncoronary atherosclerosis for identifying coronary artery disease: results of the Leipzig LIFE Heart Study. *Clinical research in cardiology official journal of the German Cardiac Society* 2016, *105*, 172–181, DOI: 10.1007/s00392-015-0900-x.
[9] Bis, J. C., Kavousi, M., Franceschini, N., Isaacs, A. *et al.*, Meta-analysis of genome-wide association studies from the CHARGE consortium identifies common variants associated with carotid intima media thickness and plaque. *Nature genetics* 2011, *43*, 940–947, DOI: 10.1038/ng.920.
[10] den Hoed, M., Strawbridge, R. J., Almgren, P., Gustafsson, S. *et al.*, GWAS-identified loci for coronary heart disease are associated with intima-media thickness and plaque presence at the carotid artery bulb. *Atherosclerosis* 2015, *239*, 304–310, DOI: 10.1016/j.atherosclerosis.2015.01.032.
[11] Della-Morte, D., Wang, L., Beecham, A., Blanton, S. H. *et al.*, Novel genetic variants modify the effect of smoking on carotid plaque burden in Hispanics. *Journal of the neurological sciences* 2014, *344*, 27–31, DOI: 10.1016/j.jns.2014.06.006.
[12] Dichgans, M., Malik, R., Konig, I. R., Rosand, J. *et al.*, Shared genetic susceptibility to ischemic stroke and coronary artery disease: a genome-wide analysis of common variants. *Stroke; a journal of cerebral circulation* 2014, *45*, 24–36, DOI: 10.1161/STROKEAHA.113.002707.
[13] NINDS Stroke Genetics Network (SiGN), International Stroke Genetics Consortium (ISGC), Loci associated with ischaemic stroke and its subtypes (SiGN): A genome-wide association study. *The Lancet Neurology* 2016, *15*, 174–184, DOI: 10.1016/S1474-4422(15)00338-5.
[14] Adams, H. P., JR, Bendixen, B. H., Kappelle, L. J., Biller, J. *et al.*, Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993, *24*, 35–41.
[15] Loeffler, M., Engel, C., Ahnert, P., Alfermann, D. *et al.*, The LIFE-Adult-Study: objectives and design of a population-based cohort study with 10,000 deeply phenotyped adults in Germany. *BMC public health* 2015, *15*, 691, DOI: 10.1186/s12889-015-1983-z.
[16] Beutner, F., Teupser, D., Gielen, S., Holdt, L. M. *et al.*, Rationale and design of the Leipzig (LIFE) Heart Study: phenotyping and cardiovascular characteristics of patients with coronary artery disease. *PloS one* 2011, *6*, e29070, DOI: 10.1371/journal.pone.0029070.
[17] Stein, J. H., Korcarz, C. E., Hurst, R. T., Lonn, E. *et al.*, Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *Journal of the American Society of Echocardiography official publication of the American Society of Echocardiography* 2008, *21*, 93-111; quiz 189-90, DOI: 10.1016/j.echo.2007.11.011.
[18] Affymetrix, I., Axiom Genotyping Solution Data Analysis Guide.
[19] Laurie, C. C., Doheny, K. F., Mirel, D. B., Pugh, E. W. *et al.*, Quality control and quality assurance in genotypic data for genome-wide association studies. *Genetic epidemiology* 2010, *34*, 591–602, DOI: 10.1002/gepi.20516.
[20] Wang, J., An estimator for pairwise relatedness using molecular markers. *Genetics* 2002, *160*, 1203–1215.
[21] Patterson, N., Price, A. L., Reich, D., Population structure and eigenanalysis. *PLoS genetics* 2006, *2*, e190, DOI: 10.1371/journal.pgen.0020190.
[22] Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S. *et al.*, Second-generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience* 2015, *4*, 7, DOI: 10.1186/s13742-015-0047-8.
[23] Shaun Purcell, C. C., *PLINK*.

[24] Konig, I. R., Loley, C., Erdmann, J., Ziegler, A., How to include chromosome X in your genome-wide association study. *Genetic epidemiology* 2014, *38*, 97–103, DOI: 10.1002/gepi.21782.
[25] Auton, A., Brooks, L. D., Durbin, R. M., Garrison, E. P. *et al.*, A global reference for human genetic variation. *Nature* 2015, *526*, 68–74, DOI: 10.1038/nature15393.
[26] Delaneau, O., Howie, B., Cox, A. J., Zagury, J.-F. *et al.*, Haplotype estimation using sequencing reads. *American journal of human genetics* 2013, *93*, 687–696, DOI: 10.1016/j.ajhg.2013.09.002.
[27] Howie, B. N., Donnelly, P., Marchini, J., A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS genetics* 2009, *5*, e1000529, DOI: 10.1371/journal.pgen.1000529.
[28] Welter, D., MacArthur, J., Morales, J., Burdett, T. *et al.*, The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic acids research* 2014, *42*, D1001-6, DOI: 10.1093/nar/gkt1229.
[29] Kirsten, H., Al-Hasani, H., Holdt, L., Gross, A. *et al.*, Dissecting the genetics of the human transcriptome identifies novel trait-related trans-eQTLs and corroborates the regulatory relevance of non-protein coding locidagger. *Human molecular genetics* 2015, *24*, 4746–4763, DOI: 10.1093/hmg/ddv194.
[30] Yu, G., Wang, L.-G., Yan, G.-R., He, Q.-Y., DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. *Bioinformatics (Oxford, England)* 2015, *31*, 608–609, DOI: 10.1093/bioinformatics/btu684.
[31] Kircher, M., Witten, D. M., Jain, P., O'Roak, B. J. *et al.*, A general framework for estimating the relative pathogenicity of human genetic variants. *Nature genetics* 2014, *46*, 310–315, DOI: 10.1038/ng.2892.
[32] Geisel, M. H., Coassin, S., Hessler, N., Bauer, M. *et al.*, Update of the effect estimates for common variants associated with carotid intima media thickness within four independent samples: The Bonn IMT Family Study, the Heinz Nixdorf Recall Study, the SAPHIR Study and the Bruneck Study. *Atherosclerosis* 2016, *249*, 83–87, DOI: 10.1016/j.atherosclerosis.2016.03.042.
[33] Schunkert, H., Konig, I. R., Kathiresan, S., Reilly, M. P. *et al.*, Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nature genetics* 2011, *43*, 333–338, DOI: 10.1038/ng.784.
[34] Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science (New York, N.Y.)* 2015, *348*, 648–660, DOI: 10.1126/science.1262110.
[35] Westra, H.-J., Peters, M. J., Esko, T., Yaghootkar, H. *et al.*, Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nature genetics* 2013, *45*, 1238–1243, DOI: 10.1038/ng.2756.
[36] A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. *Nature genetics* 2011, *43*, 339–344, DOI: 10.1038/ng.782.
[37] Chinn, S., A simple method for converting an odds ratio to effect size for use in meta-analysis. *Statistics in medicine* 2000, *19*, 3127–3131.
[38] Holdt, L. M., Hoffmann, S., Sass, K., Langenberger, D. *et al.*, Alu elements in ANRIL non-coding RNA at chromosome 9p21 modulate atherogenic cell functions through trans-regulation of gene networks. *PLoS genetics* 2013, *9*, e1003588, DOI: 10.1371/journal.pgen.1003588.
[39] Holdt, L. M., Sass, K., Gabel, G., Bergert, H. *et al.*, Expression of Chr9p21 genes CDKN2B (p15(INK4b)), CDKN2A (p16(INK4a), p14(ARF)) and MTAP in human atherosclerotic plaque. *Atherosclerosis* 2011, *214*, 264–270, DOI: 10.1016/j.atherosclerosis.2010.06.029.
[40] Holdt, L. M., Stahringer, A., Sass, K., Pichler, G. *et al.*, Circular non-coding RNA ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans. *Nature communications* 2016, *7*, 12429, DOI: 10.1038/ncomms12429.

[41] Holdt, L. M., Beutner, F., Scholz, M., Gielen, S. *et al.*, ANRIL expression is associated with atherosclerosis risk at chromosome 9p21. *Arteriosclerosis, thrombosis, and vascular biology* 2010, *30*, 620–627, DOI: 10.1161/ATVBAHA.109.196832.
[42] Holdt, L. M., Teupser, D., Recent studies of the human chromosome 9p21 locus, which is associated with atherosclerosis in human populations. *Arteriosclerosis, thrombosis, and vascular biology* 2012, *32*, 196–206, DOI: 10.1161/ATVBAHA.111.232678.

Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018

Figures and Tables

Figure 1: QQ-Plot and Manhattan-Plot. (A) QQ-Plot of *p* values in our meta-analysis. SNPs with info score less than 0.8 are plotted as triangles, and those with MAF less than 0.05 are colored red. There is no strong inflation (λ = 1.0041). (B) Distribution of log transformed *p* values in our meta-analysis with trait PS. The bold line marks genome-wide significance (p=5x10^{-8}). Two loci reach genome-wide significance, one at chromosome 9 (*CDKN2B-AS1*), and the other one at chromosome 10 (*SFXN2-WBP1L*).

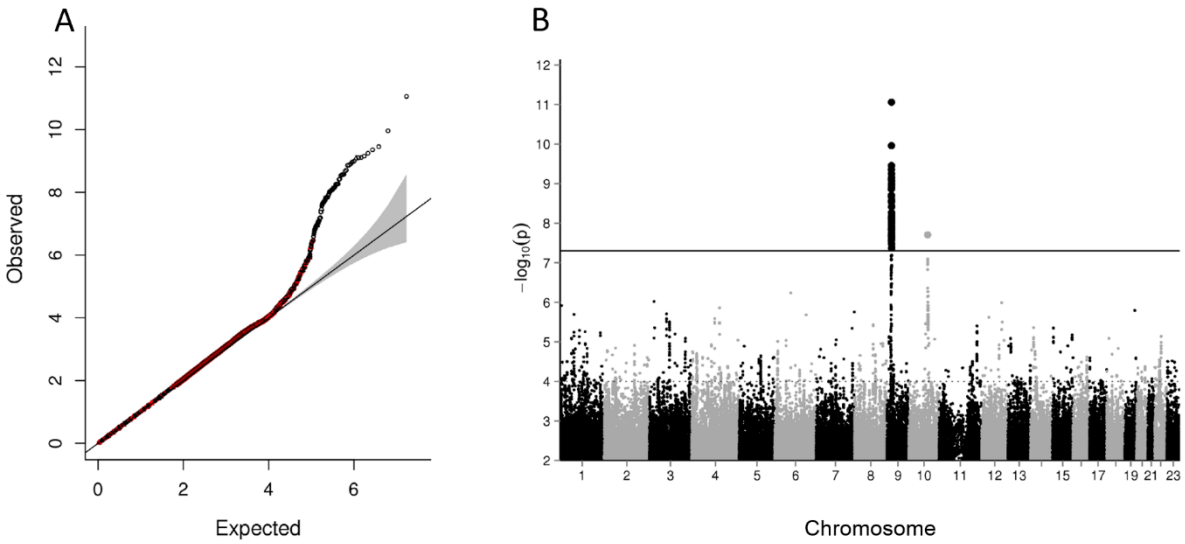
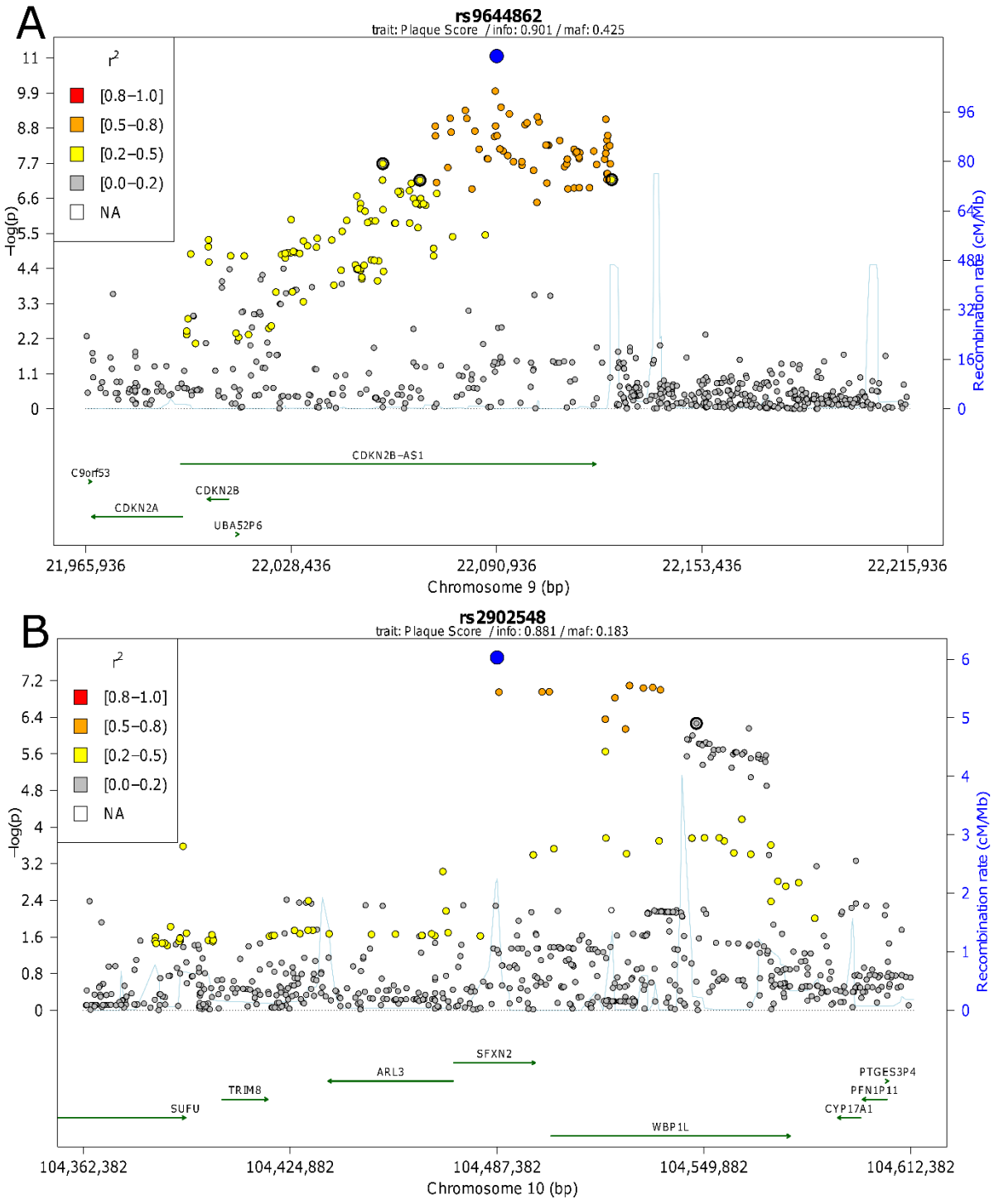


Figure 2: Regional Association Plot. Regional association plots of the two genome-wide significant loci at chromosome 9 (A) and 10 (B). The lead SNP of each locus is colored blue (rs9644862 and rs2902548 in A and B respectively). All other SNPs are colored according to their linkage disequilibrium to the lead SNP. Additional suggestive SNPs (p<1x10^{-6} and pairwise r^2<0.5) are encircled in black.

Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018



Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018

Table 1: Patient and participants' characteristics of LIFE-Heart and LIFE-Adult cohort. All parameters are restricted to patients/participants genotyped for the autosomes and with valid PS. For the continuous parameters, the unit is in parenthesis and the arithmetic mean and standard deviation is given. All categorical parameters were tested with a chi-squared test, continuous parameters were tested with a Mann-Whitney U test. Plaque Score was tested with proportional odds regression. All parameters except for age show significant differences between the studies. CAD status was not compared due to different definitions (LIFE-Heart: coronary angiography, LIFE-Adult: self-reported).

Parameter		LIFE-Heart	LIFE-Adult	*p* value
Women / Men (with Chr X)		1111 / 2041 (1110 / 2039)	2182 / 1855 (2179 / 1852)	< 0.001
Age (years)		61.7 ± 10.7	62.1 ± 11.3	ns
BMI (kg/m²)		29.7 ± 5.0	27.2 ± 4.3	< 0.001
Diabetes; known, in therapy or acute		963	422	< 0.001
Smokers; former /current		1330/659	1154/672	< 0.001
SBP/DBP (mmHg)		139/84	131/76	< 0.001
Hypertension; known, in therapy or acute		2839	2290	< 0.001
Lipid Therapy		1192	649	< 0.001
Cholesterol (mmol/l)		5.44 ± 1.21	5.71 ± 1.07	< 0.001
HDLC (mmol/l)		1.34 ± 0.41	1.66 ± 0.47	< 0.001
LDLC (mmol/l)		3.33 ± 1.04	3.55 ± 0.95	< 0.001
CAD		1259	271	
Plaque Score (PS)	0	1155	1820	< 0.001
	1	549	956	
	2	756	902	
	3	372	245	
	4	320	114	

Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018

Table 2: Genome-wide significant and suggestive SNPs. SNPs associated with carotid plaque at genome-wide significant levels and at suggestive levels ($p<1x10^{-6}$) are shown. Beta estimates and *p* values of LIFE-Heart and LIFE-Adult were obtained by linear regression analysis of plaque score adjusting for age and sex.

SNP Infos					Combined – fixed effect model				LIFE-Heart			LIFE-Adult		
Locus	**Lead SNP**	**Nearby Gene (distance)**	**Other SNPs $r^2<0.5$ and $p<1x10^{-6}$**	**effect/ other allele**	**β**	**SE**	***p* value**	***Q***	**EAF**	***p* value**	**β**	**EAF**	***p* value**	**β**
9p21	rs9644862	*CDKN2B-AS1* (0 kb)	rs7853090, rs1333050, rs10811650	G/T	0.131	0.019	$8.73x10^{-12}$	2.341	0.453	$3.36x10^{-7}$	0.174	0.425	$1.87x10^{-6}$	0.111
10q24	rs2902548	*SFXN2* (0 kb) / *WBP1L* (16 kb)	rs9663711	T/C	-0.141	0.025	$1.97x10^{-8}$	1.681	0.184	$3.94x10^{-2}$	-0.093	0.183	$7.80x10^{-8}$	-0.163
6q13	rs141249844	*KCNQ5* (0 kb)	-	T/C	0.475	0.095	$5.79x10^{-7}$	0.431	0.022	$3.21x10^{-4}$	0.554	0.020	$4.25x10^{-4}$	0.426
3p24	rs1349287	*KCNH8* (130 kb)	-	A/T	0.208	0.042	$9.54x10^{-7}$	0.176	0.060	$1.67x10^{-3}$	0.234	0.058	$1.59x10^{-4}$	0.196

Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018

Table 3: Results of previously reported SNPs in our study. SNPs of Bis et al.[9] (trait plaque prevalence) and Nikpay et al.[2] (trait CAD) reaching at least nominal significance in our study (trait PS). Tag-SNP denotes the best SNP (minimal *p* value) in LD with the reported top-SNP (r^2>0.5). Some SNPs were reversely coded in the reference (marked with [a]). For better comparison, we transformed their OR into its inverse. After transformation, six CAD-SNPs showed a different direction of effect (marked with [b]) when compared to our PS trait. For further information, see supplementary table 4 and 5.

	SNP Infos					literature		Association of Lead SNP with PS		Best associated tag SNP		
	Locus	**Lead SNP**	**Nearby Gene**	**effect/ other**	**EAF Heart Adult**	**β ± SE / OR (95% CI)**	***p* value**	***p* value**	**β**	**tag SNP**	***p* value**	**β**
Bis et al.[9]	3p14	rs17045031	*LRIG1*	A/G	0.032 0.036	-0.297 ± 0.059	$4.00x10^{-7}$	$9.95x10^{-2}$	-0.082	rs76405716	$5.63x10^{-2}$	-0.093
	4q31	rs1878406	*EDNRA*	T/C	0.141 0.141	0.199 ± 0.029	$6.90x10^{-12}$	**$1.86x10^{-2}$**	0.061	rs6820938	$6.37x10^{-4}$	0.081
	7q22	rs17398575	*PIK3CG*	A/G	0.250 0.243	0.162 ± 0.023	$2.30x10^{-12}$	**$4.40x10^{-2}$**	0.043	rs12705390	$3.63x10^{-3}$	0.065
	19p13	rs6511720	*LDLR*	T/G	0.086 0.099	-0.178 ± 0.033	$1.00x10^{-7}$	**$4.66x10^{-2}$**	-0.063	rs17242381	$8.51x10^{-3}$	-0.082
	19q13	rs445925	*APOC1*	A/G	0.100 0.113	0.82 (0.76, 0.89)	$4.00x10^{-6}$	**$7.34x10^{-3}$**	-0.098	rs7412	$1.41x10^{-3}$	-0.124
Nikpay et al.[2]	1p32	rs9970807[a]	*PPAP2B*	T/C	0.080 0.080	0.88 (0.85, 0.91)	$5.00x10^{-14}$	$2.47x10^{-1}$	-0.039	rs72664358	$1.49x10^{-2}$	-0.060
	1q41	rs67180937	*MIA3*	G/T	0.743 0.747	1.08 (1.06, 1.11)	$1.01x10^{-12}$	$5.58x10^{-2}$	0.040	rs28787398	$1.23x10^{-3}$	0.065
	2p21	chr2:44074126:D[a]	*ABCG5-ABCG8*	C/CGT	0.246 0.251	0.94 (0.92, 0.96)	$2.60x10^{-8}$	$2.05x10^{-1}$	-0.027	rs7598542	$4.31x10^{-2}$	-0.045
	2p11	rs7568458	*VAMP5-VAMP8-GGCX*	A/T	0.445 0.460	1.06 (1.04, 1.08)	$3.62x10^{-10}$	$3.45x10^{-1}$	0.017	rs6747828	$1.29x10^{-2}$	0.050
	4q32	rs72689147[a]	*GUCY1A3*	T/G	0.190 0.201	0.93 (0.91, 0.95)	$6.07x10^{-9}$	**$4.57x10^{-2}$**	-0.046	rs11731886	$3.60x10^{-2}$	-0.045
	6q25	rs55730499[a,b]	*SLC22A3-LPAL2-LPA*	T/C	0.059 0.058	0.93 (0.92, 0.96)	$1.85x10^{-8}$	$8.88x10^{-2}$	0.067	rs10455872	$4.66x10^{-2}$	0.078

Published in final edited form as: Pott et al., Genome-wide meta-analysis identifies novel loci of plaque burden in carotid artery. Atherosclerosis 259(2017):32-40; DOI: 10.1016/j.atherosclerosis.2017.02.018

	6q26	rs4252185[a]	*PLG*	C/T	0.073 0.070	1.00 (0.98, 1.02)	$9.60x10^{-1}$	$2.23x10^{-1}$	0.049	rs10455872	$4.66x10^{-2}$	0.078
	8q24	rs2954029[a]	*TRIB1*	T/A	0.487 0.492	0.96 (0.94, 0.97)	$2.61x10^{-6}$	**$1.37x10^{-3}$**	-0.059	rs2980868	$7.18x10^{-4}$	-0.062
	9p21	rs2891168	*CDKN2B*	G/A	0.480 0.468	1.21 (1.19, 1.24)	$2.29x10^{-98}$	**$2.31x10^{-8}$**	0.101	rs9644862	$8.73x10^{-12}$	0.131
	10p11	rs2487928	*KIAA1462*	A/G	0.473 0.488	1.06 (1.04, 1.08)	$4.41x10^{-11}$	**$4.98x10^{-2}$**	0.036	rs2505084	$5.63x10^{-3}$	0.050
	10q24	rs11191416[a]	*CYP17A1-CNNM2-NT5C2*	G/T	0.111 0.109	0.93 (0.90, 0.95)	$4.65x10^{-9}$	**$5.17x10^{-3}$**	-0.081	rs284841	$1.71x10^{-4}$	0.105
	12q21	rs2681472[a,b]	*ATP2B1*	G/A	0.145 0.149	0.93 (0.92, 0.96)	$1.03x10^{-9}$	**$2.20x10^{-2}$**	0.059	rs4842666	$4.57x10^{-3}$	0.076
	13q34	rs11838776[b]	*COL4A1/A2*	A/G	0.296 0.299	1.04 (1.02, 1.06)	$7.13x10^{-5}$	$1.43x10^{-1}$	-0.030	rs9515199	$1.75x10^{-2}$	0.045
	15q25	rs4468572[a,b]	*ADAMTS7*	C/ T	0.590 0.596	0.93 (0.91, 0.95)	$4.52x10^{-9}$	$2.84x10^{-1}$	0.021	rs12899452	$4.85x10^{-2}$	0.039
	17p11	rs12936587[b]	*RAI1-PEMT-RASD1*	A/G	0.434 0.433	1.04 (1.02, 1.06)	$1.84x10^{-5}$	**$4.21x10^{-2}$**	-0.038	rs58251514	$1.72x10^{-2}$	-0.046
	19p13	rs56289821[a]	*LDLR*	A/G	0.087 0.100	0.88 (0.85, 0.90)	$4.44x10^{-15}$	$6.91x10^{-2}$	-0.058	rs17242381	$8.51x10^{-3}$	-0.082
	19q13	rs4420638	*APOE-APOC1*	G/A	0.147 0.170	1.10 (1.07, 1.13)	$7.07x10^{-11}$	**$9.27x10^{-3}$**	0.069	rs429358	$7.63x10^{-4}$	0.104

[a] – SNPs were reversely coded in the reference

[b] – SNPs with a different direction of effect