Atherosclerosis 259 (2017) 32–40

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

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

Janne Pott [a, b], Ralph Burkhardt [b, c], Frank Beutner [b, d], Katrin Horn [a], Andrej Teren [b, d], Holger Kirsten [a, b], Lesca M. Holdt [b, e], Gerhard Schuler [d], Daniel Teupser [b, e], Markus Loeffler [a, b], Joachim Thiery [b, c], Markus Scholz [a, b, *]

[a] Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany
[b] LIFE Research Center for Civilization Diseases, University of Leipzig, Leipzig, Germany
[c] Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital, Leipzig, Germany
[d] Heart Center Leipzig, Leipzig, Germany
[e] Institute for Laboratory Medicine, Ludwig-Maximilians University, Munich, Germany

ARTICLE INFO

Article history:
Received 23 September 2016
Received in revised form
13 February 2017
Accepted 22 February 2017
Available online 24 February 2017

Keywords:
Atherosclerosis
Carotid artery plaque
Genome-wide association analysis

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 to 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 = 4037 and LIFE-Heart, n = 3152) 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 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.

Conclusions: 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.

1. 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

* Corresponding author. Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany.
E-mail address: markus.scholz@imise.uni-leipzig.de (M. Scholz).

http://dx.doi.org/10.1016/j.atherosclerosis.2017.02.018
0021-9150/

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 endpoint.

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 10172 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.

2. Materials and methods

2.1. Cohort description

2.1.1. 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.

2.1.2. 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 7000 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-Heart is shown in Table 1.

2.2. 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- ($\kappa = 0.93$ for the overall agreement) and inter-rater reliability ($\kappa = 0.88$ 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 9858 participants of LIFE-Adult and 3501 patients of LIFE-Heart. Distributions of PS in both cohorts are presented in Table 1.

2.3. Genotyping

2.3.1. LIFE-Adult

A total of 5101 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 $\geq$ 0.82) and sample call rate criteria (SCR $\geq$ 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