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The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound (ATHEROREMO-IVUS) study

Sanneke P.M. de Boer[1], MD; Yael Baran[2], MSc; Hector M. Garcia-Garcia[1], MD, PhD; Itamar Eskin[2], MSc; Mattie J. Lenzen[1], PhD; Marcus E. Kleber[3], PhD; Evelyn Regar[1], MD, PhD; Peter J. de Jaegere[1], MD, PhD; Jurgen M. Ligthart[1], BSc; Robert-Jan van Geuns[1], MD, PhD; Terho Lehtimäki[4], MD, PhD; Reijo Laaksonen[4], PhD; Eric Boersma[1], PhD; Winfried März[3,5,6], MD, PhD; Eran Halperin[2,7], PhD; Patrick W. Serruys[8], MD, PhD; Wolfgang Koenig[9,10]*, MD, PhD

1. Thoraxcenter, Erasmus MC, Rotterdam, the Netherlands; 2. Department of Molecular Microbiology and Biotechnology, and School of Computer Science, Tel-Aviv University, Tel-Aviv, Israel; 3. Medical Clinic V (Nephrology, Hypertensiology, Rheumatology, Endocrinology, Diabetology), Mannheim Medical Faculty, University of Heidelberg, Mannheim, Germany; 4. Department of Science Center, University of Tampere and Tampere University Hospital, Tampere, Finland; 5. Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; 6. Synlab Academy, Synlab Services GmbH, Mannheim, Germany; 7. International Computer Science Institute, Berkeley, CA, USA; 8. International Centre for Circulatory Health, NHLI, Imperial College London, London, United Kingdom; 9. Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, Ulm, Germany; 10. Deutsches Herzzentrum München, Technische Universität München, Munich, and DZHK (German Center for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany

S.P.M. de Boer and Y. Baran share 1st author position. P.W. Serruys and W. Koenig share senior author position.

GUEST EDITOR: Amir Lerman, MD; *Department of Cardiology, Mayo Clinic, Rochester, MN, USA*

KEYWORDS

- coronary artery disease
- intravascular ultrasound
- plaque rupture

Abstract

Aims: The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound (ATHEROREMO-IVUS) study was designed as an exploratory clinical study in order to investigate the associations between genetic variation, coronary atherosclerosis phenotypes, and plaque vulnerability as determined by IVUS.

Methods and results: The ATHEROREMO-IVUS study was a prospective, observational study of 581 patients with stable angina pectoris or acute coronary syndrome (ACS) who were referred for coronary angiography to the Thoraxcenter, Rotterdam, enriched with 265 IBIS-2 participants (total population, n=846). Prior to catheterisation, blood samples were drawn for genetic analyses. During the catheterisation procedure, IVUS was performed in a non-culprit coronary artery. The primary endpoint was the presence of vulnerable plaque as determined by IVUS virtual histology (VH). In addition, we performed a genome-wide association study of plaque morphology. We observed strong signals associated with plaque morphology in several chromosomal regions: twelve SNPs (rs17300022, rs6904106, rs17177818, rs2248165, rs2477539, rs16865681, rs2396058, rs4753663, rs4082252, rs6932, rs12862206, rs6780676) in or near eight different genes (GNA12, NMBR, SFMBT2, CUL3, SESN3, SLC22A25, EFBN2, SEC62) were most significant.

Conclusions: In conclusion, we found twelve SNPs in or in the proximity of eight genes, which were possibly associated with markers of vulnerable plaque. ClinicalTrials.gov Identifier: NCT01789411

**Corresponding author: Klinik für Herz-& Kreislauferkrankungen, Deutsches Herzzentrum München, Technische Universität München, Lazarettstr. 36, 80636 Munich, Germany. E-mail: koenig@dhm.mhn.de*

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Abbreviations

ACS acute coronary syndrome
ATHEROREMO-IVUS The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound study
BMI body mass index
CAG coronary angiography
CAD coronary artery disease
HDL high-density lipoprotein
IBIS-2 Integrated Biomarker and Imaging Study-2
IQR interquartile range(s)
IVUS intravascular ultrasound
LD linkage disequilibrium
LDL low-density lipoprotein
MDS multidimensional scaling
PCI percutaneous coronary intervention
SAP stable angina pectoris
TCFA thin-cap fibroatheroma
VH virtual histology

Introduction

Genetic factors play an important role in the aetiology of coronary artery disease (CAD)[1,2], and genetic markers may potentially improve risk stratification. Genetic markers can also be instrumental in developing therapeutic targets, as they are stable, and can be objectively measured and evaluated as indicators of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic interventions. Genome-wide association studies in CAD patients are warranted to unravel further the complexity of coronary atherosclerosis[3]. The European Collaborative Project on Inflammation and Vascular Wall Remodeling in Atherosclerosis - Intravascular Ultrasound (ATHEROREMO-IVUS) study was designed to investigate the associations between the genetic profile and coronary atherosclerosis phenotype and vulnerability as determined by intravascular ultrasound (IVUS)[4].

Methods

PATIENTS

The methods of the ATHEROREMO-IVUS study have been described in detail elsewhere[4]. Briefly, the target population consisted of patients aged 21 years or older with stable angina pectoris (SAP) or an acute coronary syndrome (ACS) who were referred for coronary angiography (CAG) or percutaneous coronary intervention (PCI). Patients had to have at least one non-culprit coronary artery without obstructive disease (<50% diameter stenosis) of at least 40 mm in length as assessed by on-line angiography to be used as the study vessel.

The initial ATHEROREMO-IVUS study cohort mainly consisted of 581 patients who were included during the period 2008-2011 at the Erasmus MC, Rotterdam, the Netherlands. This cohort was enriched with 265 patients from the Integrated Biomarker and Imaging Study-2 (IBIS-2) trial (inclusion period 2005-2006)[5], a study with identical inclusion criteria that was conducted in 23 participating centres in 10 European countries. Thus, the final analysis cohort comprised 846 patients[4].

ATHEROREMO-IVUS and IBIS-2 were approved by the medical ethics committees concerned, and written informed consent was obtained from all participants.

PHENOTYPING BY INTRAVASCULAR ULTRASOUND

Intravascular ultrasound imaging of the study vessel was conducted for plaque phenotyping. The IVUS greyscale and IVUS radiofrequency backscatter analyses, known as IVUS virtual histology (VH), were performed using pcVH 2.1 and qVH software (Volcano Corp., San Diego, CA, USA). IVUS images were analysed offline by an independent core laboratory (Cardialysis BV, Rotterdam, the Netherlands), which was blinded to patient characteristics as well as to genetic and clinical outcomes. Study segment plaque burden was determined, whereas atherosclerotic plaque was categorised into four different tissue types: fibrous, fibro-fatty, dense calcium and necrotic core[6]. In consensus sessions with three investigators, the lesions were further classified into different lesion types **(Table 1)**, including thin-cap fibroatheroma (TCFA)[7]. Remodelling of a lesion was assessed by means of the remodelling index.

GENOTYPING

Ethylenediamine tetraacetic acid (ETDA) blood samples were drawn from the arterial sheath prior to the CAG or PCI procedure, and these were then transported to the Erasmus MC laboratory for processing and storage at a temperature of at least -70°C. Blood samples were transported to the genotyping facility at the Mannheim Medical Faculty, Germany, for batch DNA extraction and genotyping. The Affymetrix GeneChip® Human Mapping 6.0 Array (Thermo Fisher Scientific, Waltham, MA, USA) was used for genome-wide analysis of 906,600 single nucleotide polymorphisms (SNPs).

Table 1. Study segment characteristics.

Study segment characteristics	AtheroRemo (N=718)
Length, mm	45.3 (35, 56.9)/687
Lumen volume, mm^3	352.5 (243.3, 508.2)/687
Vessel volume, mm^3	604.8 (427.9, 839.3)/687
Plaque volume, mm^3	242.5 (155.3, 347.4)/687
Plaque burden, %	40.6 (32, 47.1)/687
Fibrotic tissue volume, mm^3	61.5 (32.6, 105.3)/705
Fibro-fatty tissue volume, mm^3	11.7 (5.1, 24.7)/705
Necrotic core volume, mm^3	19.4 (7.6, 35.4)/705
Dense calcium volume, mm^3	8.8 (2.7, 18.6)/705
Fibrotic tissue percentage, %	58.7 (51.7, 65.3)/701
Fibro-fatty tissue percentage, %	11.3 (7.4, 17.1) /701
Necrotic core percentage, %	18.7 (12.8, 24.2)/701
Dense calcium percentage, %	8.2 (4.4, 13.5)/701
Number of lesions, n (ratio per patient)	820 (1.14)
TCFA lesion, n (%)	264/820 (32.2%)¶
Lesion remodelling	0.95 (0.81, 1.02)/819¶

¶Data on lesion level.

QUALITY CONTROL

The data set was filtered to include only autosomal SNPs with non-complementary alleles and genotyping rate ≥0.9. PLINK's multidimensional scaling (MDS) analysis was performed jointly on the study samples and the European samples from the POPRES data set[6]. First, the POPRES SNPs were pruned to decrease linkage disequilibrium (LD) using PLINK, so that no pair of SNPs with r2 ≥0.8 remained within sliding windows of 50 SNPs, offset by five. Additional SNPs or samples with genotyping rate ≤0.1 were removed. Next, the study SNP set and the POPRES SNP set were intersected, and MDS was performed on the resulting set. Eighty-nine outliers (individuals who were located outside of the main European cluster in the MDS plot) were detected in this step and discarded from further analysis. The results of both the European MDS and a global MDS (also using non-European POPRES samples) appear in **Figure 1**. The first four axes of variation from the European MDS were used as covariates in all association tests.

ADDITIONAL SAMPLE FILTERING AND IMPUTATION

PLINK's IBD analysis was performed and six samples were removed so as to eliminate any pair with an estimated proportion of IBD (PLINK's PI_HAT) greater than 0.1875. In addition, following PLINK's heterozygosity analysis, 17 samples with outlier inbreeding factor (<–0.15 or >0.1) were removed. The genotypes used for imputation were filtered to exclude SNPs with a genotyping rate ≤0.04 or a minor allele frequency ≤0.0125. Imputation was performed with the 1,000 genomes European reference panel[7] using BEAGLE genetic analysis software, version 3.3.2[8]. Prior to that, strand consistency between the two data sets was verified using BEAGLE's strand-checking utility.

ASSOCIATION

Samples in which reported sex did not match the sex detected by PLINK (15 samples) were removed to yield a final sample size of 719 individuals **(Figure 2)**. SNPs with minor allele frequency ≤10/719=0.0139 were discarded.

We used 28 IVUS measurements as phenotypes: 14 quantities describe the vascular segment that was analysed, and 14 quantities described the lesions detected in that segment, up to four lesions per individual. For each such phenotype, individuals with missing measurements or outliers (distant from the mean by over three standard deviations) were excluded. Phenotypes representing length or tissue percentages were square root-transformed. Phenotypes corresponding to volumes were cubic-root transformed and divided by the vascular segment length.

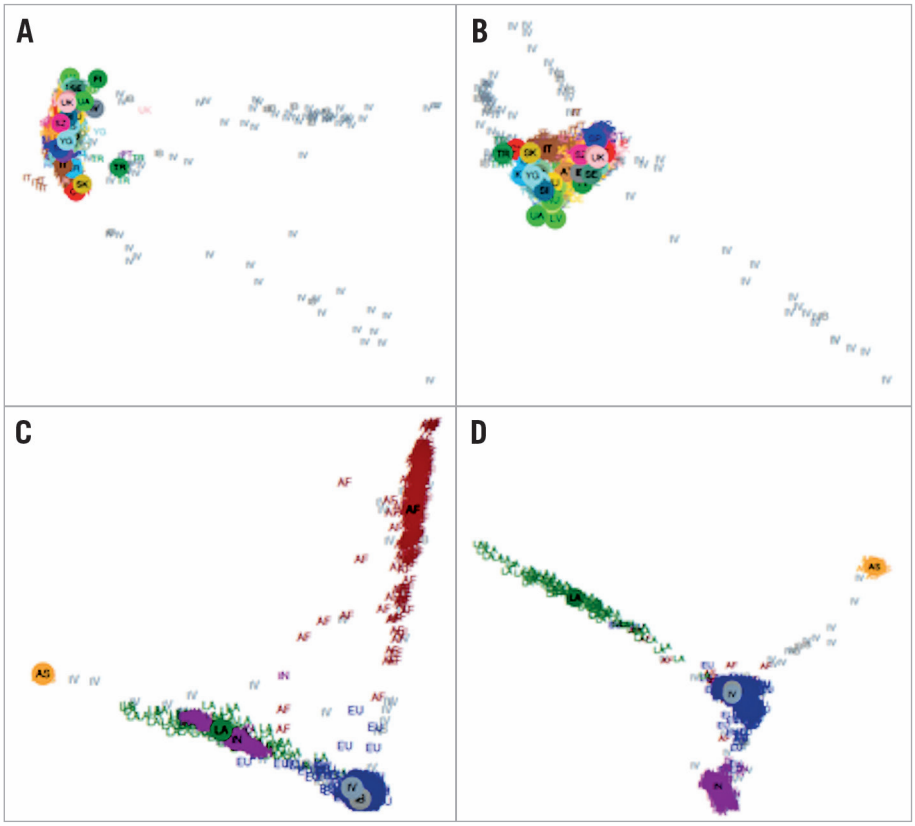

Figure 1. *The European MDS and a global MDS (also using non-European POPRES samples). The plots display the first four axes of variation of the MDS analysis performed with the European (top) and global (bottom) POPRES diversity panels. A) European, axes 1-2. B) European, axes 3-4. C) Global, axes 1-2. D) Global, axes 3-4. AF: African-American; AS: Asian; EU: European; IB: IBIS; IN: Indian; IV: IVUS; LA: Latino; TR: Turkey*

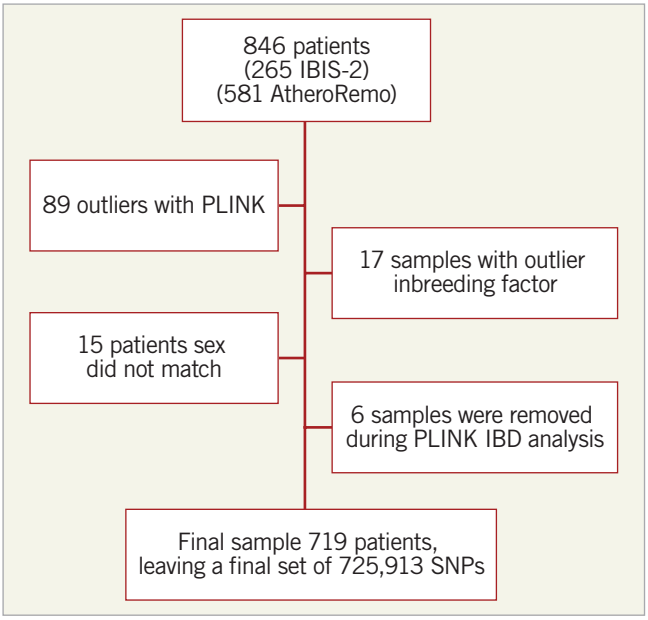

Figure 2. *Flow chart.*

Linear and logistic regression models assuming an additive genetic model were used for testing association between SNPs and quantitative and categorical phenotypes, respectively. Each model included, in addition to the SNP and the phenotype, the following covariates: site (Erasmus MC/IBIS-2), sex, age, body mass index (BMI), segment length, and the sample's coordinates along the first four MDS axes. Missing BMI values were imputed by the sex-specific median sample BMI.

In addition to the basic SNP vs. phenotype association tests, we examined the extended models, which included an interaction effect between SNPs and sex. We used a two degrees of freedom test for the combined marginal and interaction effects. An alpha error of 5.0e-08 was considered significant in this genome-wide association study.

TREATING SPECIAL PHENOTYPES

In order to increase the statistical power for the lesion-specific phenotypes, we employed two different strategies. The first approach involved considering as a phenotype the weighted average of multiple lesions in each individual. We used 1, 1.5, 2 and 3 as the weights for lesions 1, 2, 3 and 4, respectively. The second approach was to duplicate individuals with multiple discovered lesions, ending up with one lesion per individual. To account for the strong relatedness introduced by this step, we tested association with this phenotype using EMMAX[9], which uses a mixed model that corrects for relatedness.

Association with lesion type was tested by dividing the lesions into two categories: the benign category included adaptive intimal thickening, pathological intimal thickening, fibrous and fibrocalcific, and the severe category included fibroatheroma, calcified fibroatheroma, TCFA and calcified TCFA.

DEMOGRAPHICS

Continuous variables are presented as arithmetic mean values and corresponding standard deviations (±SD), or medians together with corresponding interquartile ranges (IQR). Categorical variables are expressed as numbers and percentages. All demographic data were analysed with SPSS software, Version 20.0 (IBM Corp., Armonk, NY, USA).

LURIC AND CARDIoGRAM

Additionally, we studied within the LURIC and CARDIoGRAM data set the association between the SNPs we found and different phenotypes, biomarkers, and outcome. LURIC is a prospective study in 3,316 patients of German ancestry who underwent coronary angiography. All participants underwent comprehensive assessment of cardiovascular and metabolic phenotypes. A ten-year follow-up for mortality and causes of death has been completed[10]. CARDIoGRAM combines data from all published and several unpublished genome-wide association studies (GWAS) in individuals with European ancestry, and includes 22,000 cases with CAD, MI, or both[11].

Results

PATIENT CHARACTERISTICS

Baseline and procedural characteristics are presented in **Table 1** and **Table 2**. Our patients were mostly men (78%) and the mean age was 61 years. Most patients were treated for stable angina

Table 2. Patient baseline characteristics.

Baseline characteristics	AtheroRemo (N=718)
Age, years	61.4 (53.1, 69.9)
Male, n (%)	558 (77.7)
Diabetes mellitus, n (%)	109 (15.2)
Hypertension, n (%)	404/717 (56.3)
Hypercholesterolaemia, n (%)	411/717 (57.3)
Low-density lipoprotein, mmol/l	2.56 (2.00, 3.38)/579
High-density lipoprotein, mmol/l	1.13 (0.95, 1.37)/592
Total cholesterol, mmol/l	4.5 (3.7, 5.3)/599
Current smoker, n (%)	219 (30.5)
Previous MI, n (%)	147/472 (31.1)¶
Previous PCI, n (%)	237 (33.0)
Previous CABG, n (%)	18/472 (3.8)¶
Previous stroke, n (%)	24/472 (5.1)¶
Peripheral artery disease, n (%)	40 (5.6)
Presentation with ACS, n (%)	334/711 (47.0)
Coronary artery disease	
No significant vessel disease, n (%)	142/615 (23.0)¶
1-vessel disease, n (%)	15/615 (36.5)¶
2-vessel disease, n (%)	198/615 (23.0)¶
3-vessel disease, n (%)	60/615 (7)¶

¶ Not included in the data set for the subset of IBIS-2 patients.

pectoris (53%), but prior MI (31%) and prior PCI (33%) were common. The mean length of the studied segment was 45.3 mm, with a mean plaque burden of 40.6%. On average, the plaques consisted mostly of fibrotic tissue (59%), and 19% had a necrotic core; 32% of plaques were classified as TCFA.

GWAS ANALYSIS OF VULNERABLE PLAQUE

Multiple genomic locations were shown to be potentially associated with vulnerable plaque **(Figure 3, Figure 4)**. We observed strong association signals in several chromosomal regions: twelve SNPs in or near eight different genes were most significant **(Table 3)**.

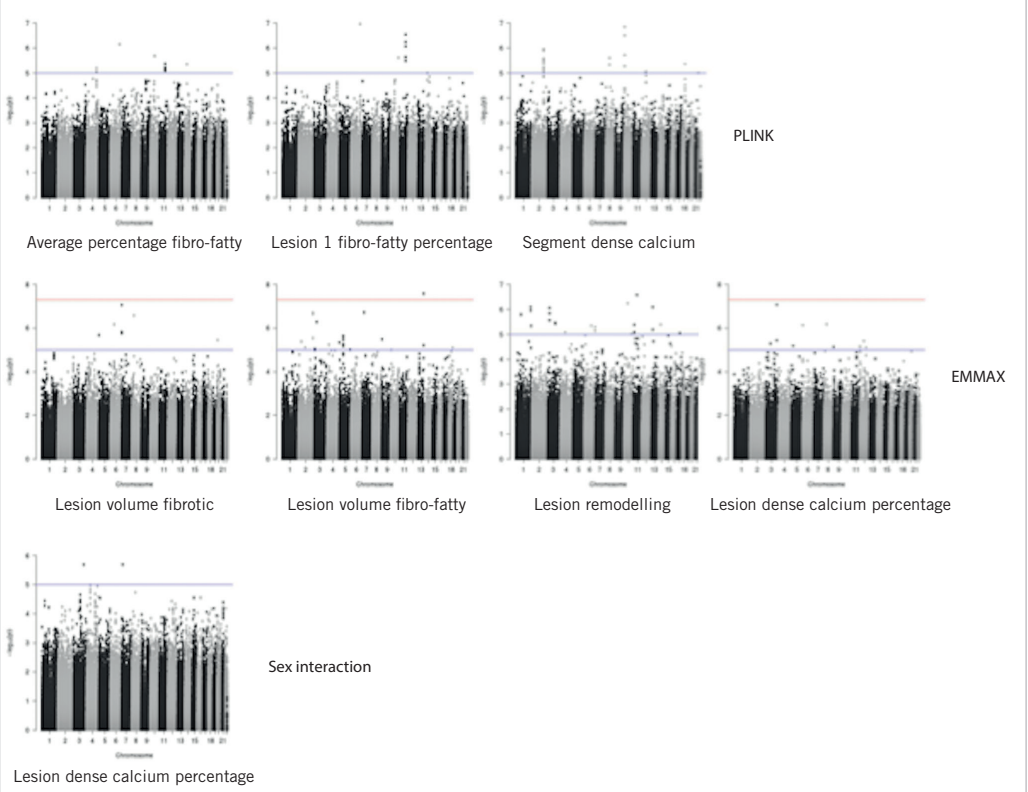

Figure 3. *Manhattan plots: potential association between multiple genomic locations and vulnerable plaque.*

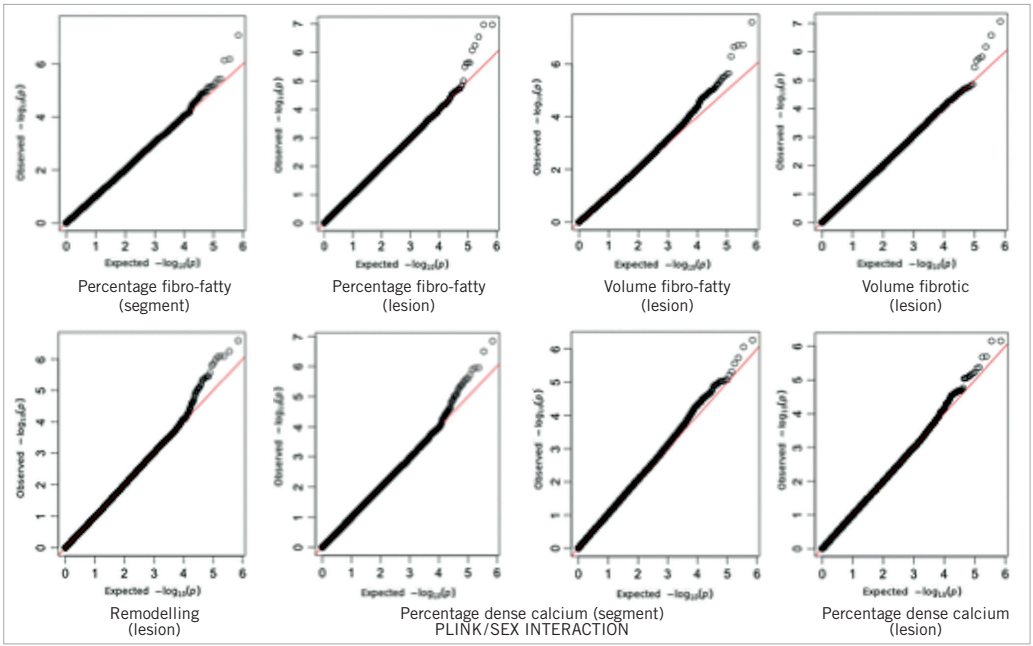

Figure 4. *QQ-plots: potential association between multiple genomic locations and vulnerable plaque.*

Table 3. Most significant associations between single-nucleotide polymorphisms (SNPs) and plaque phenotypes.

SNP	MAF	Chromo-some	Position	Test performed	Plaque phenotype	*p*-value	Beta	HWE *p*-value	Closest gene	Distance	Second closest gene	Distance
rs17300022	0.1161	7	2808517	EMMAX	Volume fibrotic (lesion)	8.61E-08	–0.57475	0.02797	GNA12			
rs6904106	0.2872	6	141898808	PLINK (VOL/LEN)	Percentage fibro-fatty (lesion)	1.09E-07	0.3016	0.5236	NMBR	530 kb		
rs6904106	0.2872	6	141898808	PLINK (VOL/LEN)	Percentage fibro-fatty (segment)	7.1E-07	0.2671	0.5236	NMBR	530 kb		
rs17177818	0.2872	6	141912038	PLINK (VOL/LEN)	Percentage fibro-fatty (lesion)	1.09E-07	0.3016	0.5236	NMBR	530 kb		
rs17177818	0.2872	6	141912038	PLINK (VOL/LEN)	Percentage fibro-fatty (segment)	7.1E-07	0.2671	0.5236	NMBR	530 kb		
rs2248165	0.3428	10	7539534	PLINK (VOL/LEN)	Percentage dense calcium (segment)	1.42E-07	–0.3179	0.7406	SFMBT2	46 kb		
rs2248165	0.3428	10	7539534	SEX INTERACTION	Percentage dense calcium (segment)	8.84E-07	–0.1468	0.7406	SFMBT2	46 kb		
rs2477539	0.3477	10	7544816	PLINK (VOL/LEN)	Percentage dense calcium (segment)	3.12E-07	–0.3096	0.6811	SFMBT2	46 kb		
rs16865681	0.07024	2	225084573	EMMAX	Volume fibro-fatty (lesion)	1.94E-07	–0.27607	0.0183	CUL3	41 kb		
rs2396058	0.06885	2	225045264	EMMAX	Volume fibro-fatty (lesion)	2.25E-07	–0.27576	0.01443	CUL3	41 kb		
rs4753663	0.1975	11	94712679	PLINK (VOL/LEN)	Percentage fibro-fatty (lesion)	2.89E-07	0.3453	0.1251	SESN3	108 kb		
rs4082252	0.1982	11	94710862	PLINK (VOL/LEN)	Percentage fibro-fatty (lesion)	5.74E-07	0.3345	0.1599	SESN3	108 kb		
rs6932	0.01599	11	62656316	EMMAX	Remodelling (lesion)	2.62E-07	–0.22988	0.1637	SLC22A25	31 kb		
rs12862206	0.09192	13	105515048	EMMAX	Volume fibro-fatty (lesion)	2.61E-08	–0.26156	0.1801	EFNB2	425 kb		
rs6780676	0.01624	3	171174616	EMMAX	Percentage dense calcium (lesion)	8.43E-08	–8.59549	1	SEC62	7 kb	SAMD7	34 kb
											GPR169	64 kb

Rs17300022 in the GNA12 gene was significantly associated with fibrotic volume of the vulnerable plaque, p=8.61E-08. The SNP rs6780676 on chromosome 3 was associated with the percentage of dense calcium within the plaque, p=8.43E-08. A gene in the proximity of rs6780676 is SEC62 at a distance 7 kb. SEC 62 is part of the protein translocation apparatus in the membrane of the endoplasmic reticulum, and has been linked to prostate and lung cancer[12]. The rs6904106 and rs17177818 were associated with the percentage fibro-fatty material within the vulnerable plaque and are in the proximity (530 kb) of the NMBR gene. The NMBR gene, a neuromedin B receptor, binds to neuromedin B protein, and is involved in a number of physiological processes including immune defence, thyroid, adrenocortical function and cognition. NMB is also aberrantly expressed by a variety of cancers and is involved in tumour proliferation[13].

Associated with dense calcium percentage of the plaque are rs2248165 and rs2477539, which are in the proximity (46 kb) of the SFMBT2 (Scm-like with four mbt domains 2) gene, which is a protein-coding gene that has been linked to prostate cancer[14] and placental development[15]. Rs16865681 and rs2396058 are linked to the volume of fibro-fatty material of the plaque, and are close to the CUL3 gene, which encodes a member of the cullin protein family. Rs4753663 and rs4082252 are associated with the percentage of fibro-fatty material of the plaque and are in close proximity to the SESN3 gene. This gene encodes for a member of the sestrin family of stress-induced proteins. Rs6932 is associated with remodelling and is in closeness to the SLC22A25 gene, which encodes for the solute carrier family 22 member 25 protein. SLC22A25 is also known as organic anion transporter UST6. Rs12862206 is associated with the volume of fibro-fatty material of the plaque and is in the proximity of the EFBN2 gene, which encodes for the Ephrin-B2 protein.

LURIC

Within the LURIC data set we studied the association between the SNPs we found and clinical biomarkers and outcomes. Several associations with significant p-values were found **(Table 4)**.

CARDIoGRAM

To study the found associations further, we used the CARDIoGRAM[11] data set and analysed the association between the identified SNPs and MI as well as CAD. We found a statistically

Table 4. The association between the SNPs we found and clinical biomarkers and outcomes within the LURIC data set. Only the significant associations are presented.

	SNP	Effect allele	BETA	SE	*p*-value
1,2,3 vessel disease	rs6932	G	–0.241	0.120	0.045
Friesinger score[24,25]	rs12862206	A	–0.387	0.181	0.033
Myocardial infarction	rs6932	G	–0.456	0.212	0.031
HDL-C (mg/dl)	rs6904106	G	0.672	0.312	0.031
	rs17177818	T	0.672	0.312	0.031
TG (mg/dl)*	rs6904106	G	–0.031	0.014	0.025
	rs17177818	T	–0.031	0.014	0.025
IL-6 (ng/l)*	rs6780676	C	0.725	0.363	0.046
MPO (g/l)*	rs17300022	C	–0.066	0.033	0.046
	rs2248165	G	–0.050	0.023	0.032
	rs4082252	C	0.056	0.027	0.040
	rs4753663	A	0.056	0.027	0.041

We found no significant association between the identified SNPs and CAD (stenosis ≥50%), CAD (stenosis ≥20%), Gensini score, all-cause mortality, cardiovascular mortality, NT-proBNP (ng/ml), total cholesterol (mg/dl), LDL-C (mg/dl), hsCRP (mg/l)*, LpPLA2 (ng/ml)*. *value log transformed before analysis. CAD: coronary artery disease; hsCRP: high-sensitivity C-reactive protein; LpPLA2: lipoprotein associated phospholipase A2; MPO: myeloperoxidase; NT-proBNP: N-terminal prohormone of brain natriuretic peptide

significant association between rs4082252 (OR 1.05- lnSE 0.02, p=0.009), rs475366 (OR 0.96- lnSE 0.02, p=0.012) and MI/CAD **(Table 5)**.

eQTL AND HaploReg

We searched the Genotype-Tissue Expression (GTEx) project[16] and HaploReg[17] for an association of our SNPs with the expression of nearby genes or the alteration of regulatory sequences. While we did not find any direct association with gene expression, a number of SNPs could alter regulatory motifs **(Table 6)**.

Discussion

In this exploratory study of 719 CAD patients we found a strong association between IVUS-derived indicators of vulnerable plaque and twelve SNPS, in or near eight different genes.

We further studied these SNPs in the LURIC data set and additionally decided to explore our results further in the CARDIoGRAM data, in which we found two further significant associations between SNPs and CAD/MI; these two SNPs encode for two genes, namely GNA12 and SESN3.

The GNA12 gene was significantly associated with vulnerable plaque (dense calcium) and CAD/MI. The GNA12 gene encodes for the guanine nucleotide-binding protein subunit alpha-12 proteins, which are involved as modulators or transducers in various transmembrane signalling systems, and may play a role in the control of cell migration through the TOR signalling cascade[18].

The SESN3 gene encodes for a member of the sestrin family of stress-induced proteins. The encoded protein reduces the levels of intracellular reactive oxygen species induced by activated Ras downstream of RAC-alpha serine/threonine-protein kinase (Akt) and FoxO transcription factor. The protein is required for normal regulation of blood glucose, insulin resistance, and plays a role in lipid storage in obesity[19].

Limitations

There may simply be no direct causal link between the SNPs and vulnerable plaque, due to the phenotypic complexity of atherosclerotic vascular disease. Alternatively, there could indeed be a causal relationship which we were not able to detect. First, in this study we visualised 40 mm of a non-culprit vessel, and we might have missed the patient's dominant phenotypic characteristic if this phenotype was only expressed in a coronary segment that had not been imaged (e.g., the culprit lesion). However, *ex vivo* as well as *in vivo* studies using IVUS

Table 5. CARDIoGRAM: the association between the identified SNPs and MI as well as CAD.

Name	Chromosome	Position	Allele1	Allele2	Effallele	Effallelefreq	N	Npops_After	Mode	*p*-value_FE	OR_FE	lnSE_FE
rs2396058	2	224753508	A	G	G	0,907626184	83573,02	13	FE	0,37086123	1,02634805	0,029062514
rs16865681	2	224792817	T	G	G	0,092520665	84206,42	14	FE	0,322107938	0,972056808	0,02862339
rs6904106	6	141898808	A	G	G	0,275894117	83134,76	13	FE	0,826089896	1,003410579	0,015496036
rs17177818	6	141912038	T	C	C	0,724759311	83621,9	13	FE	0,807499315	0,996236958	0,015473377
rs17300022	7	2775043	T	C	C	0,146119256	84163,66	14	FE	0,043813659	0,958650948	0,02094791
rs2248165	10	7539534	T	G	G	0,371467547	84071,35	14	FE	0,206727061	1,018034992	0,014156548
rs2248176	10	7539760	A	G	G	0,3623663	82252	12	FE	0,1697191	1,01980500	0,01428222
rs6932	11	62412892	G	C	C	0,982570612	30834	7	FE	0,108253644	1,15456147	0,089484644
rs4082252	11	94710862	T	C	C	0,219089343	83603,63	13	FE	0,008599617	1,046644155	0,017350227
rs4753663	11	94712679	A	G	G	0,781175506	83655,65	13	FE	0,012120483	0,95739369	0,017356413
rs12862206	13	104313049	A	G	G	0,881090456	79051,33	11	FE	0,501262965	0,982228088	0,026664127

SNP	Proxy	Distance	Rsquared
rs2477539	rs2248176	5056	0.965
rs6780676		No matching proxy SNPs found	

Table 6. Association of our SNPs with the expression of nearby genes or the alteration of regulatory sequences within Genotype-Tissue Expression (GTEx) project and HaploReg.

SNP	eQTL	Regulatory chromatin states (ENCODE)	Regulatory motifs altered
rs2396058	none	none	none
rs16865681	none	7_Weak_Enhancer (umbilical vein endothelial cells)	AP-1_known1; ATF4
rs6780676	none	4_Strong_Enhancer (B-lymphocyte, lymphoblastoid); 6_Weak_Enhancer (lung fibroblasts); 7_Weak_Enhancer (H1 Cell Line)	CEBPG; Foxd3; HNF6; Hdx; Pbx-1_1, Pbx-1_4
rs6904106	none	none	none
rs17177818	none	5_Strong_Enhancer (umbilical vein endothelial cells); 7_Weak_Enhancer (hepatocellular carcinoma)	E2A_2; Myc_known9
rs17300022	none	none	Foxj1_1; Pbx-1_1; Sox_7
rs2248165	none	4_Strong_Enhancer (B-lymphocyte, lymphoblastoid)	AP-1_disc3; AP-1_known4; BCL_disc2; Bach1; Bach2; Maf_known1; NFE2_disc1;
			Nrf-2_2; PRDM1_disc2; STAT_disc2
rs6932	none	none	GATA_known8; HNF4_known5; Mef2_disc3; RXRA_known3; Rad21_disc10;
			ZBTB7A_disc1
rs4082252	none	5_Strong_Enhancer (H1 Cell Line, mammary epithelial cells, epidermal keratinocytes); 7_Weak_Enhancer (lung fibroblasts, B-lymphocyte, lymphoblastoid)	Cphx; Myc_disc5; Pbx-1_1; Pbx-1_2; Pbx-1_3; Pbx-1_4; p300_disc2
rs4753663	none	none	none
rs12862206	none	none	GATA_disc4
rs2477539	none	none	HNF1_5

have demonstrated the presence of TCFAs in other than the culprit lesion or even in the culprit artery. Furthermore, as we demonstrated earlier, the presence of IVUS-VH-derived TCFA lesions in a non-culprit coronary artery is strongly predictive of adverse cardiac events within one year, particularly of death and ACS[20]. Therefore, we consider the non-culprit artery a good reflection of disease burden of the larger coronary vasculature. Second, one might question whether IVUS-VH is suitable for the detection of vulnerable plaques. We believe it is, despite the fact that the spatial resolution of IVUS-VH (150 μm) is insufficient to replicate exactly histopathological definitions of a thin fibrous cap (<65 μm), and IVUS-VH therefore tends to overestimate the number of histopathological TCFA lesions[21]. As mentioned above, the presence of VH-TCFA was associated with impaired prognosis in our cohort[20], an association also found in other studies[22,23]. Furthermore, TCFAs with a large plaque burden carry higher risk than small TCFA lesions, especially in the short term[20].

Since all these patients have a culprit artery and the analysis concerns the non-culprit artery, there may be a bias in the selection of the patient population. Ideally, in the future, the best analysis or the best population for the genetic analysis would be a mixed population with and without PCI. However, this limitation does not invalidate the current findings in patients with culprit lesions.

Conclusions

We found twelve SNPs in or in the proximity of eight genes, which are possibly associated with indicators of vulnerable plaque.

Impact on daily practice

We identified two genes in particular, which, besides being related to vulnerable plaque, were also related to clinical outcome. Our results are hypothesis-generating, and need to be replicated in other, larger populations.

Guest Editor

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Conflict of interest statement

The authors have no conflicts of interest to declare. The Guest Editor has no conflicts of interest to declare.

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