Current Research in Translational Medicine 71 (2023) 103374

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

Original article

Causal analysis of plasma IL-8 on carotid intima media thickness, a measure of subclinical atherosclerosis

Ilais Moreno Velásquez[a,b], Anders Malarstig[c,d], Damiano Baldassarre[e,f], Yan Borne[g], Ulf de Faire[h], Gunnar Engström[g], Per Eriksson[c,i], Philippe Giral[j], Steve E. Humphries[k], Sudhir Kurl[l], Karin Leander[h], Lars Lind[m], Anders Lindén[n,o], Nicola Orsini[p], Matteo Pirro[q], Angela Silveira[c,i], Andries J. Smit[r], Elena Tremoli[s], Fabrizio Veglia[f,s], Rona J. Strawbridge[c,t,u], Bruna Gigante[c,v,*]

[a] *Gorgas Memorial Institute for Health Studies, Panama City, Panama*
[b] *Max Delbrück Center for Molecular Medicine in the Helmholtz-Association, Molecular Epidemiology Research Group, Berlin, Germany*
[c] *Division of Cardiovascular Medicine, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden*
[d] *Emerging Science and Innovation, Pfizer Worldwide Research, Development and Medical, Stockholm, Sweden*
[e] *Department of Medical Biotechnology and Translational Medicine, Università di Milano, Milan, Italy*
[f] *Centro Cardiologico Monzino, IRCCS, Milan, Italy*
[g] *Department of Clinical Sciences, Malmö, Lund University, Malmö, Sweden*
[h] *Cardiovascular and Nutritional Epidemiology Unit, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden*
[i] *Karolinska University Hospital Solna, Stockholm, Sweden*
[j] *Sorbonne Université, INSERM UMR1166, Cardiovascular prevention unit, AP-HP, Groupe Hôpitalier Pitié-Salpetriere, Paris, France*
[k] *Cardiovascular Genetics, Institute Cardiovascular Science, University College London, United Kingdom*
[l] *Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio Campus, Finland*
[m] *Department of Medical Sciences, Clinical Epidemiology, Uppsala University, Uppsala, Sweden*
[n] *Unit for Lung and Airway Research, Institute of Environmental Medicine, Stockholm, Sweden*
[o] *Karolinska Severe COPD Center, Department of Respiratory Medicine and Allergy, Karolinska University Hospital, Stockholm, Sweden*
[p] *Department of Global Public Health, Karolinska Institutet, Stockholm, Sweden*
[q] *Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Medicine, University of Perugia, Perugia, Italy*
[r] *Department of Medicine, University Medical Center Groningen, Groningen & Isala Clinics Zwolle, Department of Medicine, the Netherlands*
[s] *Maria Cecilia Hospital, Cotignola, RA, Italy*
[t] *Institute of Health and Wellbeing, University of Glasgow, Glasgow, United Kingdom*
[u] *Health Data Research, United Kingdom*
[v] *Division of Cardiovascular Medicine, Department of Clinical Sciences, Danderyd University Hospital, Stockholm, Sweden*

ARTICLE INFO

Article History:
Received 25 March 2022
Accepted 29 November 2022
Available online 6 December 2022

Keywords:
Chemokines
Genetic association studies
Subclinical atherosclerosis
Carotid intima media thickness
Cohort studies

ABSTRACT

Background: We investigated the causality of IL-8 on carotid intima-media thickness (c-IMT), a measure of sub-clinical atherosclerosis.
Methods: The IMPROVE is a multicenter European study (n = 3,711). The association of plasma IL-8 with c-IMT (mm) was estimated by quantile regression. Genotyping was performed using the Illumina CardioMetabo and Immuno chips. Replication was attempted in three independent studies and a meta-analysis was performed using a random model.
Results: In IMPROVE, each unit increase in plasma IL-8 was associated with an increase in median c-IMT measures (all p<0·03) in multivariable analyses. Linear regression identified rs117518778 and rs8057084 as associated with IL-8 levels and with measures of c-IMT. The two SNPs were combined in an IL-8-increasing genetic risk that showed causality of IL-8 on c-IMT in IMPROVE and in the UK Biobank (n = 22,179). The effect of IL-8 on c-IMT measures was confirmed in PIVUS (n = 1,016) and MDC—CC (n = 6,103). The association of rs8057084 with c-IMT was confirmed in PIVUS and UK Biobank with a pooled estimate effect (β) of -0·006 with 95%CI (-0·008- -0·003).
Conclusion: Our results indicate that genetic variants associated with plasma IL-8 also associate with c-IMT. However, we cannot infer causality of this association, as these variants lie outside of the *IL8* locus.

* Corresponding author.
E-mail address: bruna.gigante@ki.se (B. Gigante).

https://doi.org/10.1016/j.retram.2022.103374

Introduction

The chemokine interleukin 8 (IL-8, also known as CXCL8) participates in the inflammatory response in the vessel wall [1], a response that leads to the formation and progression of atherosclerotic lesions.

Mechanistic studies have shown that IL-8 participate in the recruitment of neutrophils in the sub-intimal space [2], it is expressed and released from macrophages in atherosclerotic plaque [3] and act as a mitogen for vascular smooth cells [4]. IL-8 mRNA and protein have been shown in carotid atherosclerotic plaques [5] and CXCR2, a common receptor for IL8 and growth-regulated oncogene alpha (GRO-α or CXCL1) a chemokine sharing most of IL-8 biological properties, has been found in endothelial and intimal cells of advanced human atherosclerotic lesion [6]. Consistently, a reversible CXCR2 inhibitor is currently tested [7] as an anti-inflammatory treatment in coronary heart disease (CHD) and monoclonal IL-8 antibodies delivered to atherosclerotic plaques have been shown to reduce inflammation and increase plaque stability [8]. However, it is not known whether IL-8 expression in the atherosclerotic plaque is a consequence of the ongoing inflammatory process or reflects a causal effect on the development of atherosclerotic lesions.

We and others have investigated the association of circulating levels of IL-8 with the risk of atherosclerosis related cardiovascular diseases (CHD and ischemic stroke) with discordant results [9–12]. This may partly be explained by the observation that chemokines act in the vessel wall through the formation of concentration gradients rather than acting at the systemic level [13] and that endothelial cells have a selective chemokine expression pattern in the different vascular beds [14]. Hence, circulating chemokine levels may mirror vascular more than systemic inflammation in atherosclerosis.

To gain further insights in the role of IL-8 in the atherosclerotic process and to assess supportive evidence for causality of IL-8, we have analyzed the association of IL-8 with carotid intima-media thickness (c-IMT), a measure of vascular wall remodeling indicative of sub-clinical atherosclerosis. We have also looked at the association of GRO-α with c-IMT, as these two chemokines bind the CXCR2 receptor and share biological properties in the vascular wall. We performed our study in the c-IMT and c-IMT Progression as Predictors of Vascular Events in a High Risk European Population (IMPROVE) study, a large European cohort, and replicated the main findings in three independent populations, the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS), the Malmö Diet and Cancer Cardiovascular Cohort (MDC—CC) and the UK Biobank.

Methods

Study populations

In the present study we performed all analyses in the IMPROVE (discovery cohort) and validated our results in three validation cohorts (PIVUS, MDC—CC and UK Biobank). The three validation cohorts are described in the Supplementary Material and the c-IMT measurements are summarized for all the four studies in Supplemental Table I.

Discovery cohort. The IMPROVE was described in detail elsewhere [15,16]. Briefly, from March 2004 to April 2005 seven centers in five European Countries (Finland, France, Italy, Sweden and The Netherlands) recruited men and women (N = 3711, age 55 −79 years) who had at least three established CV risk factors [i.e., men, women at least 5 years after menopause, dyslipidemia, hypertension, diabetes, smoking and family history of CV disease] and yet had no overt CV disease. At baseline, comprehensive information on medical history, lifestyle habits, CV risk factors and co-morbidities were collected, anthropometric measures recorded, blood samples withdrawn and stored at −80 °C until analysis.

Validation cohorts. Main results were replicated in three independent cohorts, the MDC—CC (n = 6103) [17], the PIVUS (n = 1016) [18] and the UK Biobank (n = 22,179) [19], described in details in the Supplementary Material. These studies were contacted since IL-8 measurements by Proximity Extension Assays (PEA) in plasma (PIVUS, MDC—CC), c-IMT measurements (PIVUS, MDC—CC and UK Biobank) and genotyping arrays (PIVUS, MDC—CC and UK Biobank) were available.

Ethical statement. Ethical approvals to conduct all studies are detailed in the Supplemental information.

Carotid ultrasonography

A detailed description of the protocol, the validation and the precision of the c-IMT ultrasonographic measures in the IMPROVE have been reported elsewhere [16]. Briefly, the c-IMT was measured at the far wall of the right and left carotid artery at 4 different segments (common carotid, 1 cm proximal to the carotid artery bifurcation, carotid bifurcation, internal carotid artery). The mean and maximum c-IMT values were measured at each segment and utilized to derive the averaged value c-IMT_{mean}, c-IMT_{max} and the average of maximum c-IMT measured at the 8 segments, the c-$IMT_{mean\text{-}max}$ for the whole carotid tree. C-IMT values are reported in mm. In 8 study participants, carotid ultrasound measurements were not recorded because the carotid artery tree was not properly visualized. Carotid plaques, defined as c-IMT ≥1.5 mm, were included in the measurement.

The protocols for ultrasound measurements of the carotids in the MDC—CC, PIVUS and UK Biobank have been previously described [20–22]. The c-$IMT_{mean\text{-}max}$ measure was available in the PIVUS and UK Biobank but not in the MDC—CC dataset. Supplemental Table I summarizes the ultrasonographic measures available in each study.

Chemokine measurements

Plasma IL-8 (IMPROVE, PIVUS and MDC—CC) and GRO-α (IMPROVE) were measured using PEA, within the Proseek Multiplex CVD I 96×96 panel (Olink Proteomics, Uppsala, Sweden). The Proseek CVD panel includes 92 biomarkers of relevance for CV diseases. A full description of the PEA method is reported elsewhere [23]. Briefly, binding of paired oligonucleotide-labeled antibodies to the target biomarker allows the formation of a polymerase chain reaction (PCR) target that is subsequently amplified and quantified by real-time quantititative PCR. The concentration of the biomarker is expressed in arbitrary units (AU) after normalization of protein expression by internal extension controls.

Of the 3703 IMPROVE study participants with ultrasound data, measurements were missing for 175 subjects and a further 161 samples failed measurement quality control [24]. In total, 3367 samples with IL-8 and GRO-α measurements in plasma were available and included in the study.

IL-8 measurements were available in 4695 study participants from MDC—CC and 930 from PIVUS. IL-8 measurements were not available in the UK Biobank.

Genotyping

Genomic DNA from IMPROVE study participants was genotyped with two arrays, the CardioMetaboChip 200K [25] and the Immunochip [26], each one analysing approximately 200,000 genetic variants. The CardioMetaboChip 200 K is a custom Illumina iSelect genotyping array including single nucleotide polymorphisms (SNPs) mapping to genetic regions identified in genome wide association (GWA) studies as potentially relevant for cardiometabolic diseases [25]. The Immunochip is a custom Illumina Infinium HD array designed to densely genotype immune-mediated diseases using loci identified by GWA studies [26]. Standard quality control procedures for genetic data

were conducted on the individual genotyping chip as well as on the combined chips (CardioMetabo-Immuno) [24]. Multidimensional scaling (MDS) components were calculated using PLINK version 1.07 [27] (using default settings) to identify possible non-European ethnicity [28]. MDS components can also be used to adjust for population stratification, an important potential confounder when recruiting from several countries. SNPs were excluded for deviation from Hardy-Weimberg equilibrium (p<0.0000001), call rate <95% or minor allele frequency (MAF) <1%. Subjects with call rate <95%, cryptic relatedness, ambiguous sex or identified as outliers by MDS were also excluded. After exclusions, a total of 251,108 SNPs and 3325 IMPROVE study participants were available for genetic analysis Supplemental Figure I illustrates the flowchart for the inclusion/exclusion of IMPROVE study participants in the present analysis.

Lead SNPs identified in the IMPROVE were used in the replication analysis.

Statistics

Demographic characteristics, anthropometric and biochemical measures and CV risk factors at baseline in the IMPROVE are reported according to IL-8 and GRO-α quartiles. Quartile boundaries (AU) for IL-8 were (Q1≤30.59; Q2>30.6 ≤ 41.18; Q3 >41.18, ≤55.25; Q4>55.25) and for GRO-α (Q1 ≤40.1; Q2 >40.1, ≤82.2; Q3 >82.2, ≤159.2; Q4>159.2). Binary variables are reported as numbers per quartile whereas continuous variables as median and interquartile range (IQR).

The association between plasma IL-8 and GRO-α (modeled as continuous variables) and c-IMT was estimated using linear quantile regression models and expressed as beta coefficients (β) and standard error (SE). Two models were assessed: crude, adjusted by sex, age, latitude and model 1, crude model plus common CV risk factors (hypertension, diabetes, current smoking, BMI, LDL-cholesterol levels) and treatment with statins and anti-platelet agents. Hypertension was defined as blood pressure ≥140/90 mm Hg at the visit and/or antihypertensive drug therapy and/or self-reported. Diabetes was defined in the presence of fasting glucose ≥7 mmol/L visit and/or treatment with insulin and/or hypoglycemic agents and/or self-reported. No correction for multiple comparisons was performed since the various c-IMT measures are highly correlated [29].

We tested the association of CardioMetabo-Immuno chip SNPs with log transformed chemokines and c-IMT measures using a linear regression analysis in the IMPROVE. The analyses assumed an additive model of inheritance and models consistent with those defined above were applied, with the exception that MDS components 1−3 were substituted for latitude. Of note, latitude is strongly correlated with MDS component 1 (Spearmans rank rho= 0.94 P<0.0001). The 251,108 SNPs on the combined CardioMetabo-Immuno chip represent 83,263 independent signals (using the −indep-pairwise function in PLINK 1.07, with default settings). Bonferroni correction for this number of SNPs was considered, but was deemed to be overly conservative, given that the SNPs genotyped on the CardioMetabochip and Immuno chips [25,26]were selected because they reside in loci previously associated with cardiovascular, metabolic, immune and inflammatory traits. Therefore, p value $<1 \times 10^{-5}$ was chosen a priori to indicate significance threshold, which is an approach used to analyze quantitative traits [30,31].

Conditional analyses included the lead SNP at each locus as an additional covariate, to assess independence of the signals. Mendelian randomization was used to assess whether IL-8 levels had a causal effect on measures of c-IMT. IL-8 lead SNPs on chromosome 8 and chromosome 16, were combined in a genetic score, by summing the number of IL-8-increasing alleles. The IL-8 genetic score was first tested for association with log transformed c-IMT measures by linear regression and then used as an instrument to assess the impact of IL-8 on c-IMT measures using the two stages least squares method (2SLS). The validity of the IL-8 genetic score as endogenous variable was tested by Durbin score.

Lead SNPs were tested for association with IL-8 in the MDC—CC and PIVUS studies and with c-$IMT_{mean\text{-}max}$ in the PIVUS and UK-Biobank, using the same analytical strategy described above for the IMPROVE study with a pre-specified p value (p<0.05). A meta-analysis of the results was performed using a random effect model. Results are expressed as pooled estimated effect (β) and 95%CI. As for the discovery cohort, the IL-8 lead SNPs on chromosome 8 and chromosome 16, were combined in a genetic score, in the UK Biobank. The polygenic score was assessed for impact on c-$IMT_{mean\text{-}max}$ using multivariate regression models adjusting for age, sex, population structure (MDS1−8) and genotyping chip, hypertension, diabetes, smoking and BMI. Derivation of these variables has been described previously [22].

Genetic association analyses were performed using Plink v 1.07 [27]. Statistical analyses were performed using STATA v 15 (Stata Corp College Station, TX, USA).

Data mining

To investigate whether the SNPs of interest have been implicated in any other relevant phenotypes as well as if other genes had been associated with circulating IL-8 levels, we used multiple publicly available databases: the LDtrait [32] (https://ldlink.nci.nih.gov/?tab=ldtrait) from the GWAS catalog (https://www.ebi.ac.uk/gwas/), the open GWAS project (https://gwas.mrcieu.ac.uk/, accessed 20212209) and the PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/, accessed 20211511). We also explored the possible genotype-specific gene expression patterns using the GTEx dataset (https://www.gtexportal.org/home/, accessed 20211207) in the arterial wall.

Data sharing statement. Legal obligations prevent us from sharing individual level data for the IMPROVE cohort. Summary statistics are upon available upon reasonable request if it complies with the current ethical regulations.

Role of the funding source: The study was supported by the Strategic Research Area in Epidemiology (SFO) at KI, Apotekare Hedbergs fond and ALF (FoUI 2021 960053) (to BG). IMV was supported by the Sistema Nacional de Investigación (SNI, SENACYT, Panama). AL was supported by the Swedish Medical Research Council (#2016-01653) and the Heart-Lung Foundation (#2018-0315). Representatives for these funding sources were not involved in the design of the study, collection, analysis and interpretation of data, manuscript draft, or decision to submit the article for publication. The UK Biobank was established by the Wellcome Trust, Medical Research Council, Department of Health, Scottish Government and Northwest Regional Development Agency. UK Biobank has also had funding from the Welsh Assembly Government and the British Heart Foundation. Data collection was funded by UK Biobank. This project was completed using UK Biobank applications 6533 (PI. DJS) and 1755 (PI. JPP). RJS is supported by a University of Glasgow LKAS fellowship and a UKRI Innovation-HDR-UK Fellowship (MR/S003061/1).

Results

Baseline characteristics of the IMPROVE study participants are reported according to the IL-8 and GRO-α quartiles and summarized in Table 1 and Supplemental Table II, respectively. Study participants with high IL-8 and GRO-α levels in plasma were older, had a higher burden of CV risk factors and lived at higher latitudes. On average, c-IMT values at baseline were higher in the presence of high IL-8 and GRO-α levels.

Table 1
Baseline characteristics of the IMPROVE study participants included in the present study according to quartiles of IL-8 levels.

	Q1 (*N* = 841)	Q2 (*N* = 842)	Q3 (*N* = 842)	Q4 (*N* = 842)
Men/Women	370/471	421/421	402/440	431/411
Age (years)	63·17 (58·97 – 66·89)	64·47 (59·70- 66·91)	65.23 (59.71–67·44)	65·83 (60·34- 67·93)
Latitude (°)	45 (45–53)	53 (45–59)	59 (48–62)	59 (52–63)
BMI (kg/m^2)	26·00 (23·63- 28·39)	26·83 (24·24- 29·26)	27·09 (24·39- 30·02)	27·40(25·05- 30·31)
Carotid Ultrasound Measures				
c-IMT$_{mean}$	0·81 (0·71–0·94)	0·84 (0·73–0·99)	0·85 (0·75–1·00)	0·87 (0·76–1·02)
c-IMT$_{max}$	1·67 (1·33–2·22)	1·84 (1·39–2·50)	1·93 (1·45–2·51)	2·03 (1·48–2·61)
c-IMT$_{mean-max}$	1·13 (0·99–1·30)	1·18 (1·02–1·39)	1·21 (1·04–1·44)	1·24 (1·06–1·46)
Risk Factors, n (%)				
Hypertension	587 (70)	655 (77)	694 (82)	728 (86)
Diabetes	142 (17)	198 (23)	238 (28)	300 (35)
Smoke	113 (13)	111 (13)	137 (16)	134 (16)
Biochemical Measurements				
LDL (mmol/L)	3·71 (3·05–4·40)	3·45 (2·84–4·19)	3·49 (2·78–4·15)	3·37 (2·69–4·13)
HDL (mmol/L)	1·21 (1·02–1·48)	1·21 (1·02–1·47)	1·20 (1·00–1·48)	1·18 (0·98–1·43)
CRP (mg/L)	1·71 (0·71–3·40)	1·77 (0·69–3·44)	1·85 (0·82–3·64)	2·10 (0·9–3·85)
Pharmacological treatment, n (%)				
Statin	119 (14)	114 (13)	152 (18)	181 (21)
Antiplatelet	346 (41)	375 (44)	337 (40)	298 (35)

Data are presented as median and IQR for continuous variables and as number for binary variables. LDL: LDL-cholesterol; HDL: HDL-cholesterol. Missing values: c-IMT$_{mean}$, *n* = 2; c-IMT$_{max}$, *n* = 2; LDL, *n* = 66; HDL, *n* = 6; CRP, *n* = 2; diabetes, *n* = 54.

Chemokines and c-IMT at baseline

The associations of plasma IL-8 and GRO-α in c-IMT at baseline in the IMPROVE study are reported in Table 2 and Supplemental Table III, respectively.

Each unit increase in IL-8 plasma level was associated with an increase in the median c-IMT$_{mean}$, c-IMT$_{max}$ and c-IMT$_{mean-max}$ values. This association was consistently observed for all the carotid ultrasound measures after adjustments for the common CV risk factors and treatment with statins and anti-platelet agents (Table 2).

The levels of GRO-α, were positively associated with c-IMT$_{mean}$ and c-IMT$_{mean-max}$ values at baseline. However, the association did not retain statistical significance after adjustment for latitude (Supplemental Table III), therefore the association of GRO-α with c-IMT was not analyzed further.

Supplemental Table IV summarizes the results of the association of IL-8 with c-IMT measures in the PIVUS and MDC—CC studies. IL-8 was associated with an increase in the median c-IMT$_{max}$ in the PIVUS and with c-IMT$_{mean}$ in the MDC—CC study.

Genetic determinants of plasma IL-8

Analysis of the combined CardioMetabo-Immuno chip (crude model) for association with IL-8 levels identified two loci, on chromosome 8 and chromosome 16 (Fig. 1). Further adjustment had negligible effects on either locus (Supplemental Table V). These SNPs were previously reported in secondary analyses in the IMPROVE as associated with IL-8 but not investigated further [24] (please see Data mining section and Supplemental Table VIII and IX)

The chromosome 8 locus includes one significant SNP, rs117518778, which has little or no linkage disequilibrium (LD) with surrounding SNPs (Fig. 2, Panel A and Supplemental Table V). The minor (effect) allele (EA, G) of rs117518778 was associated with higher IL-8 levels (EA frequency 0·02, β:0·079, SE 0·017, $p = 2{\cdot}80\times10^{-6}$) in the fully adjusted model.

In contrast, the chromosome 16 locus (Fig. 2, Panel B and Supplemental Table V) contains 14 SNPs passing the significance threshold. For the lead SNP, rs8057084, the EA (T) was associated with significantly lower IL-8 levels (EA frequency 0·37, β: −0·023, SE 0·005, $p = 5{\cdot}72\times10^{-6}$). Similarly, in the PIVUS study rs8057084 was associated with lower IL-8 levels (EA frequency 0·37, β: −0·005, SE 0·0015, $p = 8{\cdot}30\times10^{-4}$).

A genetic variant at the duffy antigen receptor for chemokines (DARC), previously associated with serum but not plasma IL-8 [33], was not associated with IL8 levels in the IMPROVE (Supplemental Table VI). Similarly, SNPs in the IL-8 gene locus were not associated with plasma IL-8 in the IMPROVE (Supplemental Table VII).

Conditional analysis

The chromosome 16 locus is particularly interesting, as this region was associated with baseline c-IMT measures and risk of CV disease in the IMPROVE [28]. Specifically, the EA of rs4888378 (A) at the *BCAR1*(breast cancer antiestrogen resistance 1)-*CFDP1 (*Craniofacial Development Protein 1)-*TMEM170A* (transmembrane protein 170a) locus was associated with lower c-IMT baseline measures and reduced risk of CHD [28]. The lead IL-8-associated SNP on chromosome 16 (rs8057084) and the lead c-IMT-associated SNP (rs4888378) are 161 kb apart but in moderate LD (r^2=0·72).

Table 2
Association between unit increase of plasma IL8 and c-IMT ultrasound measures in the IMPROVE study.

	Crude			Model 1		
	N	β (SE)	p	N	β (SE)	p
c-IMT$_{mean}$	3365	$1{\cdot}13\times10^{-4}$ ($4{\cdot}1\times10^{-5}$)	0·006	3246	$8{\cdot}77\times10^{-5}$ (4.2×10^{-5})	0·035
c-IMT$_{max}$	3365	$6{\cdot}61\times10^{-4}$ ($2{\cdot}1\times10^{-4}$)	0·002	3246	$6{\cdot}91\times10^{-4}$ (2.1×10^{-4})	0·001
c-IMT$_{mean-max}$	3364	$1{\cdot}84\times10^{-4}$ ($6{\cdot}2\times10^{-5}$)	0·003	3245	$1{\cdot}72\times10^{-4}$ (6.3×10^{-5})	0·007

Crude: adjusted by age, sex, latitude. Model 1: crude + body mass index, hypertension, diabetes, current smokers and LDL-cholesterol, treatment with statins and antiplatelet agents. For each model the total number of study participants, the coefficient of association and the p-value are reported. Missing values as in Table 1.

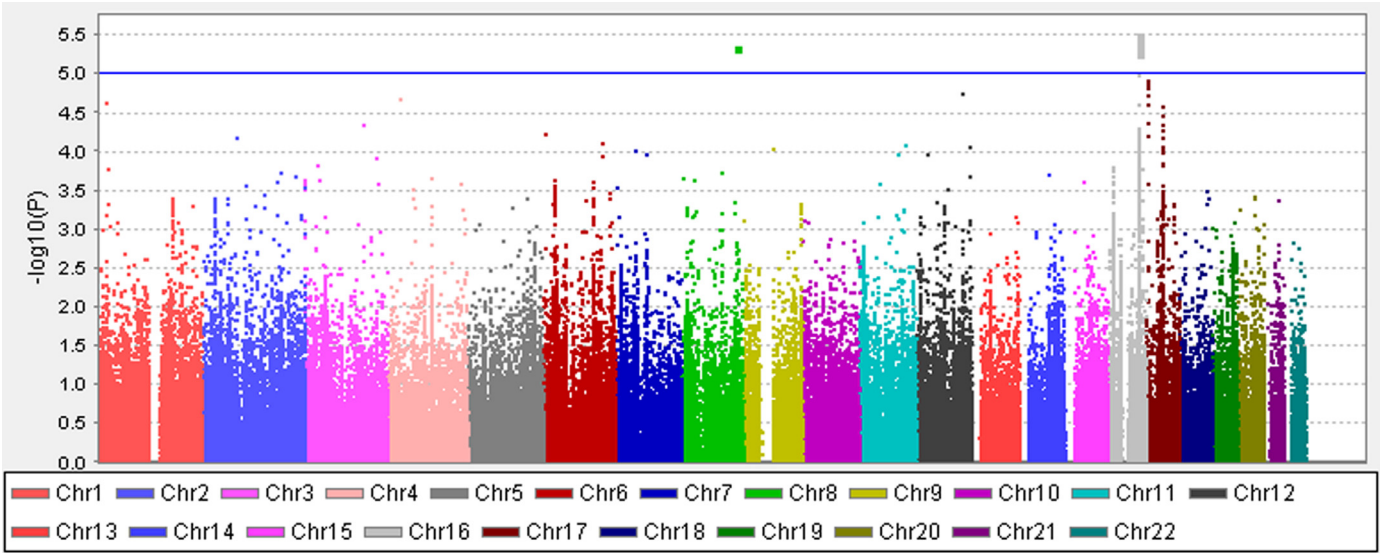

Fig. 1. Association of CardioMetabo-Immuno chip SNPs with IL8 levels in plasma (adjusted for age, sex, MDS1−3). Two loci meet the threshold for significance ($p<1 \times 10^{-5}$), indicated by the blue horizontal line.

To test the hypothesis that these two SNPs represent the same genetic signal, we analyzed the association of the lead c-IMT$_{mean\text{-}max}$ SNP, rs4888378, with circulating IL-8 levels and the association of the IL-8 lead SNP, rs8057084, with c-IMT$_{mean\text{-}max}$ in the IMPROVE study.

As shown in Table 3, the EA of rs4888378, the c-IMT lead SNP, associated with lower c-IMT$_{mean\text{-}max}$ values and lower levels of IL-8 (β −0·018, SE 0·005, $p = 4{\cdot}07\times10^{-4}$), but the association did not meet the threshold for statistical significance ($p<1 \times 10^{-5}$). Similarly, the EA of rs8057084, the lead IL-8 SNP, was associated with a lower c-IMT$_{mean\text{-}max}$. Finally, to confirm that these two SNPs represent the same genetic signal, the association of rs8057084 with IL-8 levels was additionally adjusted (IL-8 conditional in Table 3) for rs4888378, and the association of rs4888378 with c-IMT$_{mean\text{-}max}$ was additionally adjusted for rs8057084 (c-IMT$_{mean\text{-}max}$ conditional in Table 3). If the two SNPs are independent, then adjusting each analysis for the other lead SNP should not alter the association. As expected from the degree of LD between rs4888378 and rs8057084, both signals demonstrated weaker, but still, nominally significant associations with their respective traits (Table 3), suggesting that these two SNPs might represent the same genetic association.

Instrumental variable analysis

Supplemental Figure II illustrates the distribution of plasma IL-8 levels and c-IMT$_{mean\text{-}max}$ measures according to the IL-8-increasing genetic score, in the IMPROVE. Consistent with the effects of increased IL-8 levels on c-IMT, an IL-8-increasing genetic score was associated with increased c-IMT measures (Table 4). The IL-8 genetic score was then used as an instrumental variable to confirm the causality of the association of IL-8 with c-IMT measures using the 2SLS analysis (Table 4). Fig. 3

Replication of the association of the IL-8 lead SNP with c-IMT$_{mean\text{-}max}$

The IL-8 lead SNP (rs8057084) was tested for association with c-IMT$_{mean\text{-}max}$ in the PIVUS and UK-Biobank studies (MDC—CC was not included here as it lacks the phenotyping of c-IMT$_{mean\text{-}max}$). Consistent with our findings in IMPROVE, the EA was associated with lower c-IMT$_{mean\text{-}max}$ in both UK Biobank (β: −0·0050, SE 0·001, $p = 8{\cdot}3 \times 10^{-4}$) and (non-significantly) PIVUS (β: −0·0113, SE 0·010, $p = 0{\cdot}25$). A meta-analysis of the association of the IL-8 lead SNP on chromosome 16 with c-IMT$_{mean\text{-}max}$, including also the results obtained in the IMPROVE, showed a pooled estimate β of −0·006 with (95%CI) (−0·008 - −0·003), $p = 0{\cdot}0001$, as summarized in Fig. 4.

When the IL-8 increasing alleles on chromosome 8 and 16 were combined in a genetic score in the UK Biobank, the IL-8 increasing genetic score associated with c-IMT in the UK Biobank (β: 0·00,518, SE: 0·00,157, $p = 0{\cdot}001$) in the direction observed in the IMPROVE in a multivariable adjusted model (Supplemental Figure III).

Data mining

We have screened several on-line data repositories to interpreter our data in the context of all available information. Rs4888378 has been reported to have effect on SBP, anti-hypertensive medication use and coronary artery disease (GWAS catalog, PhenoScanner, Supplemental Table VIII); rs8057084 on platelet crit and platelet count for (GWAS catalog, Supplemental Table VIII). Additional associations were identified using the open GWAS project (Supplemental Table IX), including the association with lipid biomarkers. However, many of these should be interpreted with caution, as not all results are peer-reviewed/published and some use non-standard methods with limited data cleaning.

We have also explored genotype specific expression patterns at the two loci, on chromosome 8 and on chromosome 16. While no pattern is reported for rs117518778 on chromosome 8, rs8057084 and rs4888378 on chromosome 16 were reported to be associated with changes in the expression of RP11−252K23.2, a long non-coding transcript lying in the *CFDP1* intron and with opposite changes in the expression pattern of BCAR1 in the artery wall (Supplemental Table X).

In addition, while no association between *IL-8* and circulating IL-8 levels was observed in the IMPROVE, this association has been shown in other populations included in a recently performed meta-analysis [34]. At the same time, SNPs located at multiple loci have been identified as associated with IL-8 in independent cohorts [35−37].

Discussion

This is the first study analyzing the association of IL-8, a chemokine that participates in the inflammatory response in atherosclerosis [2,38], with c-IMT, a measure of vascular wall remodeling indicative

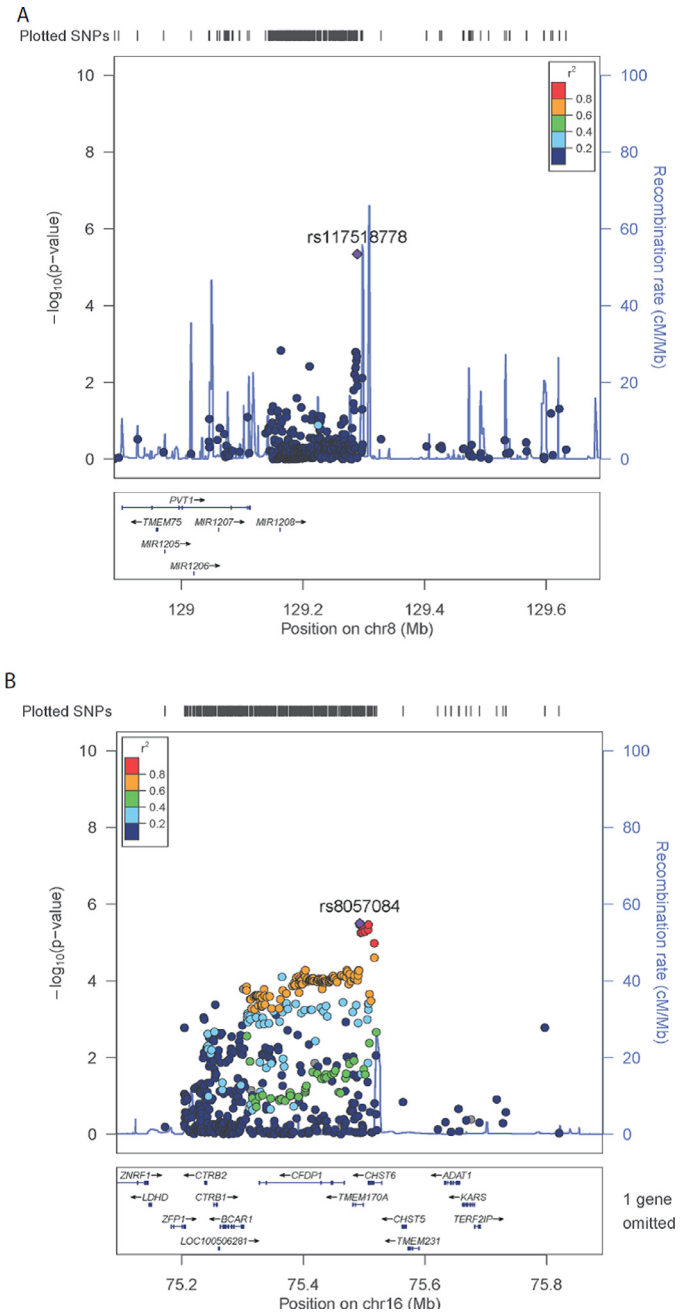

Fig. 2. Regional plots of IL-8-associated loci. A) Chromosome 8. The locus consists of a single significant SNP, rs117518778; B) Chromosome 16. The locus consists of 78 SNPs in linkage disequilibrium with the lead SNP, rs8057084. Threshold for significance was defined as $p<1 \times 10^{-5}$ or $-\log10\ p > 5{\cdot}0$.

of sub-clinical atherosclerosis. Our results indicate that IL-8 associates with changes in c-IMT, but GRO-α, a chemokine that shares structural and functional properties with IL-8, does not.

We identified two loci associated with plasma IL-8 levels and c-IMT. The signal on chromosome 8 maps to a locus with no prior associations reported with c-IMT. In contrast, the signal on chromosome 16 maps to the *BCAR1-CFDP1-TMEM170A* locus, previously identified as a genetic determinant of c-IMT in the IMPROVE [28]. Both the c-IMT and the IL-8 lead SNPs at this locus were associated with both lower levels of circulating IL-8 and thinner c-IMT.

Both lead SNPs seem to regulate the expression of *BCAR1-CFDP1-TMEM170A* with both effect alleles associated with a lower expression of BCRA1 and higher expression of a novel intron at CFDP1 according to the data from the GTex dataset as well as published data [39]. In particular, it has been shown that rs4888378 regulates the activity of an enhancer acting on different promoters possibly mediating a reduced expression level of BRCA1 in the presence of the effect allele A. Experiments performed in an experimental model of carotid artery ligation indicate that down regulation of BCAR1 expression is associated with a reduced migration of vascular smooth muscle cells after PDGF-BB stimulation and with a reduced neointima formation after carotid artery ligation [40]. Notably, the association of the *BCAR1-CFDP1-TMEM170A* locus with c-IMT has been confirmed in a large meta-analysis [4]. In the present study, we have identified a SNP, rs8057084, mapping at the same locus on chromosome 16 and associated with plasma IL-8 levels. The functional relation between BCAR1 and IL-8 has been previously reported [41]. In particular, the signaling pathway leading to the IL-8-mediated chemotaxis of neutrophils through the CXCR1, the IL-8 receptor not shared by GRO-α, is dependent by the phosphorylation of proline-rich tyrosine kinase 2 (Pyk2), a kinase able to interact with BCAR1 [42]. Taken together these results suggest that the *BCAR1-CFDP1-TMEM170A* locus exerts a functional role in regulating c-IMT. This regulation can occur through the reduction of the proliferation of vascular smooth cells and the inhibition of the IL-8-mediated migration of neutrophils in the early stages of atherosclerosis. Thus, this effect seems to be specific for IL-8 since GRO-α does not bind CXCR1.

To assess the causality of IL-8 on subclinical atherosclerosis, we have used a mendelian randomization approach, created a genetic score as instrumental variable and tested the association of the IL-8 genetic score with c-IMT in the IMPROVE and in the large UK Biobank. The instrumental variable analysis using the IL-8 genetic score supports a causal association of IL-8 on c-IMT. We have interrogated the GTex portal and we did not find any indication of a trans effect of the chromosome 16 and 8 loci. Whilst the bulk of our data, support a causal role for IL-8 on c-IMT, we cannot exclude genes located at the *IL-8* locus or at other loci may contribute to the regulation of IL-8 circulating levels and that the SNPs we report may be involved in atherosclerosis through pathways that are independent from IL-8. To rule out horizontal pleiotropy, we assessed possible pleiotropic

Table 3
Association of lead IL-8 and c-IMT $_{\text{mean-max}}$ SNPs with plasma IL-8 levels and c-IMT$_{\text{mean-max}}$ and conditional analysis.

Lead SNP		Phenotype tested						Conditional analysis					
		IL-8 levels			c-IMT$_{\text{mean-max}}$			IL-8 conditional			c-IMT$_{\text{mean-meax}}$ conditional		
	EA	β	SE	p	β	SE	p	β	SE	p	B	SE	p
rs8057084 (IL-8)	T	−0·024	0·005	$3{\cdot}17\times10^{-6}$	−0·009	0·004	$6{\cdot}97\times10^{-5}$	−0·031	0·009	0·0017	–	–	–
rs4888378 (c-IMT)	A	−0·018	0·005	$4{\cdot}07\times10^{-4}$	−0·010	0·002	$6{\cdot}46\times10^{-6}$	–	–	–	−0·009	−0·004	0·0322

EA: Effect allele; IL-8: association of the two lead SNPs associated with IL8 (rs8057084) and c-IMT $_{\text{mean max}}$ (rs4888378) with IL8 adjusted for age, sex MSD1−3. c-IMT$_{\text{mean-max}}$: association of the two lead SNPs with c-IMT$_{\text{mean-max}}$ adjusted for age, sex MSD1−3; IL-8 conditional: association of the IL8 lead SNP (rs8057084) with circulating IL8 levels adjusted for age, sex MSD1−3 and rs4888378; c- IMT $_{\text{mean-max}}$: conditional association of the c-IMT mean max lead SNP (rs4888378) with c-IMT adjusted for age, sex MSD1−3 and rs8057084. Of note, the results presented here for chromosome 16 association with c-IMT$_{\text{mean-max}}$ differ from those presented by Gertow et al. [28], in that the previous report was based on the CardioMetabochip only, whereas here is the CardioMetabo-Immuno chip chromosome 16 region.

Table 4
Instrumental analysis: IL-8 genetic score as instrument to assess the causality of the association between plasma IL-8 levels and c-IMT measures.

	Linear regression IL-8 genetic score and c-IMT			2SLS IL-8 genetic score as instrumental variable						
	β	SE	p	β	SE	p	Endogeneity score	Endogeneity p	Partial R2	Eigenvalue
c-IMT$_{-mean}$	0·009	0·002	<0·0001	0·316	0·089	<0·0001	18·21	<0·0001	0·01	33·39
c-IMT$_{-max}$	0·017	0·004	<0·0001	0·621	0·170	<0·0001	18·93	<0·0001	0·01	33·39
c-IMT$_{-mean-max}$	0·010	0·002	<0·0001	0·344	0·096	<0·0001	19·03	<0·0001	0·01	33·39

Linear regression analysis shows the association of the IL-8 genetic score with c-IMT measures. Two stage least square analysis (2SLS) shows the association of IL-8 values with c-IMT using the IL-8 genetic score as an instrumental variable. Estimates are expressed as □ coefficients and SE and adjusted by age, sex and MDS 1−3. The assumption of endogeneity was tested by the Durbin score which confirmed that IL-8 genetic score and IL-8 can be considered endogenous variables in the analysis. Partial R square estimates the correlation between IL-8 and IL-8 SNP score after are age, sex and MDS 1−3 are partied out. The eigenvalue statistic rejected the hypothesis the instruments are weak (threshold value for rejection a = 0·05, 16·36).

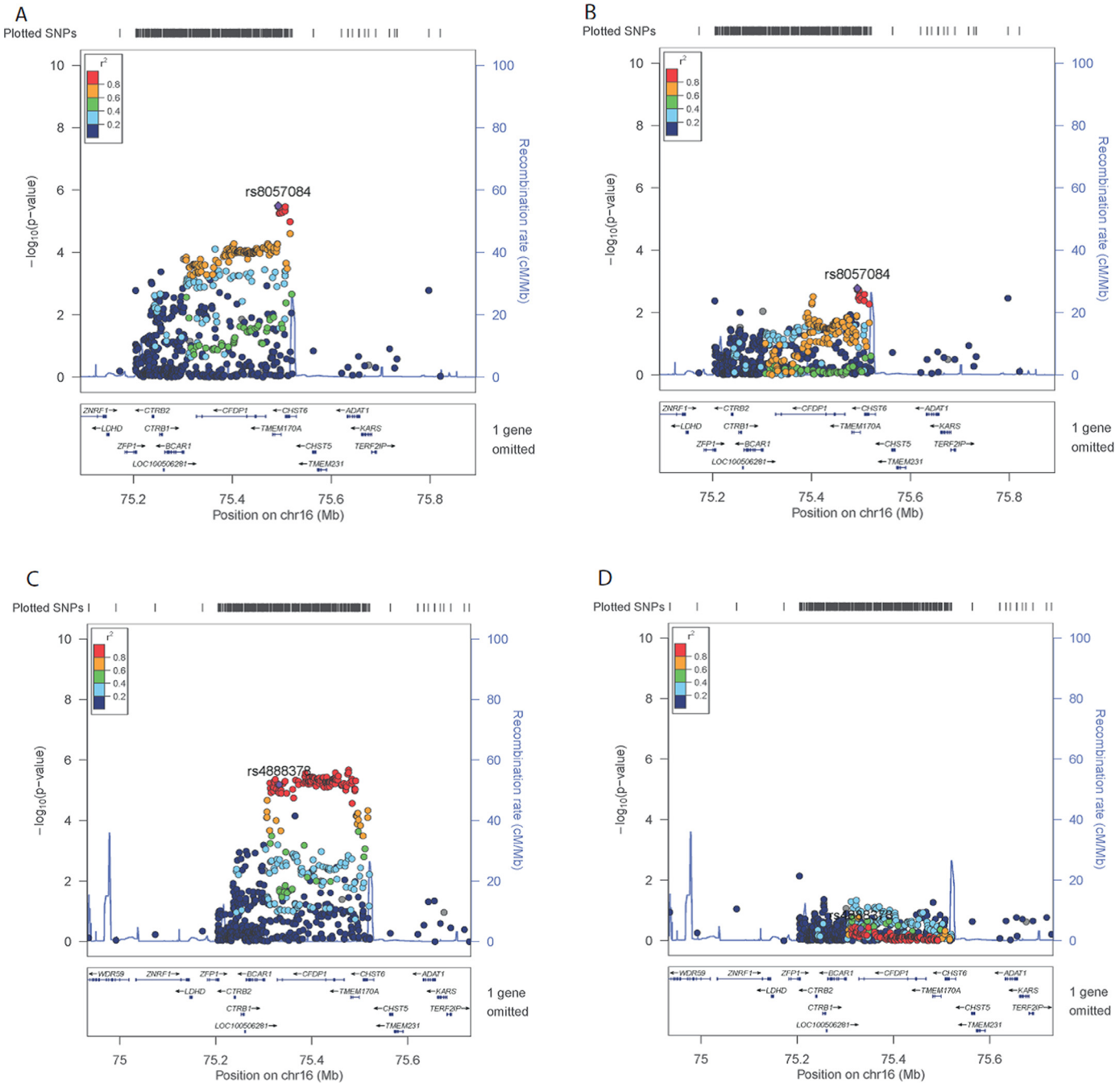

Fig. 3. Chromosome 16 locus associated with circulating IL-8 levels (lead SNP rs8057084) and with c-IMT (lead SNP rs4888378). Regional association plots demonstrating the association between rs8057084 and IL-8 without (A) and with (B) adjustment for rs4888378 and between IMT $_{mean-max}$ and rs4888378 without (C) and with (D) adjustment for rs8057084. Threshold for significance was defined as $p<1 \times 10^{-5}$ or −log10 p >5·0.

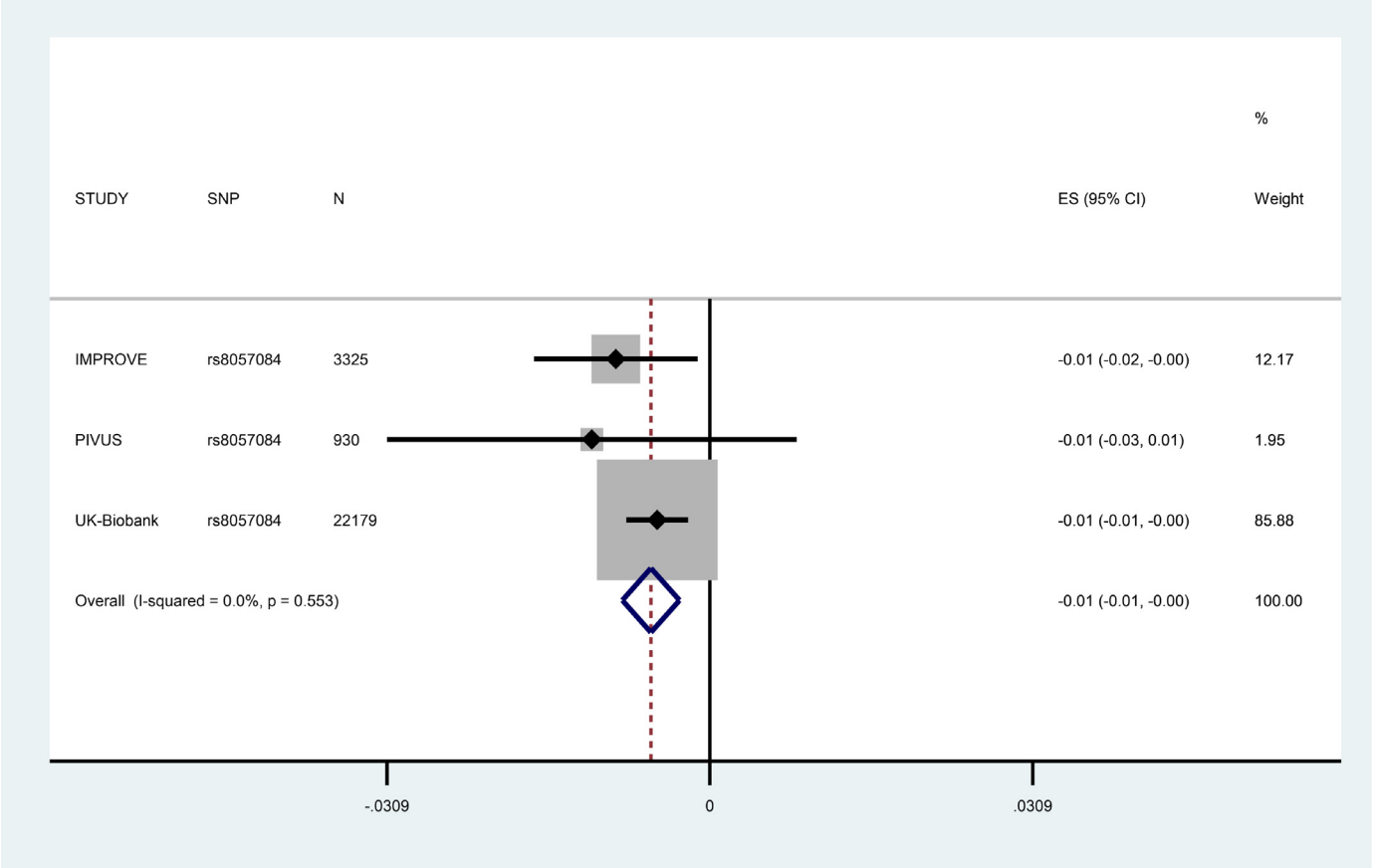

Fig. 4. Meta-analysis of the association between the lead IL-8 SNP on chromosome 16 identified in the IMPROVE study and c-IMT$_{mean-max}$ in the IMPROVE, PIVUS and UK Biobank studies.

associations of the loci by searching multiple databases. Data mining of the available data repositories indicate that the lead IL-8 and c-IMT SNPs may contribute to the regulation of blood pressure, lipid metabolism and to the risk of coronary heart disease. Hence, our data have to be interpreted with caution and warrant further investigation.

An intrinsic limitation of our approach is that the SNPs we have identified do not map to the IL8-locus. This may challenge one assumption underlying mendelian randomization, that the SNP has direct effects on the phenotype measured (IL-8), since we cannot exclude the possibility that these loci indirectly influence circulating IL-8 levels through the regulation of a transcription factor or other chemokines/mechanisms.

The association of one SNP, rs6836038, at the *IL-8* locus with circulating IL8 levels has been observed in some studies (e.g. STABILITY n = 2967: ORCADES n = 971; INTERVAL n = 4987) of the 13 cohorts of European ancestry participating in the SCALLOP to which IMPROVE contributed [34] and other studies have identified several SNPs associated with IL-8 [35–37]. We do not have evidence of such associations in the IMPROVE. Similarly, the association of genetic variants with c-IMT differs across studies. Three large meta-analyses [43–45] have reported several SNPs on chromosome 8 associated with c-IMT, none of these were in LD with the SNP reported in the present study (all r^2<0·01). On the other hand, the lead IL-8 SNP on chromosome 16 is in moderate LD (r^2=0·73) with SNPs recently associated with c-IMT [45] as formerly reported in the IMPROVE [28]. Both loci identified in the presented study were not found to be associated at genome-wide significance level either with c-IMT in large GWAS meta-analyses [43] and in the UK Biobank GWAS of c-IMT [22]. In summary, the regulation of both IL-8 circulating levels and c-IMT may be omnigenic, i.e. dependent on the concerted action of a network of genetic variants within and outside the *IL-8* locus [46]. Differences in the selection of study participants (e.g. IMPROVE participants were selected according to a high CV risk while participants in the INTERVAL study where healthy blood donors [34]), in the methods used to measure circulating levels of IL-8, in imputation strategies and indirect genetic effects secondary to differences in environmental exposure may contribute to explain these controversial results.

Several additional limitations also need to be acknowledged. The lead SNPs did not meet the threshold for genome-wide significance established by the conservative Bonferroni correction in the IMPROVE; however, as that assumes 1 million independent tests, it is overly conservative in this setting. In addition, the use of a large-scale candidate locus approach means that there is prior probability for immunological and cardiometabolic traits, therefore our use of a suggestive *a priori* threshold to define significance in IMPROVE was appropriate. Genetic instrumental variables are robust, but typically demonstrate modest effect sizes, as observed here. An additional limitation is the non-standard methods for measuring c-IMT. We acknowledge that the differences in measurements might influence the findings, however the results in the different datasets are convergent, with gives us confidence in the comparability of the findings. In addition, different c-IMT measures have previously been combined in meta-analyses [28,43], with some results being externally validated in UK Biobank [22]. Differences in c-IMT measures may dilute the strength of the association by increasing the heterogeneity of the trait, possibly leading to an underestimation more than to an overestimation of the observed association. Finally, the method used to measure circulating IL-8 does not discriminate free IL-8 from IL-8 eventually bound to microvesicles derived from endothelial or

circulating cells and does not differentiate IL-8 levels according to the cellular source.

In conclusion, our study indicates that (1) plasma levels of IL-8 were associated with subclinical atherosclerosis assessed by c-IMT and (2) that the EA at the lead IL-8 SNP associated with both a thinner c-IMT lower IL-8 circulating levels, i.e. with an atheroprotective phenotype. However, we cannot infer causality of this association, as these variants lie outside of the *IL-8* locus and multiple loci have been associated with circulating levels of IL-8 and c-IMT in different populations. We can only warrant further observational and mechanistic studies to address the question of a possible causality behind the observed association.

Contributors

IMV, RJS, BG: literature search, study design, data analysis and interpretation, drafting of the manuscript. AM: data collection, data analysis and interpretation. DB, ET, FV, KL, PE, AS, SHE, SK, AL, AJS, PG, MP: study design, data collection and interpretation. YB, GE, LL, NO: data analysis and interpretation

Declaration of Competing Interest

None

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.retram.2022.103374.

References

[1] Rolin J, Maghazachi AA. Implications of chemokines, chemokine receptors, and inflammatory lipids in atherosclerosis. J Leukoc Biol 2014;95:575–85.
[2] Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. Circulation 2010;122:1837–45.
[3] Apostolopoulos J, Davenport P, Tipping PG. Interleukin-8 production by macrophages from atheromatous plaques. Arterioscler Thromb Vasc Biol 1996;16:1007–12.
[4] Yue TL, et al. Interleukin-8. A mitogen and chemoattractant for vascular smooth muscle cells. Circ Res 1994;75:1–7.
[5] Marino F, et al. Production of IL-8, VEGF and elastase by circulating and intraplaque neutrophils in patients with carotid atherosclerosis. PLoS ONE 2015;10:e0124565.
[6] Papadopoulou C, Corrigall V, Taylor PR, Poston RN. The role of the chemokines MCP-1, GRO-alpha, IL-8 and their receptors in the adhesion of monocytic cells to human atherosclerotic plaques. Cytokine 2008;43:181–6.
[7] Joseph JP, et al. CXCR2 Inhibition - a novel approach to treating coronary heart disease (CICADA): study protocol for a randomised controlled trial. Trials 2017;18:473.
[8] Yang H, et al. The effects of ultrasound-targeted microbubble destruction (UTMD) carrying IL-8 monoclonal antibody on the inflammatory responses and stability of atherosclerotic plaques. Biomed Pharmacother 2019;118:109161.
[9] Boekholdt SM, et al. IL-8 plasma concentrations and the risk of future coronary artery disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. Arterioscler Thromb Vasc Biol 2004;24:1503–8.
[10] Herder C, et al. Chemokines and incident coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. Arterioscler Thromb Vasc Biol 2006;26:2147–52.
[11] Moreno Velasquez I, et al. Interleukin-8 is associated with increased total mortality in women but not in men-findings from a community-based cohort of elderly. Ann Med 2015;47:28–33.
[12] Moreno Velasquez I, et al. Serum IL8 is not associated with cardiovascular events but with all-cause mortality. BMC Cardiovasc Disord 2019;19:34.
[13] Rot A. Chemokine patterning by glycosaminoglycans and interceptors. Front Biosci (Landmark Ed) 2010;15:645–60.
[14] Hillyer P, Mordelet E, Flynn G, Male D. Chemokines, chemokine receptors and adhesion molecules on different human endothelia: discriminating the tissue-specific functions that affect leucocyte migration. Clin Exp Immunol 2003;134:431–41.
[15] Baldassarre D, et al. Measurements of carotid intima-media thickness and of interadventitia common carotid diameter improve prediction of cardiovascular events: results of the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) study. J Am Coll Cardiol 2012;60:1489–99.
[16] Baldassarre D, et al. Cross-sectional analysis of baseline data to identify the major determinants of carotid intima-media thickness in a European population: the IMPROVE study. Eur Heart J 2010;31:614–22.
[17] Berglund G, Elmstahl S, Janzon L, Larsson SA. The malmo diet and cancer study. Design and feasibility. J Intern Med 1993;233:45–51.
[18] Lind L, Fors N, Hall J, Marttala K, Stenborg A. A comparison of three different methods to evaluate endothelium-dependent vasodilation in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Arterioscler Thromb Vasc Biol 2005;25:2368–75.
[19] Bycroft C, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature 2018;562:203–9.
[20] Rodriguez-Macias KA, Lind L, Naessen T. Thicker carotid intima layer and thinner media layer in subjects with cardiovascular diseases. An investigation using non-invasive high-frequency ultrasound. Atherosclerosis 2006;189:393–400.
[21] Rosvall M, et al. Occupational status, educational level, and the prevalence of carotid atherosclerosis in a general population sample of middle-aged Swedish men and women: results from the Malmo Diet and Cancer Study. Am J Epidemiol 2000;152:334–46.
[22] Strawbridge RJ, et al. Carotid intima-media thickness: novel loci, sex-specific effects, and genetic correlations with obesity and glucometabolic traits in UK biobank. Arterioscler Thromb Vasc Biol 2020;40:446–61.
[23] Assarsson E, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS ONE 2014;9:e95192.
[24] Folkersen L, et al. Mapping of 79 loci for 83 plasma protein biomarkers in cardiovascular disease. PLoS Genet 2017;13:e1006706.
[25] Voight BF, et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. PLoS Genet 2012;8:e1002793.
[26] Trynka G, et al. Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. Nat Genet 2011;43:1193–201.
[27] Purcell S, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–75.
[28] Gertow K, et al. Identification of the BCAR1-CFDP1-TMEM170A locus as a determinant of carotid intima-media thickness and coronary artery disease risk. Circ Cardiovasc Genet 2012;5:656–65.
[29] Baldassarre D, et al. Progression of carotid intima-media thickness as predictor of vascular events: results from the IMPROVE study. Arterioscler Thromb Vasc Biol 2013;33:2273–9.
[30] Bonomi A, et al. Analysis of the genetic variants associated with circulating levels of sgp130. Results from the IMPROVE study. Genes Immun 2020;21:100–8.
[31] Cai DC, et al. A genome-wide search for quantitative trait loci affecting the cortical surface area and thickness of Heschl's gyrus. Genes Brain Behav 2014;13:675–85.
[32] Lin SH, Brown DW, Machiela MJ. LDtrait: an Online tool for identifying published phenotype associations in linkage disequilibrium. Cancer Res 2020;80:3443–6.
[33] Moreno Velasquez I, et al. Duffy antigen receptor genetic variant and the association with Interleukin 8 levels. Cytokine 2015;72:178–84.
[34] Folkersen L, et al. Genomic and drug target evaluation of 90 cardiovascular proteins in 30,931 individuals. Nat Metab 2020;2:1135–48.
[35] Ahola-Olli AV, et al. Genome-wide association study identifies 27 loci influencing concentrations of circulating cytokines and growth factors. Am J Hum Genet 2017;100:40–50.
[36] Kim DK, et al. Genome-wide association analysis of blood biomarkers in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012;186:1238–47.
[37] Melzer D, et al. A genome-wide association study identifies protein quantitative trait loci (pQTLs). PLoS Genet 2008;4:e1000072.
[38] Moreau M, et al. Interleukin-8 mediates downregulation of tissue inhibitor of metalloproteinase-1 expression in cholesterol-loaded human macrophages: relevance to stability of atherosclerotic plaque. Circulation 1999;99:420–6.
[39] Boardman-Pretty F, et al. Functional analysis of a carotid intima-media thickness locus implicates BCAR1 and suggests a causal variant. Circ Cardiovasc Genet 2015;8:696–706.
[40] Chen CH, et al. Cysteine-rich protein 2 alters p130Cas localization and inhibits vascular smooth muscle cell migration. Cardiovasc Res 2013;100:461–71.
[41] Chehade R, et al. Reduced BRCA1 transcript levels in freshly isolated blood leukocytes from BRCA1 mutation carriers is mutation specific. Breast Cancer Res 2016;18:87.
[42] Di Cioccio V, et al. Key role of proline-rich tyrosine kinase 2 in interleukin-8 (CXCL8/IL-8)-mediated human neutrophil chemotaxis. Immunology 2004;111:407–15.
[43] Franceschini N, et al. GWAS and colocalization analyses implicate carotid intima-media thickness and carotid plaque loci in cardiovascular outcomes. Nat Commun 2018;9:5141.
[44] O'Donnell CJ, et al. Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction. Circulation 2011;124:2855–64.
[45] Yeung MW, et al. Twenty-five novel loci for carotid intima-media thickness: a genome-wide association study in >45 000 individuals and meta-analysis of >100 000 individuals. Arterioscler Thromb Vasc Biol 2022;42:484–501.
[46] Boyle EA, Li YI, Pritchard JK. An expanded view of complex traits: from polygenic to omnigenic. Cell 2017;169:1177–86.