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Association of MMP9 with adverse features of plaque progression and residual inflammatory risk in patients with chronic coronary syndrome (CCS)

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ABSTRACT

Background and aims: MMP-9 is a predictor of atherosclerotic plaque instability and adverse cardiovascular events, but longitudinal data on the association between *MMP9* and coronary disease progression are lacking. This study is aimed at investigating whether *MMP9* is associated with atherosclerotic plaque progression and the related molecular basis in stable patients with chronic coronary syndrome (CCS).

Methods: MMP9 serum levels were measured in 157 CCS patients (58 ± 8 years of age; 66% male) undergoing coronary computed tomography angiography at baseline and after a follow up period of 6.5 ± 1.1 years to assess progression of Total, Fibrous, Fibro-fatty, Necrotic Core, and Dense Calcium plaque volumes (PV). Gene expression analysis was evaluated in whole blood using a transcriptomic approach by RNA-seq.

Results: At multivariate analysis, serum *MMP9* was associated with annual change of Total and Necrotic Core PV (Coefficient 3.205, SE 1.321, $P = 0.017$; 1.449, SE 0.690, $P = 0.038$, respectively), while *MMP9* gene expression with Necrotic Core PV (Coefficient 70.559, SE 32.629, $P = 0.034$), independently from traditional cardiovascular risk factors, medications, and presence of obstructive CAD. After transcriptomic analysis, *MMP9* expression was linked to expression of genes involved in the innate immunity.

Conclusions: Among CCS patients, *MMP9* is an independent predictive marker of progression of adverse coronary plaques, possibly reflecting the activity of inflammatory pathways conditioning adverse plaque phenotypes. Thus, blood *MMP9* might be used for the identification of patients with residual risk even with optimal management of classical cardiovascular risk factors who may derive the greatest benefit from targeted anti-inflammatory drugs.

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1. Introduction

Coronary artery disease (CAD) is a leading cause of morbidity and mortality worldwide [1]. Patients with CAD remain at high risk for future cardiovascular events, even with optimal risk factor modification, lipid-lowering drugs and antithrombotic regimens [2]. Precursors of plaque destabilization and evolution into a plaque at a high risk of rupture in the milieu of stable CAD remain unclear [3]. Several inflammatory pathways contribute to progression of the atherosclerotic burden and plaque destabilization, often referred to as residual inflammatory risk [4], and numerous previous studies have suggested the role of vascular remodelling in the development of CAD [5,6]. Clinical studies have evaluated matrix metalloproteinases (MMP) levels and plaque structure and morphology in patients with acute coronary syndrome [7] or in patients with unstable angina [8,9]. However, longitudinal data on the association between *MMP9* and coronary disease progression are lacking. Coronary computed tomography angiography (CTA) is a well-established non-invasive imaging tool with high diagnostic performance for coronary atherosclerosis and predictive value for adverse CV events [10,11]. Therefore, we aimed to examine the association between baseline *MMP9* serum levels and progression of coronary atherosclerosis and plaque features assessed by serial coronary CTA in a population of stable patients with chronic coronary syndrome (CCS) who were already treated using guideline-directed preventative therapies. Moreover, in order to evaluate the molecular mechanisms associated with *MMP9* modifications in patients with CCS, a gene expression study was performed by RNA sequencing using the whole blood samples at follow-up. In particular, we investigated the association between *MMP9* gene expression in whole blood and coronary plaque features. Finally, a correlation analysis was used to evaluate the association between *MMP9* gene expression and other genes known to be involved in the atherosclerotic process.

2. Methods

2.1. Study population

The "Simulation Modeling of coronary ARTery disease: a Tool for clinical decision support" (SMARTool) study was a prospective, international, multicenter observational study in which patients who previously underwent coronary CTA for suspected CAD, as part of the EVINCI (FP7–222915) and of the ARTreat (FP7–224297) clinical studies were prospectively included to undergo clinical, molecular and coronary CTA follow-up. The list of eligibility, inclusion, exclusion and exit criteria of SMARTool Clinical Study were previously reported in detail [12]. Blood samples were collected from patients in a fasting state during the clinical visit before coronary CTA, and aliquots were stored at IFC Biobank. Among the 212 patients whose CTA scan were evaluated by quantitative analysis [12], 157 patients with available information on clinical and molecular profiles, including data on *MMP9* plasma levels, were included in the present study. A subgroup of 152 patients having also available whole blood samples at follow-up was used for gene expression analysis. The study flow chart is reported in Supplementary Material (Fig. S1). The protocol of the SMARTool study was approved by local ethics committees and conformed to the ethical guidelines of the 1975 Declaration of Helsinki, all patients gave their written informed consent to participate, and the procedures followed were in accordance with institutional guidelines.

2.2. Coronary CTA analysis

The methodology for coronary CTA imaging acquisition and analysis has been previously reported in detail [12]. First, a visual, side-by-side analysis of the baseline and follow-up coronary CTAs was performed to assess presence and severity of coronary plaques. Obstructive CAD was defined as presence of lumen stenosis >50% in all segments. Subsequently, quantitative CTA analysis was performed for all visually determined plaques, using a dedicated software package (QAngio CT Research Edition version 3.1.2.0). Baseline and follow-up coronary lesions were matched using fiduciary landmarks (e.g. side branches, distance from the ostium) and analyzed side-by-side. The complete workflow of quantitative CTA analysis has been described in detail previously [12]. For each coronary lesion, total plaque volume (TPV) and plaque volume (PV) according to the plaque composition (Fibrous, Fibrous-Fatty, Necrotic Core, and Dense Calcium) were determined [12].

CAD progression was defined as the absolute increase in plaque volume by quantitative CTA analysis on a per patient basis. Per-patient plaque volume was calculated by summation of the plaque volumes of individual coronary plaques. Total, Necrotic Core, Fibro-fatty, Fibrous plaque, and Dense Calcium PV progression were assessed on a per-patient basis and were adjusted for the time interval between the baseline and follow-up coronary CTA (i.e., the interscan period). Accordingly, the annual PV change was calculated as follows: (PV at follow-up − PV at baseline) / (interscan period).

2.3. Clinical and bio-humoral variables

Information on cardiovascular risk factors, including age, gender, family history of CAD, smoking status, diabetes mellitus, hypertension, obesity, medication use and molecular profiles were collected [13]. *MMP9* plasma levels were evaluated by a dedicated ELISA (Quantikine ELISA kit, R&D Systems). Bio-humoral markers associated with lipid and glucose metabolism, and systemic inflammation were determined at baseline and at follow-up in all patients. Analytical methods and biomarker ranges of normality were previously reported [13–15].

2.4. RNA extraction, sequencing and analysis

Total RNA was extracted from whole blood samples from patients at follow up using MagMax for stabilised Blood Tubes RNA isolation kit (Thermofisher). mRNA profiling analysis was carried out providing sequencing of blood. mRNA Sequencing libraries were prepared from 500 ng of total RNA. First, mRNA selection was performed using oligo (dT) beads. Then mRNA was converted into sequencing library according to the IlluminaTruSeq stranded mRNA protocol. Sequencing was performed on HiSeq2500 platform, using paired end sequencing (2x50bp). Raw data were aligned to the human reference genome hg19/GRCh37 and gene expression levels quantified using STAR and gene models of the Ensembl release 75. Gene expression values were calculated as RPKM (reads per kilobase per million mapped reads) and median-normalized across the cohort to ensure comparability between samples.

2.5. Statistical analysis

Categorical variables are presented as numbers (percentage), continuous variables as mean ± standard deviation (SD). No normally distributed data after Shapiro-Wilk test were Ln transformed.

The comparison between clinical, bio-humoral and coronary CTA data, obtained at the time of the first scan and of the second scan, was performed by the paired *t*-test or McNemar test as appropriate.

Patients were divided in groups according to *MMP9* Tertiles at baseline (Supplementary Table S1) and according to MMP9 gene expression Tertiles in peripheral blood at follow up (Supplementary Table S2). Total, Necrotic Core, Fibro-fatty, Fibrous, and Dense Calcium PV at baseline and follow up, and annual change of PV, were compared across *MMP9* Tertiles using Kruskal-Wallis or χ^2 test as appropriate. Mann-Whitney test was used for post-hoc pairwise comparisons among Tertiles.

Univariate and multivariate linear regression were used to estimate the association between baseline *MMP9* and the annual increase in

plaque volumes. Models were developed starting from the univariate association and then, to account for possible confounding effects, adjusting for clinical variables including, age, sex, risk factors, anti-ischemic (beta blockers and calcium antagonists), anti-hypertensive (ACE inhibitors, ARBs, diuretics), antiplatelets and anti-diabetic drugs, statins, LDL-C, TG/HDL-C ratio, hs-CRP, and Obstructive CAD. Again, univariate and multivariate linear regression were used to estimate the association between *MMP9* gene expression in whole blood and plaque volumes at follow-up. Models were developed starting from the univariate association and then adjusting for possible confounding effects (as previously listed). Analyses were performed using the SPSS 23 software. A 2-sided *p*-value of $P < 0.05$ was considered statistically significant.

In gene expression study, correlation analysis was used to evaluate coefficient (r) and relative statistical significance (*P* value) for the association in gene expression between *MMP9* and other genes involved in the "lipid and atherosclerosis" KEGG pathway (hsa05417) [16]. Correlations were considered statistically significant with a P value <0.05. mRNAs count matrices were analyzed using R software. *MMP9*-correlated genes were functionally characterized through Gene Ontology (GO) enrichment analysis. GO Biological Processes (BPs) over-representation analysis was conducted using clusterProfiler R package (v 4.3.1) [17,18]. The results were adjusted for multiple testing within the default framework and GO terms with an adjusted P value <0.01 were considered significant for the analysis. The top 30 statistically significant results of functional enrichment were visualized in bar plots and visual combination of genes with related biological processes were created with enrichplot R package (version 1.15.1) to enhance graphical representation of biological processes related to innate immunity.

3. Results

3.1. Patient characteristics and coronary plaque features at baseline and follow-up

Clinical characteristics, bio-humoral profiles, and CTA coronary plaque features at the time of the 1st and at the time of the 2nd CTA scan (after a period of follow up of 6.5 ± 1.1 years) are summarized in Table 1. Study participants ($n = 157$) were aged 58 ± 8 at baseline (65 ± 8 years at follow up), 67% were men, and family history of CAD was present in 44% of the study population. Among risk factors, frequency of hypertension significantly increased and of smoking significantly decreased from baseline to follow-up. Among medications, the use of calcium antagonist, anti-diabetics, and statins significantly increased. While LDL-C significantly decreased from baseline to follow-up, there was a significant increase of plasma HDL-C, TG, and TG/HDL-C ratio. Frequency of obstructive CAD did not change from baseline to follow-up, but a significant increase of TPV was observed. Among coronary plaque features, Necrotic Core and Dense Calcium PV significantly increased. Fibrous PV significantly decreased and Fibrous-fatty PV did not show any changes from baseline to follow up.

3.2. MMP9 serum levels, coronary plaque features and progression

Clinical characteristics and bio-humoral profiles of study samples divided according to Tertiles of serum *MMP9* at baseline are reported in Table S1. Age, sex, risk factors and frequency of obstructive CAD were similar across *MMP9* Tertiles. Use of statins and beta-blockers was progressively lower, while Total-C, LDL-C, and HDL-C levels were progressively higher.

Total PV, Fibrous, Fibrous-Fatty, and Necrotic Core PVs were not different among baseline *MMP9* Tertiles. On the other hand, annual changes of Fibrous-Fatty and Necrotic Core PVs showed a significant trend to increase among baseline *MMP9* Tertiles (Table 2). At univariate regression analysis, baseline *MMP9* serum levels were significantly associated with annual change of TPV, Fibrous-Fatty PV and Necrotic Core PV, but not with changes of Fibrous and Dense Calcium PV (Fig. 1). The association between *MMP9* serum levels and TPV and Necrotic Core PV annual change remained significant at multivariable linear regression analysis after adjustment for baseline confounding factors (Fig. 1).

Table 1
Clinical, bio-humoral and coronary CTA data of the study population at Baseline and Follow-up.

	Baseline n = 157	Follow-up n = 157	p Value
Clinical features			
Demographic			
Age, years	58 ± 8	65 ± 8	**<0.001**
Male gender	104 (66)	104 (66)	–
Risk factors			
Family history	69 (44)	69 (44)	–
Diabetes	47 (30)	50 (32)	0.648
Hypertension	104 (66)	117 (74)	**0.019**
Smoking	26 (16)	16 (10)	**0.031**
Obesity	33 (21)	41 (26)	0.200
Medications			
Beta-blockers	69 (43)	77 (49)	0.229
Calcium antagonists	22 (14)	41 (26)	**0.001**
ACE inhibitors	59 (38)	62 (39)	0.771
ARBs	26 (16)	31 (20)	0.442
Diuretics	29 (18)	33 (21)	0.585
Anti-diabetic	25 (16)	42 (27)	**>0.001**
Statins	83 (53)	110 (70)	**>0.001**
Anti-platelets	107 (68)	99 (63)	0.366
Bio-humoral Profile			
Total-C, mg/dL	182 ± 48	178 ± 44	0.382
LDL-C, mg/dL	107 ± 41	94 ± 40	**<0.001**
HDL-C, mg/dL	52 ± 15	55 ± 15	**<0.001**
Triglycerides, mg/dL	120 ± 63	146 ± 101	**0.002**
Triglycerides/HDL-C	2.66 ± 1.98	3.05 ± 3.02	**0.048**
FPG, mg/dL	110 ± 27	109 ± 28	0.561
TyG index	8.65 ± 0.57	8.80 ± 0.68	**0.014**
hs-CRP, mg/dL	0.37 ± 0.54	0.32 ± 0.51	0.255
MMP9, ng/mL	134 ± 186	86 ± 80	**0.031**
Imaging measurements			
Obstructive CAD	56 (36)	48 (31)	0.093
Coronary PV			
Total PV, mm^3	632 ± 583	710 ± 646	**<0.001**
Fibrous PV, mm^3	261 ± 256	245 ± 230	**0.032**
Fibrous-fatty PV, mm^3	132 ± 127	130 ± 123	0.657
Necrotic Core PV, mm^3	161 ± 154	190 ± 173	**<0.001**
Dense Calcium PV, mm^3	60 ± 92	114 ± 138	**<0.001**

Continuous variables are presented as mean ± standard deviation, categorical variables as absolute N and (%). **Bold** *p values* < 0.05 show statistically significant differences.

3.3. Gene expression of MMP9 in whole blood and coronary plaque features at follow-up

Clinical characteristics and bio-humoral profiles of study sample divided according to Tertiles of *MMP9* gene expression in whole blood at follow-up are reported in Table S2. Presence of obstructive CAD, use of anti-diabetic drugs, and circulating levels of MMP9 were progressively higher across Tertiles of *MMP9* gene expression. Serum and gene expression levels of MMP9 were significantly associated (Fig. 2A). At univariate regression analysis, *MMP9* gene expression was significantly associated with TPV and Necrotic Core PV, but not with Fibrous-Fatty, Fibrous and Dense Calcium PV (Fig. 2B). At multivariable linear regression analysis after adjustment for confounding factors, *MMP9* gene expression was associated only with Necrotic Core PV (Fig. 2B).

3.4. MMP9-related genes and functional annotation of correlations

MMP9 gene expression in whole blood at follow-up was evaluated

Table 2
Baseline and changes in plaque phenotypes at coronary CTA according to *MMP9* Tertiles.

	Tertile I <38.90 *n* = 52	Tertile II 38.90–102.40 n = 52	Tertile III >102.40 *n* = 53	*p Value*
Baseline Plaque Volume, mm^3				
Total PV	679 ± 682	542 ± 474	676 ± 577	0.560
Fibrous PV	277 ± 299	217 ± 213	289 ± 248	0.253
Fibrous-Fatty PV	151 ± 153	115 ± 98	130 ± 125	0.783
Necrotic Core PV	183 ± 181	142 ± 116	160 ± 158	0.865
Dense Calcium PV	51 ± 72	50 ± 108	79 ± 92$^{\#}$	**0.042**
Annual change Plaque Volume, mm^3/year				
Total PV	9.88 ± 14.13	9.34 ± 10.69	16.78 ± 19.13	0.074
Fibrous PV	−2.44 ± 11.97	−3.09 ± 14.61	−2.07 ± 17.57	0.787
Fibrous-Fatty PV	−1.05 ± 4.33	−1.24 ± 6.06	2.01 ± 7.83*$^{\#}$	**0.003**
Necrotic Core PV	2.12 ± 6.99	4.60 ± 8.56*	7.03 ± 10.33*	**0.002**
Dense Calcium PV	9.37 ± 11.52	6.51 ± 8.16	8.25 ± 13.49	0.577

Continuous variables are presented as mean ± standard deviation, categorical variables as absolute N and (%). **Bold** *p values* < 0.05 show statistically significant differences. *$p < 0.05$ I vs. III tertiles; $^{\#}p < 0.05$ II vs. III tertiles.

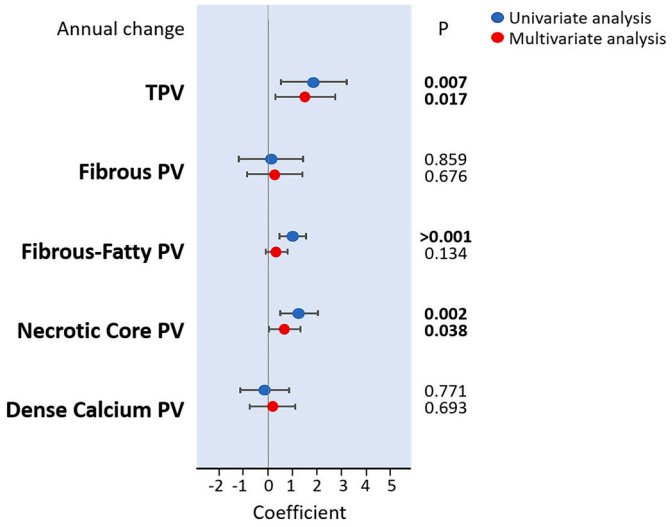

Fig. 1. Association of *MMP9* serum levels and plaque progression. In univariate and multivariate models, data are depicted as β coefficients with 95% CIs for the annual change in plaque volume from baseline to follow-up coronary CTA.

with respect to the expression of genes known to be involved in atherosclerotic process as reported in the "lipid and atherosclerosis" KEGG pathway. Expression levels for 64 genes showed a significant correlation with *MMP9* expression (*P* value <0.05): 56 genes were positively correlated, while 8 genes were negatively correlated (Fig. 3A). The most *MMP9*-correlated gene was NCF4 ($r = 0.80$), a neutrophil cytosolic factor involved, along with NCF2 ($r = 0.73$) and NCF1 ($r = 0.70$), in leukocyte migration and neutrophil extracellular trap formation. Moreover, genes involved in the innate immunity (TRLs/MYD88/IRAK4) (TLR2, $r = 0.54$; TLR4, $r = 0.32$; TLR6, r = 0.32; MYD88, $r = 0.41$; IRAK4, $r = 0.22$), inflammasome complex, including NLRP3 ($r = 0.20$) and CASP1 ($r = 0.28$), and their downstream pro-inflammatory factors, IL-1β ($r = 0.36$) and IL-18 ($r = 0.23$), were significantly associated with *MMP9* gene expression. Different MAPK signaling effectors and several mediators involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis were also positively correlated with *MMP9* expression.

Based on GO functional enrichment analysis, the 64 genes associated with *MMP9* expression were functionally characterized using the biological processes ontology and the 30 most significant terms are presented in Fig. 3B. *MMP9*-correlated genes were found to be involved in biological processes related with innate immunity (e.g., Toll-like receptor pathways, stress-activated MAPK cascade, oxidative stress, phagocytosis, lipopolysaccharide-mediated signaling pathway, positive regulation of NF-kappa B signaling) and atherogenic inflammatory response (myeloid leukocyte migration, regulation of inflammatory response, aging) (Fig. 3B). Visual combination of genes with related biological processes, suggested functional interactions between biological processes and their mediators related to innate immunity (Fig. 3C).

4. Discussion

The specificity of the present study was the demonstration of a significant association between serum *MMP9* concentrations and progression of adverse coronary plaque features, investigated using serial coronary CTA, in patients with coronary atherosclerosis. Patients with higher serum *MMP9* concentrations had an increased progression of Total, Fibrous-fatty, and Necrotic Core plaque volumes after a period of 6.5 ± 1.1 years. Conversely, *MMP9* serum levels were not associated with the progression of other plaque features. In particular the relationship between serum *MMP9* and the progression of adverse plaque features, such as the Necrotic Core plaque volume, was robust and independent from baseline traditional cardiovascular risk factors and medications, supporting for MMP9 a role as novel marker of residual risk.

Moreover, *MMP9* serum levels at follow-up were related with *MMP9* gene expression in whole blood assessed by transcriptomic analysis. The relationship between mRNA expression and MMP9 serum levels had not been previously reported in patients with CCS and, more importantly, had not been related with coronary plaque features. *MMP9* gene expression was positively associated with Necrotic Core PV also independently from traditional cardiovascular risk factors, medications, and presence of obstructive CAD. Moreover, the transcriptomic approach offered a tool to interrogate mechanisms linked to MMP9 expression in this specific population. We documented a link between the expression of *MMP9* gene and of genes involved in inflammatory pathways of innate immunity (neutrophil regulation, inflammasome complex and proinflammatory cytokines, leukocyte migration). Taken together, the present results suggest that higher circulating MMP9 levels in patients with CAD express the activity of inflammatory pathways conditioning adverse plaque phenotypes. Thus, the present results suggest that a novel approach based on the integration of MMP9 expression in blood samples with imaging features of CAD might identify patients at higher atherosclerotic inflammatory residual risk.

Common histological characteristics of culprit plaques responsible for events include inflammatory cells, a thin fibrous cap, positive remodelling, a large necrotic core, microcalcification and plaque haemorrhage [19]. Coronary CTA can reliably identify some of these adverse plaque features and their progression over time. In the present population of patients with CCS, the progression of adverse plaque features assessed by coronary CTA was significantly associated with baseline serum *MMP9* concentrations. Moreover, adverse plaque progression occurred despite the increased use of statins and other preventative medications supporting the role of *MMP9* as a marker of residual risk of coronary atherosclerosis.

The role of serum *MMP9* as independent predictive marker for the progression of adverse coronary atherosclerosis observed in this study could be explained considering that an altered *MMP9* activity is involved in the transition from stable to unstable plaques [20–22]. *MMP9* mediated mechanisms include: extra-cellular matrix (ECM) remodelling, that induces the thinning of fibrous cap leading to a

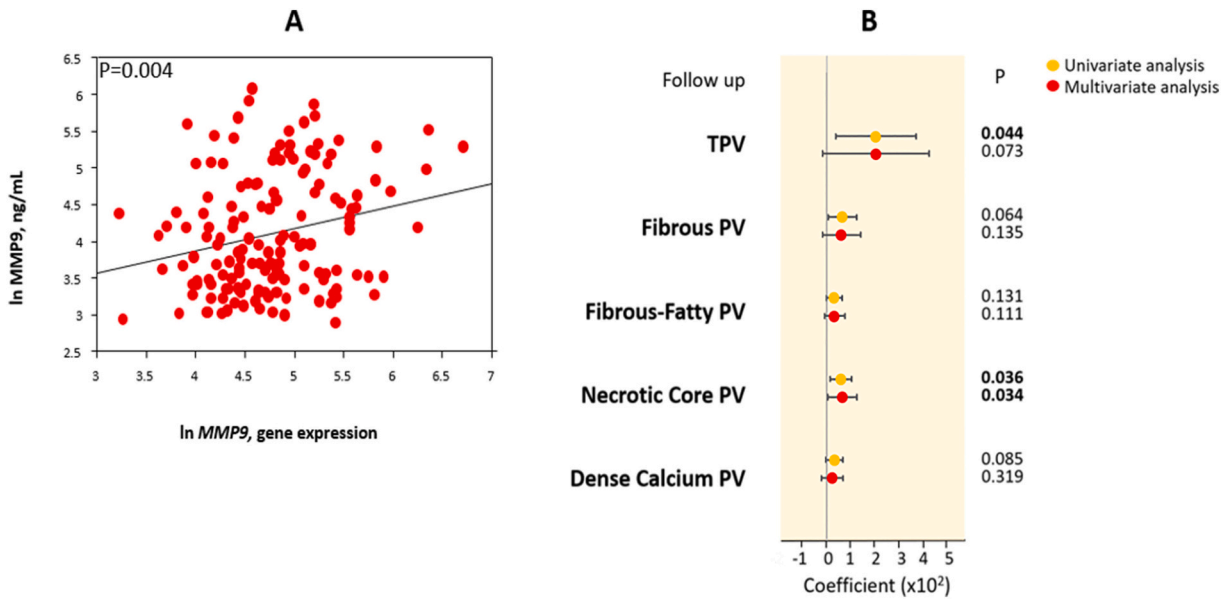

Fig. 2. Gene expression of *MMP9* in whole blood. (A) Association between circulating levels of serum MMP9 and gene expression of *MMP9* in whole blood; (B) Univariate and Multivariate association of *MMP9* gene expression and plaque volume at follow-up. Data are depicted as β coefficients with 95% CIs for the plaque volume at follow-up coronary CTA.

relative increase in the size of the necrotic core [22–24]; infiltration of monocyte/macrophages, that provokes an enhancing of the inflammatory process [25–26]; and, VSMC migration through VEGF secretion, which plays an important role in neovascularization [27]. Histopathological studies showed that MMP9 was mostly distributed in the shoulder regions, necrotic core, and the fibrous cap of the atherosclerotic plaques [25–28]. Animal experiments have shown in MMP9 deficient mice that plaque volume and length are significantly reduced and carotid plaques accumulated less smooth muscle cells, collagen, intraplaque foam cells, and macrophages [29–30].

In the present population of patients with CCS, serum *MMP9* levels were significantly correlated with *MMP9* gene expression in the whole blood which in turn was associated with the presence and extent of necrotic core plaque volume. Interestingly, most of the molecular pathways identified as associated with *MMP9* expression in the whole blood by transcriptomic analysis belong to innate immunity activation (Fig. 3). It is known that activation of innate immunity perpetuates a local inflammatory response and the production of matrix proteases that degrade the fibrous cap overlying an atherosclerotic plaque contribute to necrotic core formation [31]. Moreover, the pathways found to be associated with *MMP9* gene expression in the present study are among those suggested as potential pharmacological targets of anti-inflammatory therapies in patients with coronary disease. In particular while canakinumab acts through the selective inhibition of IL-1β [32], colchicine acts through a mechanism supposed to be related with inhibition of inflammasome and neutrophil recruitment and adhesion [33,34]. Other anti-inflammatory interventions under consideration for atherosclerosis also include inhibition of the inflammasome [35]. Future studies are warranted to confirm whether serum levels of *MMP9* are independent predictors of prognosis in patients with CCS and may be used to indicate targeted medical treatments. Among new therapeutic intervention deemed to possibly reduce the residual ASCVD risk [36], anti-inflammatory drugs receive a class IIb indication. Our results suggest that patients with higher *MMP9* gene expression could probably benefit more from a pharmacological intervention by colchicine or new drugs targeting inflammasome complex but specific trials would be needed. *MMP9* itself could be a treatment target in coronary atherosclerosis [22], also in the setting of myocardial ischemia [37], but previous clinical studies on MMP9 inhibitors in populations not selected for *MMP9* levels have provided contrasting results [22].

Limitations must be acknowledged in this study. Since whole blood samples were available only at follow-up, we could not perform a transcriptomic analysis at baseline. Due to the small sample size, further studies are necessary to confirm the role of *MMP9* in predicting adverse plaque progression in patients with CCS.

5. Conclusions

Results from this study demonstrate that *MMP9* is an independent predictive marker of progression of adverse coronary plaques and residual inflammatory risk. Transcriptomic data suggested that patients with CCS and higher *MMP9* could benefit more from a therapy targeting innate immunity, such as colchicine.

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CRediT authorship contribution statement

Chiara Caselli: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision. **Nicoletta Di Giorgi:** Methodology, Data curation, Writing – original draft, Writing – review & editing. **Rosetta Ragusa:** Methodology, Data curation, Writing – original draft, Writing – review & editing. **Valentina Lorenzoni:** Methodology, Data curation, Writing – original draft, Writing – review & editing. **Jeff Smit:** Data curation, Writing – review & editing. **Mohammed el Mahdiui:** Methodology, Writing – review & editing. **Ronny R. Buechel:** Investigation, Writing – review & editing. **Anna Teresinska:** Investigation, Writing – review & editing. **Maria N. Pizzi:** Investigation, Writing – review & editing. **Albert Roque:** Investigation, Writing – review & editing. **Rosa Poddighe:** Investigation, Writing – review & editing. **Juhani Knuuti:** Investigation, Writing – review & editing. **Moritz Schütte:** Investigation, Writing – review & editing. **Oberdan Parodi:** Investigation, Writing – review & editing, Funding acquisition.

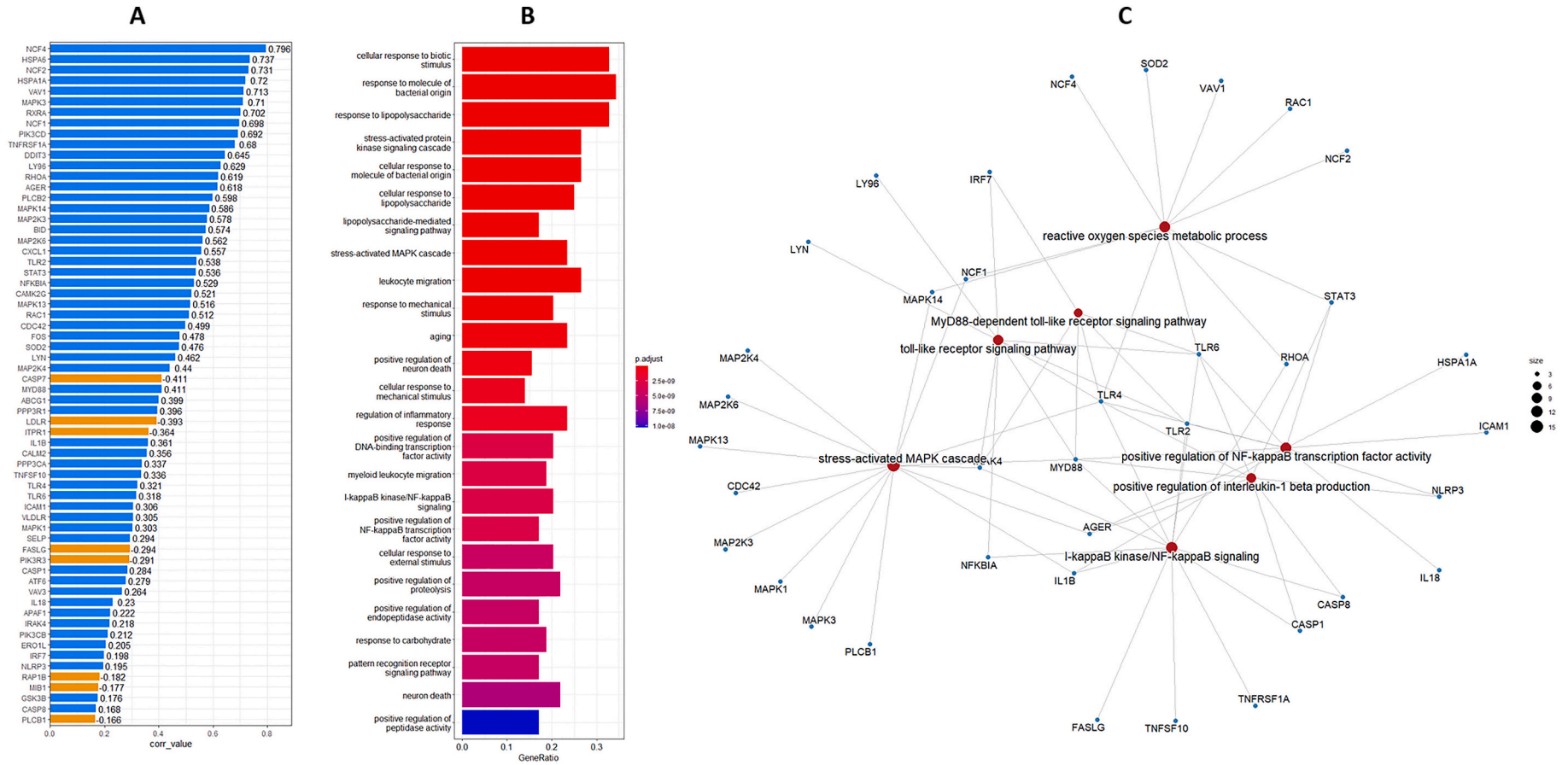

Fig. 3. *MMP9*-related genes and functional annotation of correlations. (A) Significant correlations in gene expression between *MMP9* and genes involved in "lipid and atherosclerosis" KEGG pathway (hsa05417). Barplot shows correlation coefficients (r) for significantly *MMP9*-correlated genes; (B) Functional annotation of correlations according to GO Biological Processes. The 30 most significant biological processes are shown. The GeneRatio indicates how many *MMP9*-related genes included in the analysis were annotated to the specific GO biological process. GO terms were filtered for adjusted *p*-value <0.01; (C) Visual combination of genes with related biological processes for enhanced graphical representation of functional categories related to innate immunity. Specifically, in myeloid cells such as monocytes, activation of Toll-like receptor 4 (TLR4) and TLR6 and subsequent ROS production and signaling through the NF-kB transcription factor activate NLRP3-containing inflammasome [31]. The NLRP3 inflammasome, through caspase-1 triggering, determinates IL-1β and IL-18 cleaving to their mature forms, which play varying roles in the development of atherosclerosis, including expression and activation of proteases such as *MMP9*, which ultimately leads to plaque rupture [38]. Myeloid cells such as neutrophils act through their granule proteins or through the neutrophil extracellular traps (NETs), a network consisting of neutrophil-derived DNA, and proteins of nuclear, granular, and cytosolic origin, including histones, pro-oxidant enzymes and multiple proteinases, including *MMP9* [39]. NETs have been associated with coronary stenosis in humans [40,41]. Recently, inflammasome complex has been identified as main trigger for NETs [39]. On the other hand, neutrophil secretory products attract and activate monocytes by NETs, resulting in production of IL-1β and IL18 by stimulating the NLRP3 inflammasome in plaques with signs of instability [42].

Gualtiero Pelosi: Investigation, Data curation, Writing – review & editing. **Arthur Scholte:** Investigation, Writing – review & editing. **Silvia Rocchiccioli:** Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Danilo Neglia:** Investigation, Writing – original draft, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

MS is an employee of Alacris Theranostics GmbH.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vph.2022.107098.

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