**CARD8** gene encoding a protein of innate immunity is expressed in human atherosclerosis and associated with markers of inflammation

Geena Varghese PARAMEL*, Lasse FOLKERSEN†, Rona J. STRAWBRIDGE†, Ali Ateia ELMABSOUT*, Eva SÅRNDALH, Pia LUNDMAN†, Jan-Håkan JANSSON‡, Göran K. HANSSON∥, Allan SIRSJÖ* and Karin FRANSÉN*

*Department of Clinical Medicine, School of Health and Medical Sciences, Örebro University, Örebro, Sweden
†Atherosclerosis Research Unit, Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden
‡Division of Cardiovascular Medicine, Department of Clinical Sciences, Karolinska Institutet, Danderyd University Hospital, Stockholm, Sweden
∥Department of Internal Medicine, Skellefteå Hospital and Umeå University Hospital, Umeå, Sweden
§Experimental Cardiovascular Research Unit, Center for Molecular Medicine, Department of Medicine at Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden

**Abstract**

Inflammation is a key factor in the development of atherosclerotic coronary artery disease. It is promoted through the inflammasome, a molecular machine that produces IL (interleukin)-1β in response to cholesterol crystal accumulation in macrophages. The **CARD8** (caspase recruitment domain 8) protein modulates this process by suppressing caspase 1 and the transcription factor NF-κB (nuclear factor κB). The expression of **CARD8** mRNA was examined in atherosclerotic vascular tissue and the impact on MI (myocardial infarction) of a polymorphism in the **CARD8** gene determined. **CARD8** mRNA was analysed by microarray of human atherosclerotic tissue and compared with transplant donor arterial tissue. Microarray analysis was performed for proximal genes associated with the rs2043211 locus in plaque. The **CARD8** rs2043211 polymorphism was analysed by genotyping of two Swedish MI cohorts, FIA (First Myocardial Infarction in Northern Sweden) and SCARF (Stockholm Coronary Atherosclerosis Risk Factor). The CRP (C-reactive protein) level was measured in both cohorts, but the levels of the pro-inflammatory cytokines IL-1β, IL-18, TNF (tumour necrosis factor) and MCP-1 (monocyte chemoattractant protein) were measured in sera available from the SCARF cohort. **CARD8** mRNA was highly expressed in atherosclerotic plaques compared with the expression in transplant donor vessel (P < 0.00001). The minor allele was associated with lower expression of **CARD8** in the plaques, suggesting that **CARD8** may promote inflammation. Carriers of the minor allele of the rs2043211 polymorphism also displayed lower circulating CRP and lower levels of the pro-atherosclerotic chemokine MCP-1. However, no significant association could be detected between this polymorphism and MI in the two cohorts. Genetic alterations in the **CARD8** gene therefore seem to be of limited importance for the development of MI.

**Key words:** caspase activation and recruitment domain 8 (**CARD8**), cytokines, gene polymorphism, inflammasome, myocardial infarction, rs2043211

**INTRODUCTION**

Ischaemic heart disease is the leading cause of death and is responsible for 12.2% of all deaths worldwide, with a frequency of 17 million deaths from cardiovascular diseases every year and is caused by the combinatory effects of environmental and genetic risk factors [1,2]. Among the genetic risk factors for cardiovascular diseases identified, polymorphisms in genes related to lipid metabolism, blood coagulation, glucose homeostasis and inflammation have been the focus of previous investigations. It is widely accepted that the disease process of atherosclerosis, which causes most cases of ischaemic heart disease, is an inflammatory response to lipid accumulation in the artery wall [3,4]. Innate as well as adaptive immune responses are thought to drive this inflammatory response. Whereas the latter is initiated by recognition of specific antigens, the former can be activated by a variety

Abbreviations: AMI, acute myocardial infarction; BIKE, Biobank of Karolinska Endarterectomies; CARD, caspase activation and recruitment domain; CRP, C-reactive protein; FIA, First Myocardial Infarction in Northern Sweden; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MI, myocardial infarction; NF-κB, nuclear factor κB; PBMC, peripheral blood mononuclear cell; SCARF, Stockholm Coronary Atherosclerosis Risk Factor; SNP, single nucleotide polymorphism; TNF, tumour necrosis factor.; WHO, World Health Organization.

Correspondence: Professor Allan Sirsjo (email allan.sirsjo@oru.se).
of pathogenic molecular patterns that ligate pattern recognition receptors, both inside and on the surface of cells [5]. Among them, the intracellular inflammasome includes a pattern detector, a transducer, and caspase 1 enzyme that processes a pre-form of IL (interleukin)-1 β to the bioactive cytokine [6,7]. Interestingly, the NLRP3 [NLR (nucleotide-binding domain, leucine-rich repeat-containing receptor) family, PYD domain-containing 3] inflammasome is activated by intracellular cholesterol crystals and initiates responses that promote atherosclerosis [8,9], mediated by several cytokines and other inflammatory mediators, including IL-1β, TNF (tumour necrosis factor), IFNγ (interferon γ), MCP-1 (monocyte chemoattractant protein-1) and IL-18 [4].

CARD8 [caspase activation and recruitment domain 8 (also known as TUCAN/CARDINAL/NDDP1)] is a component of innate immunity involved in the suppression of NF-κB (nuclear factor κB) activation through NOD2 (nucleotide-binding oligomerization domain 2), leading to the suppression of the immune response and suppression of inflammatory activities [10–12]. The CARD8 gene product has also been suggested to be a part of the inflammasome [6]. Several previous investigations have studied the association between a previous T30A polymorphism (rs2043211) in exon 5 in the CARD8 gene encoding the C10X variant with different inflammatory diseases including inflammatory bowel disease, rheumatoid arthritis, and Alzheimer’s disease [13–21]. Owing to the importance of inflammation in atherosclerosis and MI (myocardial infarction), we have studied CARD8 mRNA expression in human atherosclerosis and the impact of CARD8 polymorphism rs2043211 in MI in two independent Swedish cohorts, the FIA (First Myocardial Infarction in Northern Sweden) and the SCARF (Stockholm Coronary Atherosclerosis Risk Factor) cohorts.

MATERIALS AND METHODS

Study subjects

Gene expression patterns of atherosclerosis were analysed in the BiKE (Biobank of Karolinska Endarterectomies) cohort at Karolinska University Hospital, Stockholm, Sweden. mRNA was isolated from carotid artery plaque tissue and obtained at endarterectomy surgery in 106 patients with ischaemic cerebrovascular disease. A total of nine samples of iliac arteries and one sample from intima of aorta were obtained from transplant donors and transplant donor vessels in comparison with the implanted rs2043211 polymorphism

The expression of CARD8 mRNA in the samples from the BiKE project was assessed using Affymetrix HG-U133 plus 2.0 Genechip® arrays [22]. Briefly, the RNA was purified using the Qiagen RNeasy procedure and hybridized to arrays at the Karolinska Institute Affymetrix core facility. Raw data were normalized using the RMA procedure, including a log2 transformation step. Furthermore, the SNP (single nucleotide polymorphism) measurements for eQTL (expression quantitative trait loci) were obtained according to the method of Folkersen et al. [26]. In the BiKE database the rs2043211 was imputed using the MACH 1.0 algorithm with data from 1000 genomes as reference. The quality of imputation was an R² score of 0.706.

Genotyping of the rs2043211 polymorphism and the relation to MI

Genotyping of the rs2043211 polymorphism in the CARD8 gene was performed for the FIA and SCARF cohorts using TaqMan® SNP genotyping assay with the 7900 HT Fast Real-Time PCR system (Applied Biosystems). In brief, the PCR was performed using 10 ng of genomic DNA in 10 μl reaction mixtures containing 2× TaqMan® Genotyping Master mix (Applied Biosystems) and TaqMan® SNP Genotyping Assay with predesigned primer and probes C___11708080_1_ (Applied Biosystems) according to a TaqMan® standard protocol followed by allelic discrimination analysis to evaluate the genotype of each sample. The accuracy of the genotyping was verified by repeating the PCR in a random selection of 10% of the samples. Using a two-stage analysis of FIA and SCARF, we have 88% power to detect an effect (assuming an effect of 1.2 and MAF (minor allele frequency) of 0.39 as for FIA, and disease incidence of 10%).

Plasma concentration of pro-inflammatory cytokines and CRP (C-reactive protein) measurements

Plasma from the SCARF cohort was utilized for the measurement of pro-inflammatory cytokines. The levels of cytokines screening of hospital discharge records, general practitioners’ reports and death certificates, in accordance with WHO (World Health Organization) and MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) criteria [25]. Exclusion criteria for cases were stroke, AMI (acute MI) or cancer within the 5 years prior to or 1 year after AMI.

In the SCARF study, which was conducted from 1996 to 2000, the contemporary WHO criteria for MI were used. Two of the three following criteria had to be fulfilled: symptoms suggesting MI, ECG-changes suggesting ischaemia and elevated cardiac enzyme, in our institutions the CK-MB was used at that time.

All studies received ethical approval and were conducted in accordance with the ethical guidelines of the Declaration of Helsinki and written informed consent was obtained from the patients. Approximately 1.6% of the samples from the FIA cohort and 7.6% of the SCARF cohort were excluded from analysis, due to poor genotype calling.

CARD8 mRNA expression in human atherosclerotic lesions and transplant donor vessels in comparison with the implanted rs2043211 polymorphism

The expression of CARD8 mRNA in the samples from the BiKE project was assessed using Affymetrix HG-U133 plus 2.0 Genechip® arrays [22]. Briefly, the RNA was purified using the Qiagen RNeasy procedure and hybridized to arrays at the Karolinska Institute Affymetrix core facility. Raw data were normalized using the RMA procedure, including a log2 transformation step. Furthermore, the SNP (single nucleotide polymorphism) measurements for eQTL (expression quantitative trait loci) were obtained according to the method of Folkersen et al. [26]. In the BiKE database the rs2043211 was imputed using the MACH 1.0 algorithm with data from 1000 genomes as reference. The quality of imputation was an R² score of 0.706.

Genotyping of the rs2043211 polymorphism and the relation to MI

Genotyping of the rs2043211 polymorphism in the CARD8 gene was performed for the FIA and SCARF cohorts using TaqMan® SNP genotyping assay with the 7900 HT Fast Real-Time PCR system (Applied Biosystems). In brief, the PCR was performed using 10 ng of genomic DNA in 10 μl reaction mixtures containing 2× TaqMan® Genotyping Master mix (Applied Biosystems) and TaqMan® SNP Genotyping Assay with predesigned primer and probes C___11708080_1_ (Applied Biosystems) according to a TaqMan® standard protocol followed by allelic discrimination analysis to evaluate the genotype of each sample. The accuracy of the genotyping was verified by repeating the PCR in a random selection of 10% of the samples. Using a two-stage analysis of FIA and SCARF, we have 88% power to detect an effect (assuming an effect of 1.2 and MAF (minor allele frequency) of 0.39 as for FIA, and disease incidence of 10%).

Plasma concentration of pro-inflammatory cytokines and CRP (C-reactive protein) measurements

Plasma from the SCARF cohort was utilized for the measurement of pro-inflammatory cytokines. The levels of cytokines
and CRP were previously published [24,27–29]. The plasma concentration of IL-18 was determined by ELISA kits from R&D Systems [27]. The cytokines IL-1β, TNF and MCP-1 were analysed by Evidence® automated biochip array system based on sandwich technique and competitive immunoassay techniques (Randox Laboratories) [28]. High-sensitivity CRP was measured for the SCARF and FIA cohorts as described in Samnegård et al. [24] and Wennberg et al. [29] respectively.

**Statistical analysis**

The difference in CARD8 mRNA levels between carotid lesions and transplant donor vessels was analysed using a two-tailed Student’s t test assuming heteroscedasticity. Since this analysis was solely directed at the CARD8 gene testing hypotheses generated by prior publications, the significance level was here considered to be \( P < 0.05 \). The statistical analyses for cytokine profiling were done using STATISTICA 7.1 software (StatSoft). Testing for the association between genotype and the expression of CARD8 mRNA in carotid plaque was performed using a linear additive model, as previously described [26]. This analysis tested a total of 12 genes proximal to the rs2043211 SNP in two tissue types and therefore considered a \( P \) value of 0.0021 to indicate significance as calculated by Bonferroni correction. Testing for deviation of rs2043211 from Hardy–Weinberg equilibrium was performed using a web-based calculator (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The association between genotype of the rs2043211 polymorphism and MI was analysed with \( \chi^2 \) test using Yates correction using SPSS software package (SPSS Inc.) and EpiInfo Software 2008 (Centers for Disease Control and Prevention). Associations between rs2043211 and cytokine levels were investigated using linear regression assuming and additive genetic model, with adjustment for age, gender (FIA) and case-control status (SCARF), using PLINK 4.0 [30]. Where necessary, cytokine levels were log transformed for normalization prior to analysis.

**RESULTS**

**Increased CARD8 mRNA expression in human carotid plaque**

The mRNA level of CARD8 was compared between atherosclerotic arteries and transplant donor arteries. The expression of CARD8 mRNA was significantly higher in atherosclerotic lesions compared with transplant donor vessel tissue \( (P < 0.00001; \text{ Figure 1}) \).

**Association between the rs2043211 polymorphism and CARD8 mRNA in plaque and PBMCs**

To investigate whether the C10X polymorphism (rs2043211) affected CARD8 expression, the mRNA level for CARD8 in atherosclerotic plaque tissue from the BiKE project was examined. Carriers of the minor allele (X) of the rs2043211 polymorphism displayed decreased expression of CARD8 \( (P = 0.0000869; \text{ Figure 2}) \). When mRNA for genes located in the vicinity of rs2043211 (<200 kb) were analysed, the rs2043211 polymorphism was associated with increased mRNA expression of TMEM143 (gene encoding transmembrane protein 143) in plaque, which is one of the target genes for PPARγ (peroxisome-proliferator-activated receptor \( \gamma \)) [31] (borderline-significance at \( P = 0.00251 \); results not shown).

**No association between the rs2043211 polymorphism and MI**

No deviation from Hardy–Weinberg equilibrium was observed across cases or controls for the rs2043211 polymorphism. The FIA and SCARF cohorts were utilized to investigate whether the CARD8 C10X polymorphism (rs2043211) contributes to the risk for MI. However, no significant association between rs2043211 and MI was observed in either the FIA or SCARF cohorts, neither on genotype nor on allele frequency level (Table 1). As outlined by Roberts et al. [32], the frequencies of individuals homozygous for the minor allele (XX) was combined with the heterozygous individuals (CX) and compared with individuals homozygous for the major allele (CC). In the FIA cohort, a slightly higher
Table 1 Overview of the genotype frequencies of the polymorphism rs2043211 in the CARD8 gene in patients with MI and healthy controls in the FIA and SCARF cohorts

In (a), n = 539 patients and n = 1007 controls in the FIA cohort, and n = 348 patients and n = 367 controls in the SCARF cohort. In (b), n = 1078 patients and n = 2014 controls in the FIA cohort, and n = 696 patients and n = 734 controls in the SCARF cohort. CI, confidence interval.

(a) Genotype frequencies

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients (n)</th>
<th>Controls (n)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIA cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>200 (37%)</td>
<td>423 (42%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CX</td>
<td>256 (48%)</td>
<td>442 (44%)</td>
<td>1.2 (1.0–1.6)</td>
<td>0.09</td>
</tr>
<tr>
<td>XX</td>
<td>83 (15%)</td>
<td>142 (14%)</td>
<td>1.2 (0.9–1.7)</td>
<td>0.22</td>
</tr>
<tr>
<td>CX+XX</td>
<td>339 (63%)</td>
<td>584 (58%)</td>
<td>1.2 (1.0–1.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>SCARF cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>146 (42%)</td>
<td>169 (46%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CX</td>
<td>165 (47%)</td>
<td>153 (42%)</td>
<td>1.2 (0.9–1.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>XX</td>
<td>37 (11%)</td>
<td>45 (12%)</td>
<td>1.1 (0.6–1.6)</td>
<td>0.94</td>
</tr>
<tr>
<td>CX+XX</td>
<td>202 (58%)</td>
<td>198 (54%)</td>
<td>1.2 (0.9–1.6)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

(b) Allele frequencies

<table>
<thead>
<tr>
<th>Allele</th>
<th>Patients (n)</th>
<th>Controls (n)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIA cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>656 (61%)</td>
<td>1288 (64%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>422 (39%)</td>
<td>726 (36%)</td>
<td>1.1 (1.0–1.3)</td>
<td>0.10</td>
</tr>
<tr>
<td>SCARF cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>457 (66%)</td>
<td>491 (67%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>239 (34%)</td>
<td>243 (33%)</td>
<td>1.0 (0.8–1.3)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Table 2 Overview of the association of rs2043211 with CRP levels in the FIA and SCARF cohorts

The minor allele (X) was significantly associated with a lower mean CRP value in the SCARF cohort, but not in the FIA cohort. Beta indicates the magnitude of effect per allele.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Samples (n)</th>
<th>Beta</th>
<th>S.E.M.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIA</td>
<td>1363</td>
<td>−0.40</td>
<td>0.23</td>
<td>0.08</td>
</tr>
<tr>
<td>SCARF</td>
<td>696</td>
<td>−0.20</td>
<td>0.062</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3 Association between the rs2043211 polymorphism with cytokine level (log values) in serum from MI patients (SCARF) using ELISA and Evidence®® automated biochip array system

Beta indicates the magnitude of effect per allele.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Samples (n)</th>
<th>Beta</th>
<th>S.E.M.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-18</td>
<td>690</td>
<td>0.0113</td>
<td>0.02178</td>
<td>0.60</td>
</tr>
<tr>
<td>TNF</td>
<td>674</td>
<td>−0.039</td>
<td>0.02941</td>
<td>0.18</td>
</tr>
<tr>
<td>IL-1β</td>
<td>328</td>
<td>−0.003</td>
<td>0.1393</td>
<td>0.97</td>
</tr>
<tr>
<td>MCP-1</td>
<td>692</td>
<td>−0.045</td>
<td>0.02204</td>
<td>0.039</td>
</tr>
</tbody>
</table>

MCP-1 was investigated in the SCARF cohort. An association of the minor allele (X) with lower levels of MCP-1 was evident (P = 0.039), but no association was found with respect to the IL-1β, IL-18 or TNF levels (Table 3).

Association between the rs2043211 polymorphism and lower MCP-1 levels in serum

The association of the rs2043211 SNP with the serum levels of the inflammatory/immune protein mediators, IL-1β, IL-18, TNF and MCP-1 was investigated in the SCARF cohort. An association of the minor allele (X) with lower levels of MCP-1 was evident (P = 0.039), but no association was found with respect to the IL-1β, IL-18 or TNF levels (Table 3).

DISCUSSION

The inflammasome has been implicated in atherosclerosis both because of its activation by cholesterol crystals and since its activation generates the pro-inflammatory cytokine, IL-1β. CARD8 negatively regulates caspase 1, the inflammasome component that processes pro-IL-1β to bioactive cytokine [33]. In the present investigation, we show that CARD8 mRNA is significantly increased in human atherosclerotic plaques compared with its expression in vascular control tissue from transplantation donors. As CARD8 is expressed in vascular tissue [34], our data imply...
that increased CARD8 expression is associated with the atherosclerotic disease process.

We also investigated the association between expression of CARD8 mRNA and the rs2043211 polymorphism in exon 5 of the CARD8 gene, and found that the minor allele (X) was associated with lower CARD8 mRNA expression in the atherosclerotic plaque. In addition, we found a borderline association between the rs2043211 polymorphism and expression of TMEM143, a gene adjacent to the CARD8 gene. Therefore additional genes surrounding the rs2043211 locus may also be associated with this polymorphism and perhaps with the pathogenesis of atherosclerosis. However, the functional role of these genes in the pathogenesis of atherosclerosis and MI is unclear.

Our finding that the minor allele was associated with a lower mRNA expression of CARD8 is consistent with the findings of Ko et al. [21], who reported reduced expression level of CARD8 in cells carrying the minor/truncated allele of the rs2043211 polymorphism [21]. According to Ko et al. [21], the minor allele was also associated with higher levels of Salmonella-induced cell death, possibly due to loss of capacity to inhibit caspase 1 activity and NF-kB signalling [18,21,33]. The higher level of caspase 1 activity, which may be a result of lower levels of CARD8 in individuals carrying the minor allele, may therefore increase the risk of inflammatory reactions in atherosclerosis. In concordance with previous investigations, those individuals carrying the rare variant of the rs2043211 polymorphism would therefore have an increased inflammatory activity. In contrast to this, in the present investigation the minor variant was significantly associated with lower CRP values. No association was evident between the CARD8 SNP rs2043211 and mRNA levels for IL-1β, IL-18 or TNF expression; data that do not support the assumption of a higher inflammatory level in individuals with minor CARD8 allele in the atherosclerotic plaque. Furthermore, the minor CARD8 allele was also associated with lower circulating MCP-1 levels. This is in line with previous studies showing that defective inflammasome activation attenuates MCP-1 expression, leading to reduced inflammation [35]. However, cautious interpretation of the results must be undertaken, since fresh healthy carotid arteries were not utilized as the control tissue, which would have been a better alternative. Fresh healthy carotid arteries are however inaccessible and post-mortem arterial biopsies are not an option, due to the need for high-quality RNA for these analyses. In the present study, most of the control samples were from iliac arteries taken from brain dead transplant donors. Iliac arteries have similar properties as carotid arteries in terms of size, flow conditions and anatomical build. The iliac artery also commonly develops atherosclerosis in a similar way to the carotid artery and they share at least some properties in terms of gene expression [36]. In addition, systemic inflammatory response and increase of proapoptotic and antiapoptotic mRNA expression may occur after brain death, which may have affected the results [37]. Further studies will, however, be required to clarify if and how CARD8 affects the inflammatory process in the atherosclerotic plaque.

In the present study, we examined the role of the CARD8 polymorphism rs2043211 in a case-control study of 942 MI cases and 1403 controls, in the FIA and the SCARF cohorts. However, no significant association could be revealed between the CARD8 polymorphism rs2043211 and MI in either the SCARF or the FIA cohort. Our results are supported by a previous study made by García-Bermúdez et al. [38] who did not find any association between the rs2043211 polymorphism and cardiovascular events in rheumatoid arthritis patients. The small trend towards a higher MI risk for carriers of the minor allele in the FIA cohort, but not SCARF may reflect genetic divergence between the two Swedish regions from which the two cohorts were recruited. However, conflicting evidence has also been published previously regarding the rs2043211 polymorphism and several different diseases with inflammatory background, like inflammatory bowel disease, increased severity of rheumatoid arthritis, increased risk of Alzheimer's disease in women and other inflammatory diseases [14–20]. Although the role of CARD8 polymorphisms should be assessed in larger studies, the role of the rs2043211 SNP seems to be limited for the onset of MI.

Taken together, the present investigation shows that CARD8 is highly expressed in the atherosclerotic plaque and that its rs2043211 polymorphism impacts on gene expression levels. However, genetic alterations in the CARD8 gene seem to be of limited importance for the development of MI.

**CLINICAL PERSPECTIVES**

- Atherosclerosis and MI are associated with an inflammatory response but the role of CARD8 in inflammation, atherosclerosis and MI is not clear.
- CARD8 mRNA was found overexpressed in atherosclerotic lesions and a truncated variant of the CARD8 gene was associated with lower CRP and lower expression of MCP-1, two markers of inflammatory response. No association was revealed between the truncated variant and the onset of MI in two Swedish cohorts.
- Further studies of the mechanistic interplay of CARD8 in inflammation and atherosclerosis are therefore needed.

**AUTHOR CONTRIBUTION**

Geena Varghese Paramel, Jan-Håkan Jansson, Göran Hansson, Allan Sirsjö and Karin Fransén conceived and designed the study. Allan Sirsjö co-ordinated the study. Geena Varghese Paramel, Lasse Folkersen, Rona Strawbridge, Ali Ateia Elmabsout and Karin Fransén performed the experiments. Geena Varghese Paramel, Lasse Folkersen, Rona Strawbridge, Allan Sirsjo and Karin Fransén analysed the data. Eva Särndahl, Pia Lundman, Jan-Håkan Jansson, Göran Hansson and Allan Sirsjo contributed reagents/materials/analysis tools. Geena Varghese Paramel, Göran Hansson, Allan Sirsjo and Karin Fransén wrote the paper. Lasse Folkersen, Rona Strawbridge, Ali Ateia Elmabsout, Eva Särndahl, Pia Lundman, and Jan-Håkan Jansson contributed to the writing of the paper. All authors contributed to drafting of the paper and revising it critically for intellectual content, and the authors approved of the final version to be published.
ACKNOWLEDGEMENTS
We thank the individuals in the SCARF, RA and BiKE cohorts for their participation. We are grateful to Dr Gabrielle Paulsson Berne and Dr Ulf Hedin for help with the BiKE cohort, and Dr Ken Jatta for the help with parts of the genetic analysis of the FIA cohort. We also acknowledge Örebro University, Skellefteå Hospital, Umeå University Hospital and Karolinska Institute for technical facilities and support.

FUNDING
This study was supported by the Magnus Bergvalls Foundation, the Swedish Heart-Lung foundation, Swedish Research Council [grant numbers 521-2009-4203, 349-2007-8703] and Örebro University.

REFERENCES
CARD8 in cardiovascular disease


