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Plasma and genetic determinants of soluble TREM-1 and major adverse cardiovascular events in a prospective cohort of acute myocardial infarction patients. Results from the FAST-MI 2010 study

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ABSTRACT

Introduction: Triggering receptor expressing on myeloid cells (TREM)-1 is involved in the pathophysiology of ischemic heart disease. Plasma soluble TREM-1 levels (sTREM-1) has been associated with increased risk of major adverse cardiovascular events (MACE) in acute myocardial infarction (AMI) patients. However, the causative link between TREM-1 and MACE remains unknown and requires further investigation before developing potential therapeutic approaches.

Methods and results: Using the serum and DNA data bank from the prospective, nationwide French registry of Acute ST-elevation and non-ST-elevation Myocardial Infarction (FAST-MI 2010, $N = 1293$), we studied the association of plasma levels of sTREM-1 with 9 common genetic variants at the *TREM1* locus and their relationship with recurrent MACE over a 3-year follow up. Plasma levels of sTREM-1 were associated with an increased risk of MACEs (death, recurrent MI or stroke) (adjusted HR = 1.86, 95%CI = 1.06–3.26 and HR = 1.11, 95%CI = 0.61–2.02 respectively for tertiles 3 and 2 versus tertile 1, $P < 0.001$). The study of common variants identified two major genetic determinants of sTREM-1 (rs4714449: beta = -0.11, $P_{add} = 7.85 \times 10^{-5}$ and rs3804276: beta = 0.18, $P_{add} = 2.65 \times 10^{-11}$) with a potential role on maintenance and/or differentiation of hematopoietic stem cells. However, associated variants only explained 4% of sTREM-1 variance ($P = 2.74 \times 10^{-14}$). Moreover, the rs4714449 variant, individually and in haplotype, was not significantly associated with MACE (HR = 0.61, 95% CI: 0.35–1.05, $P = 0.07$).

Conclusions: Despite its relationship with increased risk of death, recurrent MI and stroke, genetic determinants of plasma levels of sTREM-1 were not found to be causal prognostic factors in patients with acute myocardial infarction.

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Nonstandard abbreviations and acronyms

AMI	Acute Myocardial Infarction
CRP	C-Reactive Protein
HR	Hazard Ratio
MACE	Major Adverse Cardiovascular Event
QTL	Quantitative Trait Loci
ROC	Receiver Operating characteristic Curve
SNP	Single-Nucleotide Polymorphism
STEMI	ST Elevation Myocardial Infarction
TREM-1	Triggering receptor expressing on myeloid cells

1. Introduction

Innate immunity plays a major role in the pathophysiology of cardiovascular diseases, including atherosclerosis, thrombotic complications of plaque rupture [1] and adverse post-ischemic myocardial remodeling [2]. Monocytes and macrophages promote both atherosclerotic plaque growth and rupture and exacerbate excessive cardiac remodeling following acute myocardial infarction (AMI) through cytokine and chemokine production and metalloproteinase secretion [2]. Neutrophils are also implicated in atherogenesis and deleterious cardiac remodeling following AMI leading to heart failure [3]. Unfortunately, therapeutic strategies that target these specific cell populations are still lacking.

Triggering receptor expressing on myeloid cells (TREM)-1 is a receptor expressed mainly by monocytes and neutrophils that is shed after engagement [4]. Our group has previously shown that TREM-1 plays a pathogenic role in atherosclerosis orchestrating cytokine production, leukocyte recruitment within inflamed tissues and lipid uptake by macrophages. In mouse models, *Trem1* genetic deletion or pharmacological blockade limits post-ischemic cardiac remodeling [5] and atherosclerosis development [6]. High plasma levels of soluble TREM-1 (sTREM-1) associate with increased-risk of recurrent major adverse cardiac events (MACE) in patients with AMI [5]. These data suggest that TREM-1 blockade could represent a promising anti-inflammatory strategy in AMI [6], although it is not known whether the relationship between TREM-1 and increased risk of MACE is causal. We thus aimed to determine the relationship between sTREM-1 plasma concentrations, the common genetic variants at the *TREM1* locus on chromosome 6, and the risk of MACE (death, recurrent MI or stroke) over a 3-year follow up in a nationwide cohort of AMI patients (FAST-MI 2010, NCT01237418).

2. Methods

2.1. Study population

The population and methods of the French registry of Acute ST segment–elevation and non–ST–segment–elevation MI (FAST-MI) have been described in detail in previous publications [7,8]. Briefly, all patients aged ≥ 18 years were included in the registry over a 1-month period (from 1st to the 31st of October 2010 in Fast-MI 2010) if they had elevated serum markers of myocardial necrosis higher than twice the upper limit of normal for creatine kinase, creatine kinase-MB, or elevated troponins, and either symptoms compatible with acute MI or electrocardiographic changes on at least 2 contiguous leads with pathological Q waves or persisting ST-elevation or depression. The time from symptom onset to intensive care unit admission had to be less than 48 h. Patients were managed according to usual practice; treatment was not affected by participation. Participation in the study was offered to all intensive care units in French institutions with the capacity to receive acute coronary syndrome emergencies. The registry was conducted in compliance with Good Clinical Practice guidelines, French law, and the French data protection law. The protocol was reviewed and approved by the Committee for the Protection of Human Subjects of Saint-Louis University Hospital, (CPP IV Ile de France). The data recorded and the

way they are handled and stored were reviewed and approved by the national authorities. Over 1 month, 4169 patients were included in 219 centers (76% of active centers in France). A biology sub-study collected blood and serum samples for the assessment of DNA and centralized measurement of biological markers. The present study was performed in all the 1293 patients who gave their written consent for both plasma and genetic database biobanks. The follow-up data was collected through contacts with the patients' physicians, the patients themselves or their relatives, and registry offices of their birthplace. The 3-year follow-up was 95% complete for MACEs. The outcome events were assessed blinded to the results of sTREM-1 measurements.

2.2. Soluble TREM-1 quantification

Plasma concentrations of soluble TREM-1 (pg/mL) were determined at admission in duplicate by enzyme linked immunosorbent assay (RnD Systems®) and the mean value was recorded. We determined the within- and between-subjects variance with repeated-subjects analysis of variance. The intra-class coefficient (ICC), which corresponds to the fraction of total variance that is not due to measurement, was used as a measure of repeatability.

2.3. Selection of genetic variants

Common genetic variants (MAF > 5%) were selected through the portal for the Genotype-Tissue Expression (GTEx, <https://www.gtexportal.org/home/>, V6p Release, accessed Oct 2016). We identified 140 expression quantitative trait loci (eQTLs, P -value $\leq 10^{-5}$) for *TREM1* gene expression, all located in chromosome 6, including 118 that were specific to whole blood tissue and were retained. SNP annotation by Variant Annotation Integrator (VAI was consulted in October 2016: <https://genome.ucsc.edu/cgi-bin/hgVai>), indicated that 39 SNPs are located in transcript binding site (TFBS) or DNase I Hypersensitivity site (DNASEV) in blood tissue or vessels (Supplementary Table 1). After linkage disequilibrium (LD) pruning we retained 10 SNPs for genotyping using the KASP™ assays (LGC genomics). LD between analyzed SNPs is presented in Supplementary Table 2.

2.4. SNPs annotation

DNase-seq and transcription factors, “Fold change over control” bigwig files, aligned on hg38 genome, were downloaded from ENCODE website (<https://www.encodeproject.org/>) or GEO repository (<https://www.ncbi.nlm.nih.gov/gds>, GSE60477 [9]), and visualized using Integrated Genome Browser (<http://bioviz.org/igb/>). Accession numbers of files are ENCF363RHA (H3K4me3), ENCF577YZK (H3K4me1), ENCF601NLG (H3K27ac), ENCF724HAH (DNase-seq), ENCF530EOD (PU.1), ENCF603CVY (GABPA) and ENCF044XMQ (RNA Pol II phosphoS5). We used PERFECTOS-APE algorithm (<http://opera.autosome.ru/perfectosape/>) to predict effects of SNPs on transcription factors binding sites, with HOCOMOCO v11 [10] as a database of human transcription factors binding motifs. MoloTool (<http://molotool.autosome.ru/>) from HOCOMOCO v11 website was used to identify potential binding sites for PU.1, TAL1, LYL1, GATA2, RUNX1, FLI1 and ERG.

2.5. Statistical analyses

Outcome events (MACE), a composite of all-cause death and non-fatal myocardial infarction and stroke, were adjudicated by a committee whose members were unaware of the patients' blood measurements. First, a stepwise backward Cox multivariate model was used for assessing predictors of 3-year MACE. Candidate variables included in the multivariate models were selected ad hoc, based upon their physiological relevance and potential to be associated with long-term outcome events. Analyses were adjusted for age, sex, body mass index, diabetes,

smoking status, hypertension, previous history of MI, stroke, chronic heart or renal failure, left ventricular ejection fraction, early GRACE score, hospital management including reperfusion therapy and recommended drug therapies including statins, and C reactive protein (CRP). A *P* value of 0.05 for inclusion and 0.10 for exclusion at each step was used. The cumulative hazard functions for each covariable were computed to assess proportionality, and collinearity was assessed by calculating variance inflation factors. MACE occurrence according to sTREM-1 tertiles were estimated using the Kaplan–Meier estimator. We assessed predictive value of sTREM-1 for MACE by the area under the Receiver Operating characteristic Curve (ROC) and determined the most discriminant threshold by maximizing the Youden index (Sensitivity+Specificity-1).

We tested the association between nine common variants located in the vicinity of *TREM1* that passed genotyping quality control, mainly call rate > 0.97 and did not deviate from Hardy-Weinberg equilibrium (*P* > 0.01). The association between genetic variants and sTREM-1 was evaluated by linear regression under the additive, dominant and recessive models using PLINK [11]. Adjustments for potential confounders included 21 covariates in the regression models namely sex, age, previous or current smoking, body mass index, family history of coronary disease, history of hypertension, previous history of myocardial infarction, heart failure, renal failure, diabetes, heart rate at admission, Killip class, left ventricular ejection fraction, C-reactive protein levels, hospital management including reperfusion therapy and recommended drug therapies including statins. Haplotype association analyses were performed for the five individually associated variants (*P* < 0.05) in the single marker analyses and included rs7769759, rs7450812, rs900823, rs4714449 and rs3804276. Independent effect of variants on the associated haplotype was assessed using conditional haplotype-based test likelihood ratio test, as recommended in the software manual [11].

Single SNPs and haplotype effects on MACE at 3 years was tested using Kaplan Meier survival analyses in the THESIAS software [12]. Multivariate analyses were restricted to the haplotype formed by rs3804276 and rs4714449, the two SNPs that independently contributed to the haplotype association with circulating sTREM-1 and was adjusted by sex and age.

The authors declare that all supporting data are available within the article and its online supplementary files.

3. Results

3.1. Study population

Among the 1293 included patients, 74% were men (*n* = 950) with a mean age of 64 ± 14 years, half (54%) had a STEMI. During their hospitalization, 1007 patients (78%) had PCI, 119 patients (9%) received fibrinolytic therapy, and 42 patients (3%) underwent coronary artery by-pass surgery (Supplementary Table 3). Included patients received during hospitalization the recommended treatments for AMI including aspirin (98%), beta-blockers (82%), statins (89%).

During the 3-year follow-up, 189 patients (14.6%) developed MACE (death, recurrent MI or stroke). At admission, patients who developed cardiovascular events had more frequent cardiovascular risk factors, more frequent co-morbidities and more severe acute heart disease. Finally, baseline CRP levels were significantly higher in patients with future MACE (34 ± 58 vs 14 ± 32 mg/L, *P* < 0.0001) (Supplementary Table 4).

3.2. Confirmation of Plasma levels of sTREM-1 relation to long-term outcomes in AMI patients

In the cohort, mean sTREM-1 plasma levels measured by ELISA were 430 ± 211 pg/mL. The within-individual standard deviation was 12 pg/mL and the ICC was 99.3% (98.9–99.5). sTREM-1 levels were significantly higher in patients who developed MACE during follow-up (695 ±

553 vs 386 ± 342 pg/mL, *P* < 0.0001) (Supplementary Table 4). Next, we analyzed baseline clinical and biological characteristics of included patients according to sTREM-1 levels (Table 1). Co-morbidities were more frequent and acute heart disease more severe in patients with high sTREM-1 levels (Table 1). Higher sTREM-1 plasma levels at the time of admission associated with higher risk of death at one-year (Supplementary Fig. 1) and higher risk of MACE at 3 years (Fig. 1). After adjustment, sTREM-1 plasma concentration remained an independent predictor of MACE (HR_{Adj} = 1.86, 95%CI: 1.06–3.26 for tertile 1 vs tertile 3 and HR_{Adj} = 1.11, 95%CI:0.61–2.02 for tertile 1 vs tertile 2 *P* = 0.03)(Supplementary Table 5). A ROC curve showed that TREM-1, at the cut-off level of 429.8 pg/mL, had its highest combined sensitivity and specificity to predict MACE, respectively 66% (95% CI, 58–72%) and 79% (95% CI, 68–73%). The area under the ROC curve was 0.74 (95% CI, 0.68–0.77) (Supplementary Fig. 2).

3.3. Common genetic variants of *TREM1* and plasma levels of sTREM-1

The frequency of the minor alleles varied between 0.02 for rs12189634 and 0.49 for rs3804276 in the FAST-MI 2010 cohort. Five variants showed significant association with sTREM-1 levels in at least one of the genetic models tested (Supplementary Table 6). The size effects of the SNPs studied varied between 0.06 pg/mL increase per allele for rs7769759, the less associated SNP, and 0.18 pg/mL for rs3804276, the most associated one (*P* = 0.04 and *P* = 2.65 × 10⁻¹¹, respectively, Supplementary Table 6).

Four haplotypes showed evidence for association with sTREM-1 levels (Table 2). The most associated haplotype (TTCCA) had a frequency of 0.39 and was associated with 0.22 pg/mL higher sTREM-1 levels in the haplotype carriers (*N* = 504) compared to non-carriers (*N* = 789), which represents 4% of variance explained by this haplotype (*P* = 2.74 × 10⁻¹⁴, Table 2). Conditional haplotype-based association was used to identify the most contributing SNPs to the haplotype association. Two variants, rs3804276 (*P* = 2.20 × 10⁻¹⁰) and rs4714449 (*P* = 5.89 × 10⁻³), had independent contributing effects (Table 2 & Supplementary Table 7).

3.4. Functional annotation of rs4714449 and rs3804276

First, rs3804276 is an intronic variant to *TREM1* while rs4714449 is intergenic downstream the coding region. To assess the regulatory properties of these most contributing variants to the association with sTREM-1, we examined publicly available data in cells with relevant biology to TREM-1 function. The examination of histone marks in human CD14+ monocytes shows the presence of robust enhancer marks (H3K27ac and H3K4me1) in the vicinity of rs4714449, as well as strong DNase-seq signal indicating a high level of DNA accessibility (Fig. 2a). Analysis of publicly available ChIP-seq datasets showed the binding to this region of many transcription factors playing a role in the maintenance and/or differentiation of hematopoietic stem cells, including ETS-like factors GABPA and FLI1 (Fig. 2b). The presence of Ser5-phosphorylated-RNA Polymerase II in the same position in HL-60 promyeloblast cells suggests a direct involvement of the enhancer in transcription initiation (Fig. 2b-c). Transcription factor motifs prediction indicates that the rs4714449-C allele strongly increases the predicted affinity for this sequence to bind GABPA or FLI1 (Fig. 2d, Supplementary Fig. 3 and Supplementary Table 7). The ancestral (rs4714449-C) allele which is highly conserved in mammals and vertebrates (Fig. 2e), is associated with higher expression of *TREM1*, and is the one predicted to improve the recognition by ETS-like transcription factors such as GABPA or FLI1.

We also observed typical enhancer marks H3K4me1, and to a lesser extent H3K27ac, in the vicinity of rs3804276 and SNPs in strong linkage disequilibrium (Fig. 2). Although TF binding sites overlapping SNPs can be observed in the region, they are not predicted to be affected by rs3804276 or any of the SNPs in high LD with it (Supplementary

Table 1
FAST MI 2010 - baseline characteristics and hospital management of patients according to sTREM1 tertiles.

	Tertile 1	Tertile 2	Tertile 3	P value
sTREM-1 (pg/mL)	< 264.5	[264.5–444.3]	≥ 444.3	
Death, AMI or stroke (3 yrs. f/w)	28 (6.5%)	42 (10%)	119 (28%)	<0.0001
Demographic and risk factors				
Male Sex, No (%)	325 (76%)	313 (73%)	312 (72%)	0.4753
Age, yr ^a	61 ± 12	63 ± 14	69 ± 15	<0.0001
Hypertension, No (%)	196 (46%)	208 (48%)	275 (64%)	<0.0001
Hypercholesterolemia, No (%)	187 (44%)	188 (44%)	179 (41%)	0.7676
Diabetes mellitus, No (%)	78 (18%)	73 (17%)	108 (25%)	0.0061
Family history of CAD, No (%)	152 (35%)	130 (30%)	71 (16%)	<0.0001
Current smokers, No (%)	138 (32%)	189 (44%)	168 (39%)	0.0017
Prior myocardial infarction, No (%)	50 (12%)	61 (14%)	74 (17%)	0.0695
Prior stroke or TIA, No (%)	14 (3%)	13 (3%)	24 (6%)	0.1066
Prior Heart Failure, No (%)	4 (1%)	12 (3%)	26 (6%)	0.0001
Prior Respiratory Failure, No (%)	14 (3%)	17 (4%)	38 (9%)	0.0004
Chronic Renal Failure, No (%)	2 (0.5%)	3 (1%)	48 (11%)	<0.0001
Clinical presentation				
Body mass index (kg/m ²) ^a	27 ± 4	27 ± 5	26 ± 5	0.011
Systolic blood pressure at admission ^a	148 ± 27	145 ± 28	143 ± 30	0.0153
Heart rate at admission ^a	77 ± 18	78 ± 18	80 ± 22	0.0527
STEMI, No (%)	235 (55%)	238 (55%)	232 (54%)	0.9029
Killip entree class ≥2, No (%)	45 (10%)	50 (12%)	146 (34%)	<0.0001
GRACE Score ^a	126 ± 28	133 ± 31	156 ± 39	<0.0001
Left ventricular ejection fraction ^a	55 ± 10	53 ± 11	48 ± 12	<0.0001
Baseline biological exams				
CRP (mg/L) ^a	9 ± 26	12 ± 25	28 ± 51	<0.0001
sTREM-1 (pg/mL) ^a	173 ± 64	346 ± 50	773 ± 521	<0.0001
In-hospital Management				
PCI, No (%)	351 (82%)	346 (80%)	310 (72%)	0.0008
Thrombolysis, No (%)	39 (16%)	42 (17%)	38 (16%)	0.9216
Coronary artery bypass surgery, No (%)	18 (4%)	11 (3%)	13 (3%)	0.3779
Statins, No (%)	390 (91%)	400 (93%)	364 (84%)	0.0001
Beta-blockers, No (%)	379 (88%)	359 (83%)	316 (73%)	<0.0001
Calcium channel blockers, No (%)	98 (23%)	124 (29%)	107 (25%)	0.1216
ACE inhibitors or ARB, No (%)	295 (69%)	298 (69%)	255 (59%)	0.002
Nitrated derivatives, No (%)	215 (50%)	223 (52%)	190 (44%)	0.0571
Aspirin, No (%)	420 (98%)	424 (98%)	414 (96%)	0.0593
Clopidogrel, No (%)	338 (79%)	335 (78%)	340 (79%)	0.9291
LMWH, No (%)	269 (63%)	262 (61%)	215 (50%)	0.0002
Diuretics, No (%)	79 (18%)	99 (23%)	183 (42%)	<0.0001
Glycoprotein IIb/IIIa inh, No (%)	166 (39%)	188 (44%)	120 (28%)	<0.0001
Digitalis glycosides, No (%)	2 (0.5%)	1 (0.2%)	7 (2%)	0.0854

ACE: angiotensin-converting enzyme inhibitors or ARB: angiotensin receptor blockers, CABG: coronary artery bypass surgery, CAD: Coronary Artery disease,

LMWH: Low Molecular Weight Heparin, PCI: percutaneous coronary angioplasty, TIA: transient ischemic attack. †p is given by Wilcoxon signed-rank test or Kruskal-Wallis test (for continuous variables) and exact Pearson χ^2 or Fisher test (for categorical variables).

^a Mean ± sd.

Table 7). Of note, several SNPs in strong LD with rs3804276 are positioned within *TREM1* 3'UTR (e.g rs12200981, Fig. 2), supporting a potential mechanism through *TREM1* transcript stability or translation.

3.5. Association between individual and haplotype *TREM1* variants with long-term major adverse cardiovascular events

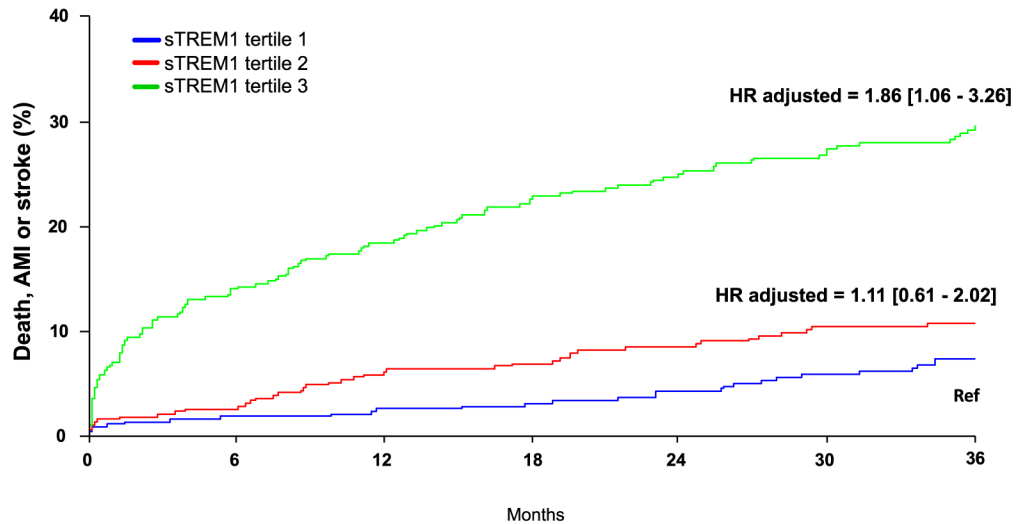
Based on experimental studies showing a critical role of TREM-1 in the pathophysiology of atherosclerosis [6], stroke [13] and post-ischemic cardiac remodeling [5], we specifically analyzed the relationship between TREM-1 variants and MACE at 3 years. We found that patients carrying the CT or TT alleles at rs4714449 in the single marker analyses ($N = 261$, supplementary Fig. 4) and those carrying the low frequency “TA” haplotype (Haplotype frequency = 0.03, supplementary Table 8) displayed lower plasma levels of sTREM-1 and tend to have lower risk of MACE at 3-year follow-up (HR = 0.61, 95%CI: 0.35–1.05, P-value = 0.07; supplementary Fig. 4).

4. Discussion

Here we investigated the clinical relevance of the TREM-1 pathogenic pathway for recurrent MACE in a nationwide cohort of AMI patients. Our data did not show a causal relationship between TREM-1 and risk of major cardiovascular events over a 3-year follow up of patients with AMI. Indeed, despite the association between higher sTREM-1 levels and increased-risk of MACE, the genetic variants in *TREM1* were not statistically associated with the risk for MACE.

The current findings replicate our previous results on the relationship between plasma sTREM-1 levels in AMI patients and long-term risk of MACE in an independent nationwide registry [5]. Similar findings have been also recently reported in a Chinese population [14]. Overall, these results, in combination with those in mouse models [5,6], support evidence for the role of TREM-1 in enhancing cardiovascular inflammation. Engagement of TREM-1 with agonist antibody has been shown to amplify the production of pro-inflammatory cytokines and chemokines in mice [4,15]. TREM-1 also stimulates rapid neutrophil degranulation and oxidative burst. At the vascular level, TREM-1 engagement accelerates atherosclerotic plaque growth, macrophage infiltration and foam cell formation [6]. At the heart level, TREM-1 promotes post-ischemic deleterious remodeling through the recruitment of innate immune cells in the myocardium and the amplification of toxic product release such as myeloperoxidase. TREM-1 pharmacological blockade is protective in non-re-perfused and re-perfused models of myocardial infarction in mouse [5], rat [5] and pig [16]. After activation, TREM-1 is shed and a soluble form of the receptor can be detected in the milieu [17]. In this study we measured sTREM-1 in the plasma but we did not specifically investigate the cellular source of TREM-1 that is known to be secreted by neutrophils, monocytes [18] and endothelial cells [19]. Differences in cell source of TREM-1 as well as in activation status of myeloid and/or endothelial cells may, at least in part, impact on sTREM-1 levels and variability between included patients.

Few studies have previously investigated the genetic determinants of sTREM-1 in the context of cardiovascular risk in large and adequately-powered cohorts. A total of eight genetic variants, most of which were tagged in the set of SNPs we studied, were reported to be marginally associated with coronary atherosclerosis in a Russian study of ~100 patients and 300 controls and no effect on sTREM1 plasma levels was described [20]. A recent study reported significant association between rs2234246, also tagged by SNPs we analyzed, and plasma levels of sTREM-1 in a population-based sample of ~500 French healthy



No.at Risk				
sTREM-1 T1	430	388	344	287
sTREM-1 T2	431	373	343	405
sTREM-1 T3	432	337	286	366

Fig. 1. MACE occurrence according to sTREM-1 tertiles during 3-year follow-up.

Table 2

Association between haplotypes formed by individually associated common SNPs and TREM-1 protein levels.

Haplotype	Frequency	Beta	R ²	P-value
TTCCA	0.39	0.22	0.04	2.74×10^{-14}
CAT TG	0.06	-0.26	0.02	7.63×10^{-06}
TTCCG	0.38	-0.12	0.01	8.80×10^{-05}
TTCTG	0.02	-0.44	0.01	1.29×10^{-04}
CATCG	0.01	-0.26	2.22×10^{-3}	0.09
CTCCG	0.03	-0.14	1.54×10^{-3}	0.16
CATTA	0.02	-0.02	3.36×10^{-5}	0.84
CTCCA	0.07	0.01	1.57×10^{-5}	0.89
Independent effect of rs3804276:				2.20×10^{-10}
Independent effect of rs4714449:				5.89×10^{-03}

Haplotypes frequencies were estimated and the quantitative association was tested by linear regression in PLINK. The independent effect of each variant was tested and is indicated for rs3804276 and rs4714449, which significantly contribute the associations of haplotypes. BETA: Regression coefficient; R²: Proportion variance explained.

individuals that explained 33% of sTREM-1 variance [21] whereas in our large cohort, only ~4% variance of plasma levels of s-TREM1 was explained by a common haplotype of genetic variants. Such discrepancy indicates an important misestimation of the effect sizes of the variants analyzed in that study, given its limited sample.

Despite the confirmation of the independent relationship between high levels of sTREM-1 and MACE, genetically raised concentrations of sTREM-1 were not related to recurrent outcomes during the 3 years follow-up of patients with AMI. In the present study, rs4714449 and rs3804276 *TREM1* variants were associated with plasma sTREM-1 levels but none of them were significantly associated with MACE at follow-up. Nonetheless, the observed trend for an association between higher risk of MACE in AMI patients with CT or TT genotypes at rs4714449 or the low frequency of "TA" haplotype combining both variants may deserve further investigation and an attempt of replication. We have developed and patented a promising TREM-1 pharmacological inhibitor, named LR-12, that protects against atherosclerosis development [6], post-ischemic cardiac remodeling [5] and sepsis-related mortality in mice [22]. Such pharmacological agent is currently under investigation in a

phase II trial in septic shock patients [23]. However, the demonstration of the causal role of TREM-1 on MACE in patient with AMI is mandatory prior to the evaluation of TREM-1 blockers in AMI patients as a preventive treatment. Nevertheless, given that soluble TREM-1 reflects myeloid cell activation and is independently associated with both 1-year mortality and 3-year MACE, we speculated that sTREM-1 could be used as an inflammatory biomarker on top of CRP to select high risk AMI patients for immunomodulatory preventive treatment [24].

This work presents however several limitations. The observational nature of this study does not allow a cause-effect demonstration, but it should be noted that the study was a prospective nationwide registry that closely represents everyday up-to-date current clinical practice. Also, sTREM-1 plasma concentrations were measured once within 48 h of the symptom. Therefore, intra-individual variations in sTREM-1 levels according to the precise timing after the onset of the acute event was not assessed in this setting. The genetic study of sTREM1 determinants was limited to eQTLs identified in whole blood and additional genetic variants, either through *trans* eQTLs located in other loci, or indirect regulators of transcript or protein levels that were not covered by this study may also play a role. Finally, given the low frequency (Freq = 0.03) of the most relevant haplotype to s-TREM1 variability, and the ratio of number of patients with events per total number of patients followed up (0.18), the study power is only adequate to detect large effects (HR ≥ 1.95) of *TREM-1* genetic variants in predicting MACE. Based on this statistical observation, we believe that studies in larger cohort on MI patients are needed to assess the relationship between TREM-1 genetic variants and outcome.

In summary, despite its relationship with increased risk of death, recurrent MI and stroke, genetic determinants of plasma levels of sTREM-1 were not found to be causal prognostic factors in patients with acute myocardial infarction in the FAST-MI study.

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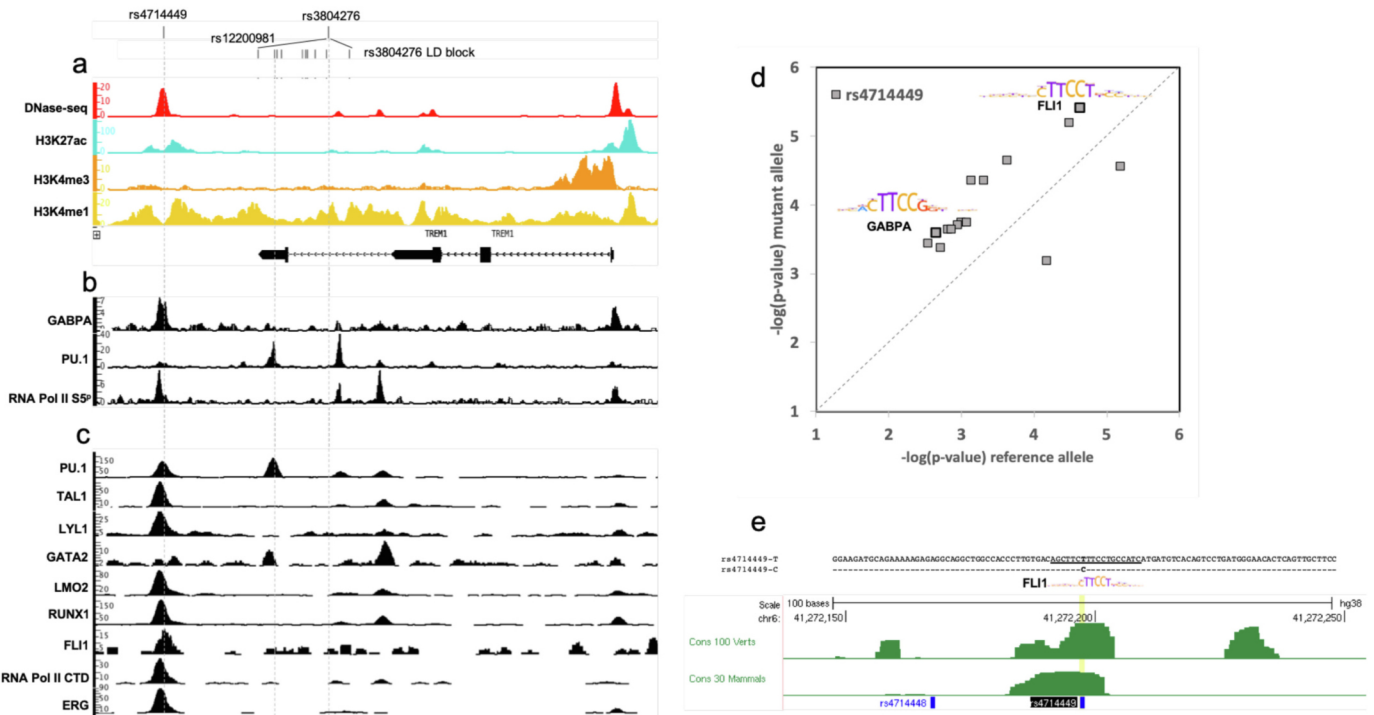


Fig. 2. Potential regulatory properties of sTREM-1 associated genetic variants.

a-c) Functional annotation based on ENCODE data from different blood cells. a) Visualization of histone marks and DNase-seq density profiles in CD14+ monocytes. Position of SNPs rs3804276 and rs4714449 are shown, as well as the position of SNPs in high ($r^2 > 0.9$) linkage with rs3804276 in European populations. b) Visualization of transcription factors (PU.1 and GABPA) and RNA Polymerase II S5-phosphorylation density profiles in HL-60 promyeloblast cell line. c) Visualization of transcription factors (PU.1, TAL1, LYL1, GATA2, LMO2, RUNX1, FLI1 and ERG) and RNA Polymerase II C-terminal domain density profiles in the acute myeloid leukemia cell line TSU-1621MT. d) In silico prediction of the effects of rs4714449 allele on binding of transcription factors. The plots represent the negative logarithm of the p -values of motif predictions using reference T allele (x-axis) or variant C allele (y-axis). Dots on the upper left part of the graph represent motifs that better match with the variant allele compared to the reference allele. Dots on the lower right part represent motifs better match with the reference allele compared to the variant allele. e) Position of the potential FLI1 binding site and sequence conservation in the sequence 50 bp upstream and downstream of rs4714449. Phastcons conservation scores for 100 vertebrates and 30 mammals are represented and were obtained from UCSC genome browser (<http://genome.ucsc.edu>).

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Disclosures

Sébastien Gibot is co-founder of INOTREM, a company developing TREM-1 inhibitors. Amir Boufenzar, Sébastien Gibot, Marc Derive, Tabassome Simon, Nicolas Danchin and Hafid Ait-Oufella applied a patent on the measurement of plasma sTREM-1 concentration to predict outcome following acute myocardial infarction and applied a patent on therapeutic strategies using inhibiting peptides derived from TREM-1.

Declaration of Competing Interest

None.

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