J Mol Med (2008) 86:1153–1161
DOI 10.1007/s00109-008-0375-6

ORIGINAL ARTICLE

Polymorphisms of the tumor necrosis factor-alpha (TNF) and the TNF-alpha converting enzyme (TACE/ ADAM17) genes in relation to cardiovascular mortality: the Athero*Gene* study

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Received: 3 March 2008 / Revised: 2 May 2008 / Accepted: 28 May 2008 / Published online: 4 July 2008

Abstract Tumor necrosis factor (TNF) is a major cytokine involved in inflammatory reaction and a mortality predictor in patients with coronary artery disease (CAD). Plasma levels of soluble TNF (sTNF) depend on the rate of its synthesis but also on its shedding from cell surface, a mechanism mainly regulated by the TNF alpha converting enzyme (TACE or ADAM17). We investigated the relationship between *ADAM17* and *TNF* polymorphisms, circulating levels of shed ADAM17 substrates (sTNF, sTNFR1 and sTNFR2), and cardiovascular risk in a prospective cohort of CAD patients. Five tag single-nucleotide polymorphisms (SNPs) of the *ADAM17* gene as well as four previously described *TNF* SNPs were genotyped in the Athero*gene* Study composed of 1,400 CAD patients among which 136 died from a cardiovascular (CV) cause. sTNF, sTNFR1, and sTNFR2 concentrations were all significantly elevated in patients with future CV death, independently of other clinical/biological variables. While none of the studied *TNF* SNPs was associated with sTNF, sTNFR1, nor sTNFR2 levels, the *ADAM17* −154A allele was found associated with a 14% increase of sTNF levels as compared to the −154C allele ($p=0.0066$). Moreover, individuals carrying the 747Leu allele displayed a borderline increased risk of future cardiovascular death

Electronic supplementary material The online version of this article (doi:10.1007/s00109-008-0375-6) contains supplementary material, which is available to authorized users.

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[odds ratio, 2.06 (1.05–4.04), p=0.03]. These results suggest a role of *ADAM17* in the regulation of sTNF plasma levels and identifies *ADAM17* gene as a candidate for CAD. Tumor necrosis factor (TNF) is a major cytokine involved in inflammatory reaction and a mortality predictor in patients with coronary artery disease (CAD). We have studied the association of *ADAM17* and *TNF* polymorphisms with circulating levels of shed ADAM17 substrates (sTNF, sTNFR1 and sTNFR2) and with cardiovascular risk in a large population of individuals with CAD (Athero*gene* Study, n=1,400). Two newly identified polymorphisms, obtained by a systematic sequencing of the *ADAM17* gene, C-154A and Ser747leu, slightly influence respectively sTNF plasma levels and the risk of cardiovascular death.

Keywords ADAM17 · Coronary artery disease · Tumor necrosis factor · Inflammation

Introduction

Among the numerous molecules involved in the inflammatory reaction, several lines of evidence support a role for tumor necrosis factor-α (TNF) in the development of atherosclerosis and its complications [1]. High levels of plasma-soluble TNF (sTNF) and of its soluble receptors (sTNFR1 and sTNFR2) are considered as predictors of cardiovascular events [2–4].

The increased levels of sTNF and of its receptors in coronary artery disease (CAD) may be attributable to environmental stimuli and/or genetic variations that affect the production of these molecules. The promoter region of the *TNF* gene contains several single-nucleotide polymorphisms (SNPs) which may influence gene expression [5, 6]; however, their influence on sTNF levels is still unclear [7]. sTNF level in plasma is not solely dependent on its synthesis but also on its shedding from cell surface. Among factors that regulate this shedding, the TNF-alpha-converting enzyme (TACE) is of great importance [8, 9]. TNF is synthesized as a 26-kDa precursor localized as a transmembrane molecule (tmTNF) which is cleaved mainly by TACE to yield the 17-kDa soluble form that circulates in plasma. TACE (referenced as *ADAM17* in Online Mendelian Inheritance in Man) belongs to the disintegrin metalloproteinases family and possesses, in addition to TNF, a large number of substrates (38 has been reported so far, generally from cell-based assays) including several molecules involved in atherosclerosis such as the TNF receptors TNFR1 and TNFR2 [10]. The *ADAM17* gene is located on chromosome 2 and comprises 19 exons spanning 55 kb. TACE is constitutively expressed and plays an essential role in development as a genetic deficiency impairing its activity leads to early death after birth in mice [11]. The influence of TACE in atherosclerosis has been poorly studied. We have shown in apoE$^{-/-}$ mice, a model of genetically induced atherosclerosis, that TACE expression increased in aortic lesions together with their development [12]. A higher vascular shedding activity of TACE substrates (TNF and its receptors) measured *ex vivo* from aortic explants of these mice was observed, which may account for the observed elevated plasma levels of sTNFR1 and sTNFR2 [12]. This underlines the predominant role of TNF shedding via TACE activity in the inflammatory reaction occurring in the lesion [13]. A possible role of TACE on vascular complications of diabetes has been also recently suggested from data obtained in mice [14]. In humans, very few data are available on the relation between TACE and CAD. Monocytes from patients with complicated CAD have been shown to express higher amounts of TACE and TNF mRNA than those without complications [15]. Thus, the ability of TACE to shed TNF could be critical for the development of complications of CAD in humans.

We hypothesized that any SNP influencing TNF or TNF receptor levels might influence the risk of complications in CAD. To test this hypothesis, we studied in a large population of CAD patients (the Athero*gene* cohort) the association of *ADAM17* and *TNF* SNPs with circulating levels of sTNF and its receptors, sTNFR1 and sTNFR2, and with the risk of future cardiovascular death. For *ADAM17*, the SNPs were selected from a prior systematic screening of the gene and for *TNF*, from those previously published [16].

Materials and methods

Study population

Detailed description of the Athero*Gene* study has been provided elsewhere [17]. Between November 1996 and June 2000, 1,400 CAD patients were recruited on the occasion of a diagnostic coronary angiography at the Department of Medicine II of the Johannes Gutenberg-University Mainz and the Bundeswehrzentralkrankenhaus Koblenz. A priori inclusion criterion was the presence of a diameter stenosis >30% in at least one major coronary artery. Exclusion criteria were evidence of significant concomitant diseases, in particular hemodynamically significant valvular heart disease, known cardiomyopathy, and malignant diseases, as well as febrile conditions. Patients were followed up during a median period of 5.9 (maximum 7.6) years, and 136 patients died from a cardiovascular cause (fatal myocardial infarction, sudden cardiac death, or death from vascular causes). Information about the cause of death was obtained from the hospital or general practitioner.

Study participants had German nationality, were inhabitants of the Rhein-Main area, and were of European ethnic

origin. The study was approved by the ethics committee of the University of Mainz. Each participant gave written informed consent.

Laboratory methods

Serum sTNF, sTNFR1, and sTNFR2 levels were measured with commercially available enzyme-linked immunosorbent assay (EASIA, Biosource Europe). C-reactive protein (CRP) was determined by a highly sensitive, latex particle-enhanced immunoassay (Roche Diagnostics). Lipid serum levels were measured immediately as previously described [18].

Molecular screening, selection and genotyping of polymorphisms

A molecular screening of the regulatory, coding, and flanking intronic regions of the *ADAM17* gene was performed by comparing 80 chromosomes from 40 unrelated healthy Caucasians living in the Marseilles area (France), by direct sequencing of polymerase chain reaction (PCR)-amplified products. Search for additional polymorphisms of the *ADAM17* gene was performed in the dbSNP database (www.ncbi.nlm.nih.gov/SNP). All polymorphisms, either identified by our molecular screening or selected from the dbSNP database, were genotyped in a sample of 474 healthy Caucasians from the Marseilles area. Haplotype analysis of these data enabled us to determine a limited set of tag SNPs characterizing the common *ADAM17* gene haplotypes. These tag SNPs were then genotyped in the Athero*Gene* study.

Four polymorphisms located in the promoter region of *TNF*, C-863A (rs1800630), C-857T (rs1799724), G-308A (rs1800629), and G-238A (rs3615525), were additionally genotyped in the Athero*Gene* study. These SNPs were chosen from a systematic screening of the promoter and coding regions of the *TNF* gene previously published [16]. Genotyping was performed by TaqMan assays [Applied Biosystems (ABI)]. Briefly, PCR primers and TaqMan MGB probes were designed with Primer Express version 2.0. Reactions were performed in 96-well microplates with GeneAmp 9700 thermal cyclers. Fluorescence was measured with an ABI Prism 7000 sequence detection system and analyzed with the ABI Prism 7000 SDS software version 1.0. Primer and probe sequences as well as amplification conditions for genotyping can be found at the GeneCanvas web site (www.genecanvas.org).

Statistical analysis

Association of SNPs with continuous sTNF, sTNFR1, and sTNFR2 levels was tested by linear regression analysis. sTNF concentrations were square-root transformed, and sTNFR1 and sTNFR2 concentrations were log-transformed to remove positive skewness. Cox proportional-hazards regression analysis was used to assess the association of SNPs, baseline sTNF, sTNFR1, and sTNFR2 levels with cardiovascular mortality. Linkage disequilibrium (LD) and haplotype analyses were performed using the THESIAS software [19]. All other analyses were performed with the SAS software, version 9.1 (SAS Institute, Cary, NC,USA).

Results

Polymorphisms of the *ADAM17* gene

Our molecular screening of the *ADAM17* gene identified 19 polymorphisms in the regions analysed, 11 being already reported in dbSNP and eight being new. Analysis of dbSNP revealed two additional polymorphisms not detected by our screening. Location of these 21 polymorphisms is represented in Fig. 1.

All nonsynonymous SNPs and any other polymorphism whose allele frequency was higher than 0.025 (i.e., those observed in more than two of 80 chromosomes) were genotyped in 474 healthy subjects to characterize the haplotype structure of the gene. Allele frequencies and LD among the polymorphisms are reported in ESM Table 1. The Arg202Gly polymorphism, previously reported in an African-descent population, was not observed in our sample. The Pro491Ser polymorphism was found to be very rare (ESM Table 1). After exclusion of rare or redundant polymorphisms, the haplotypic structure derived from 11 remaining polymorphisms was characterized (ESM Table 2). Seven common haplotypes were inferred, accounting for about 91% of all chromosomes observed. Five tag SNPs, G-703A, C-172T, C-154A, Ser608Ser (C/T), and Ser747Leu (C/T), were sufficient to characterize these haplotypes. These five polymorphisms were then genotyped in the Athero*Gene* Study.

Baseline characteristics of patients with and without cardiovascular death

The baseline characteristics of the patients according to cardiovascular mortality are reported in Table 1. Patients with future cardiovascular death were older, had a higher prevalence of diabetes, lower high-density lipoprotein (HDL)-cholesterol, and higher CRP levels than those who survived. They also had marked elevations of serum sTNF, sTNFR1, and sTNFR2 levels. sTNF was moderately correlated with sTNFR1 (p=0.18) and sTNFR2 (p=0.26), the two receptors being strongly correlated one to each other (p=0.61; ESM Table 3). They were also correlated with parameters of the metabolic syndrome and CRP levels

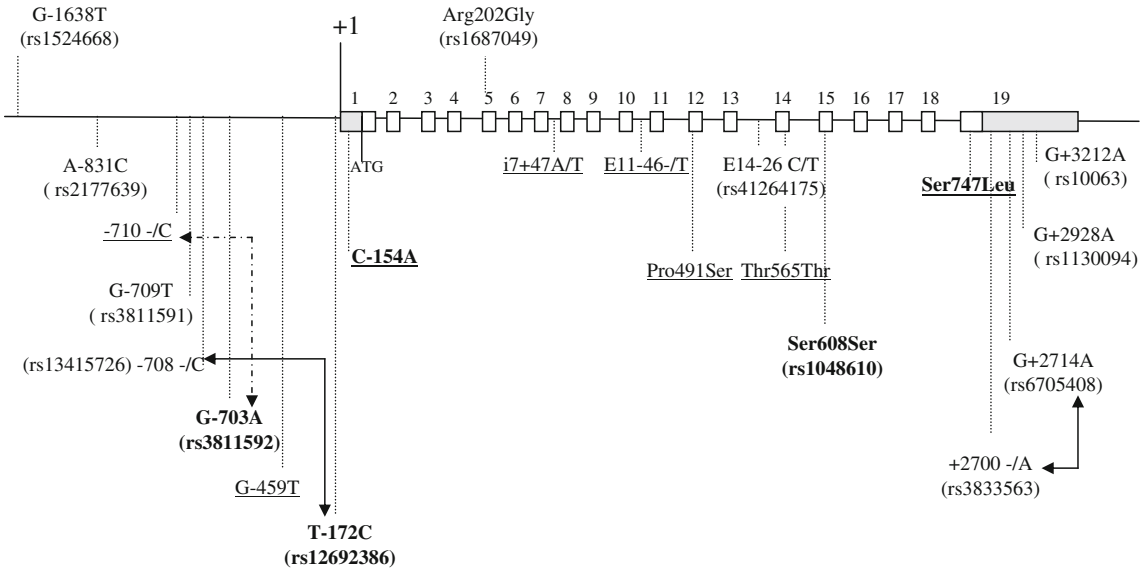

Fig. 1 Localization of identified and/or genotyped SNPs in the *ADAM17 (TACE)* gene. Exons are depicted as *white boxes* for coding sequences and *grey boxes* for untranslated sequences. SNPs found by our molecular screening are represented *below the gene* (*underlined* are SNPs that were not previously known). SNPs extracted from dbSNP are represented *above the gene*. *Double arrows* connect completely associated polymorphisms. tag SNP genotyped in the AtheroGene study are shown in *bold*

Table 1 Baseline characteristics of patients with and without cardiovascular death

Characteristic	No cardiovascular death N=1,264	Cardiovascular death N=136	p
Age, year	61 (10)	67 (9)	<0.0001
Male sex	923 (73)	100 (74)	0.90
Acute coronary syndrome	349 (30)	36 (28)	0.59
Number of stenosed coronary arteries			
1 vessel	312 (25)	27 (20)	
2 vessels	352 (28)	38 (28)	0.42
≥3 vessels	600 (47)	71 (52)	
Body mass index (kg/m^2)	27 (4)	27 (4)	0.57
Current smoker	156 (12)	24 (18)	0.08
Diabetes mellitus	181 (14)	38 (28)	0.0001
Hypertension	900 (71)	102 (75)	0.35
Medications at enrollment			
Antiplatelet therapy	1,085 (86)	110 (81)	0.12
Statins	431 (34)	32 (24)	0.01
β-Blocker	733 (58)	53 (39)	0.0001
ACE inhibitor	575 (45)	87 (64)	0.0001
Calcium antagonists	209 (17)	27 (20)	0.33
Total cholesterol (mg/dl)	221 (45)	215 (45)	0.15
HDL cholesterol (mg/dl)	49 (15)	45 (16)	0.0037
Triglycerides (mg/dl)[a,b]	140 (102–196)	156 (114–223)	0.05
CRP (mg/l)[a,b]	4.07 (1.93–10.81)	7.34 (2.34–19.90)	<0.0001
sTNF (pg/ml)[a,b]	21.6 (15.8–27.8)	25.9 (19.3–34.0)	0.0065
sTNFR1 (pg/ml)[a,b]	1,704 (1,176–2,355)	1,951 (1,445–2,932)	<0.0001
sTNFR2 (pg/ml)[a,b]	3,030 (2,300–3,989)	3,987 (2,984–4,909)	<0.0001

Values are mean (SD) or median (25th–75th percentiles) for quantitative variables, and n (%) for qualitative variables.
[a] Median
[b] For skewed variables, statistical comparison between groups was performed on log- or square root-transformed values.

Table 2 Hazard ratio [95% confidence interval (CI)] for cardiovascular mortality associated with an increase of one standard deviation of baseline circulating sTNF, sTNFR1, and sTNFR2 levels

	sTNF	sTNFR1	sTNFR2
Model 1			
HRs (95% CI)	1.27 (1.13–1.42)	1.39 (1.17–1.65)	1.52 (1.33–1.74)
	$p<0.0001$	$p<0.0001$	$p<0.0001$
Model 2			
HRs (95% CI)	1.22 (1.08–1.38)	1.30 (1.09–1.54)	1.45 (1.26–1.67)
	$p=0.0017$	$p=0.0033$	$p<0.0001$
Model 3			
HRs (95% CI)	1.23 (1.08–1.39)	1.21 (1.01–1.45)	1.41 (1.22–1.63)
	$p=0.0017$	$p=0.04$	$p<0.0001$

Model 1, adjusted for age, sex; model 2, model 1 additionally adjusted for cardiovascular risk factors (diabetes, smoking status, HDL-cholesterol, triglycerides); model 3, model 2 additionally adjusted for baseline CRP levels. Skewed variables were log (triglycerides, CRP, sTNFR1 and sTNFR2) or square root (sTNF)-transformed

and increased with diabetes (ESM Table 3). Smokers had higher levels of sTNFR1. These correlations were of similar magnitude in individuals with or without future cardiovascular death (data not shown).

Association of baseline circulating sTNF, sTNFR1, and sTNFR2 levels with cardiovascular mortality during follow-up

Baseline sTNF, sTNFR1, and sTNFR2 levels were predictive of future cardiovascular mortality. After adjustment for age and sex, the risk of cardiovascular mortality increased with increasing levels of sTNF [hazard ratio (HR), 1.27 (1.13–1.42)], sTNFR1 [HR, 1.39 (1.17–1.65)] and sTNFR2 [HR, 1.52 (1.33–1.74); Table 2]. Adjusting on risk factors related to cardiovascular mortality (age, history of diabetes, smoking status, HDL-cholesterol, triglycerides) slightly decreased these associations. Further adjustment for CRP hardly modified the associations (Table 2). These associations were observed both in individuals with acute coronary syndrome (ACS) and those with angina pectoris at baseline (data not shown).

Association of *ADAM17* gene polymorphisms with baseline serum levels of sTNF, sTNFR1, and sTNFR2

Allele frequencies of the nine studied polymorphisms (five for *ADAM17* and four for *TNF*) are reported in Table 3. Genotype distributions were compatible with Hardy–Weinberg equilibrium.

The *ADAM17* C-154A polymorphism was significantly associated with sTNF and sTNFR2 levels (ESM Table 4). Under an additive model, the −154A allele was associated with a 14% increase of sTNF ($p=0.0066$) and a 5% increase of sTNFR2 ($p=0.04$). After adjusting for sTNF, the −154A was no longer associated with sTNFR2 ($p=0.17$), while it was still associated with sTNF ($p=0.007$) when adjusting for sTNFR2. Carriers of the 747Leu allele tended to have higher sTNF levels than 747Ser/Ser individuals; however, this difference did not reach signif-

Table 3 Allele frequencies and pairwise linkage disequilibrium (D′) coefficients among *ADAM17* and *TNF* gene polymorphisms in the Athero*Gene* Study

	Minor allele frequency	G-703A	C-172T	C-154A	Ser608Ser	C-863A	C-857T	G-308A
ADAM17								
G-703A	0.16							
C-172T	0.32	−0.91***						
C-154A	0.19	−1.0***	−0.99***					
Ser608Ser	0.40	0.16**	0.98***	−0.99***				
Ser747Leu	0.02	−1.0*	−1.0***	+1.0***	−1.0***			
TNF								
C-863A	0.15							
C-857T	0.10					−1***		
G-308A	0.17					−0.89***	−1***	
G-238A	0.04					−1***	−1**	−0.81***

***$p<0.001$; **$p<0.01$; *$p<0.05$

Table 4 Effects of *ADAM17* gene haplotypes on sTNF levels adjusted for age and sex

Haplotypes[a]	Frequency	Mean level[b] (95% CI)
GCCCSer	0.33	1.79 (1.41–2.17)
GTCTSer	0.32	1.85 (1.49–2.20)
GCACSer	0.17	1.99 (1.61–2.39)
ACCCSer	0.08	1.75 (1.29–2.20)
ACCTSer	0.08	1.65 (1.16–2.13)
GCACLeu	0.02	2.32 (1.79–2.86)

Likelihood ratio test of the global haplotypic effect: $\chi^2 = 11.3$ with 5 *df*, $p = 0.04$

[a] Polymorphisms are ordered according to their position on the genomic sequence: G-703A, C-172T, C-154A, Ser608Ser (C/T), and Ser747Leu.

[b] sTNF level (square-root transformed) associated with one dose of each haplotype. The mean level of an individual is the sum of the mean levels of the two haplotypes he (she) carries.

icance ($p=0.11$; ESM Table 4). Only one individual was homozygous for the 747Leu variant, which exhibited a fourfold higher level of sTNF than homozygotes for the wild-type allele (ESM Table 4). *ADAM17* haplotypes were significantly associated with sTNF levels ($p=0.04$) and explained 1.7% of the interindividual variability (Table 4). The association was mostly due to the effect of the C-154A SNP. By comparison to the most frequent haplotype GCCCSer, the two haplotypes carrying the −154A allele, GC*A*CSer and GC*A*CLeu, were associated with an 11% ($p=0.03$) and a 30% ($p=0.01$) increase in sTNF levels, respectively, these two effects being not significantly different ($p=0.15$ for the comparison of the two effects). No association was observed between sTNFR1or sTNFR2 levels with *ADAM17* haplotypes (data not shown).

None of the *TNF* polymorphisms was associated with sTNF or sTNF receptor levels either by single locus (ESM Table 4) or by haplotype analysis (data not shown).

Association of *ADAM17* and *TNF* polymorphisms with cardiovascular mortality during follow-up

Carriers of the *ADAM17*/747Leu allele were more frequent in individuals with future cardiovascular death compared to those without (7% vs 3%, respectively, $p=0.04$, Table 5), leading to an HR of 2.06 (1.05–4.04; $p=0.03$). Adjustment for sTNF levels did not modify the association ($p=0.03$). None of the four other *ADAM17* polymorphisms were associated with cardiovascular mortality during follow-up. The effect of the 747Leu isoform was confirmed by the haplotype analysis showing that individuals carrying the GCACLeu haplotype displayed an increased risk of future cardiovascular death [HR, 2.17 (1.12–4.20)] by comparison to carriers of the reference haplotype (Table 6).

Table 5 Association of *ADAM17* and *TNF* gene polymorphisms with cardiovascular mortality during follow-up

	Patients without cardiovascular death *n* (%)	Patients with cardiovascular death *n* (%)	*p*[a]
ADAM17			
G-703A			
GG	902 (72)	89 (71)	
AG	315 (25)	34 (27)	0.53
AA	42 (3)	2 (2)	
C-154A			
CC	832 (67)	77 (61)	
CA	377 (30)	45 (35)	0.34
AA	43 (3)	5 (4)	
C-172T			
CC	586 (47)	63 (50)	
CT	541 (43)	50 (40)	0.67
TT	123 (10)	13 (10)	
Ser608Ser			
CC	436 (35)	50 (38)	
CT	639 (50)	56 (43)	0.17
TT	188 (15)	25 (19)	
Ser747Leu			
SerSer	1,224 (97)	127 (93)	
SerLeu	40 (3)	8 (6)	0.040[b]
LeuLeu	0	1 (1)	
TNF			
C-863A			
CC	840 (67)	78 (60)	
CA	361 (29)	47 (36)	0.20
AA	41 (3)	6 (4)	
C-857T			
CC	931 (80)	103 (79)	
CT	228 (19)	24 (19)	0.21
TT	7 (1)	2 (2)	
G-308A			
GG	857 (72)	96 (73)	
GA	304 (26)	33 (25)	0.73
AA	27 (2)	2 (2)	
G-238A			
GG	1,155 (91)	126 (93)	
GA	102 (8)	9 (6)	0.60
AA	4 (1)	1 (1)	

[a] Test for difference in genotype distribution (2 *df*)

[b] SerSer vs SerLeu + LeuLeu

Polymorphisms of the *TNF* gene did not exhibit any significant association with cardiovascular mortality by single-locus analysis (Table 5). However, haplotype analysis indicated that the ACGG haplotype was associated with a slight increased risk of cardiovascular death [HR, 1.41 (1.01–1.98); $p=0.04$] by comparison to the *C*CGG haplo-

Table 6 Haplotype analysis of *ADAM17* gene polymorphisms in relation to cardiovascular death

Haplotypes[a]	Haplotype frequency		Haplotypic HR (95% CI)
	Patients without CV death *N*=1,264	Patients with CV death *N*=136	
GCCCSer	0.34	0.32	–[b]
GTCTSer	0.31	0.31	1.03 (0.74–1.44)
GCACSer	0.17	0.17	1.04 (0.68–1.68)
ACCCSer	0.08	0.06	0.73 (0.38–1.39)
ACCTSer	0.08	0.09	1.15 (0.68–1.96)
GCACLeu	0.02	0.04	2.17 (1.12–4.20)

p=0.22 for the global haplotypic effect (χ^2 =5.78 with 4 *df*).
[a] Polymorphisms are ordered according to their position on the genomic sequence: G-703A, C-172T, C-154A, Ser608Ser (C/T) and Ser747Leu.
[b] Haplotype used as the reference

type taken as reference (Table 7). These two haplotypes differ only by the C/A substitution at position −836. No interaction was observed between *TNF* and *ADAM17* polymorphisms on the risk of cardiovascular death (data not shown).

Discussion

In the present study, two newly identified polymorphisms of the *ADAM17* gene, C-154A and Ser747Leu, were found to influence sTNF plasma levels and the risk of cardiovascular death, respectively.

In clinical studies, both sTNF and sTNFR plasma levels have been shown to be associated with the risk of cardiovascular event [2–4]. In the present study, baseline levels of sTNF, sTNFR1, and sTNFR2 were each found associated with the risk of cardiovascular death independently of conventional cardiovascular risk factors. The association remained significant after further adjustment for CRP levels.

TACE is considered as a key element in the TNF shedding and, by this way, might contribute to atherosclerosis and its complications [8, 9]. The gene encoding TACE, *ADAM17*, was shown to be highly polymorphic. We found that the *ADAM17*/C-154A SNP was associated with sTNF levels in a fairly additive fashion. C-154A is located in the proximal promoter region and might have a functional significance as it is strictly conserved among different species. This association remained significant (corrected p value=0.033) when the multiple testing method proposed by Li and Ji [20] was applied to correct for LD between *ADAM17* SNPs. A promoter analysis using the SNPinspector or Matinspector tools from the Genomatix suite (http://genomatix.de) did not return any significant consensus at this site. The C-154A SNP was in complete association with three other SNPs, C-831A in the promoter region, C-26T in intron 13, and G+3212A in the 3′ untranslated region region. These three polymorphisms did not seem to display any functional potentiality. It must be underlined that the C-154A SNP explained only a small fraction of sTNF variability (1.7%) and that this effect may not be of clinical relevance. However, sTNF levels may not reflect expression in tissues, and the polymorphism may have a stronger effect locally in the plaque.

The present study also suggested that carriers of the ADAM17/747Leu isoform may be at higher risk of cardiovascular death. The frequency of the 747Leu isoform was two times higher (3.7%) in individuals who died from cardiovascular death than in patients still alive by the end of

Table 7 Haplotype analysis of *TNF* gene polymorphisms in relation to cardiovascular death

Haplotypes	No events *N*=1,264	Cardiovascular death *N*=136	Haplotypic HR (95% CI)
CCGG	0.53	0.47	–[a]
ACGG	0.18	0.23	1.41 (1.01–1.98)
CCAG	0.15	0.14	1.05 (0.71–1.56)
CTGG	0.10	0.11	1.18 (0.78–1.78)
CCGA	0.04	0.04	0.96 (0.52–1.77)

Polymorphisms are ordered according to their position on the genomic sequence: C-863A, C-857T, G-308A and G-238A. p=0.31 for the global haplotypic effect (χ^2 =4.82 with 4 *df*).
[a] Haplotype used as reference

the follow-up (1.6%), while in these patients, the allele frequency was similar to that observed in a random sample of 474 healthy French Caucasians (1.8%). However, this association was no longer significant after correcting for multiple testing, and replication would be required in larger cohorts as this nonsynonymous SNP is not common.

The Ser747Leu residue is located in a potential SH3-binding domain of the cytoplasmic tail of the protein. SH3 domains are able to bind prolin-rich sequences and are involved in signal transduction regulation [21], so this residue might be important in the regulation of *ADAM17* activation. The 747Leu allele remained associated with cardiovascular death after controlling for sTNF levels, suggesting that this effect was not mediated by modifications of circulating sTNF levels. However, TACE is responsible for the shedding of other molecules than TNF that may underlie the association with the polymorphism [10].

Several SNPs located in the promoter of the *TNF* gene have been shown to modify TNF expression in vitro [5, 6]; however, their effect on plasma levels is still unclear [7]. In the present study, we found no relation between these polymorphisms and sTNF serum levels. This is in agreement with the lack of effect reported in a recent study examining a broad spectrum of cardiovascular phenotypes in relation to TNF polymorphisms [22].

In conclusion, these results suggest a role for *ADAM17* in the regulation of sTNF serum levels and identify a rare nonsynonymous polymorphism of the *ADAM17* gene (Ser747Leu) which might confer an increased risk of cardiovascular death.

Acknowledgments This work was supported by a grant of the Programme National de Recherches sur les Maladies Cardiovasculaires 2006 (A06034AS), by the Fondation de France (no. 2006002581), by the "Stiftung Rheinland-Pfalz für Innovation", Ministry for Science and Education (AZ 15202-386261/545), Mainz, by the MAIFOR grant 2001 of the Johannes Gutenberg-University Mainz, Germany, and by a grant from the French Ministry of Research (ACI IMPBIO no. 032619).

Appendix

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