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# ABCA1 impacts athero-thrombotic risk and 10-year survival in a contemporary secondary prevention setting

Jakub J. Regieli[a,b], Pieter A. Doevendans[a], Diederick E. Grobbee[b], Aeilko H. Zwinderman[c], Yolanda van der Graaf[b], John J.P. Kastelein[d], J. Wouter Jukema[e,f,*,1]

[a] University Medical Center Utrecht, Department of Cardiology, The Netherlands
[b] University Medical Center Utrecht, Juliuscenter for Health Sciences and Primary Care, The Netherlands
[c] Academical Medical Center Amsterdam, Department of Clinical Epidemiology, The Netherlands
[d] Academical Medical Center Amsterdam, Department of Vascular Medicine, The Netherlands
[e] Interuniversity Cardiology Institute of the Netherlands (ICIN/KNAW), Leiden University Medical Center, Department of Cardiology, The Netherlands
[f] Durrer Center for Cardiogenetic Research, Amsterdam, The Netherlands

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ABSTRACT

*Objectives:* We prospectively investigated the effects of ATP-binding cassette protein-1 (ABCA1) variants on long-term clinical outcome in patients with coronary artery disease (CAD).
*Background:* ABCA1 is implicated in the etiology of atherothrombosis and may offer a target to reduce cardiovascular risk. However, the impact of ABCA1 on recurrent cardiovascular disease in a secondary prevention setting is as of yet unknown.
*Methods:* We studied cause-specific 10-year mortality and quantitative coronary angiography data from the Regression GRowth Evaluation Statin Study (REGRESS), comprising 884 male CAD patients genotyped for promoter variants encompassing a proximal regulatory region (rs2422493, rs1800976, rs2740483 and rs1800977). Kaplan–Meier, proportional hazards and haplotype analyses were used to ascertain single-variant and multi-marker effects on absolute risk and extent of CAD.
*Results:* Protection from 10-year vascular death could be attributed to the rs2422493 genotype (available in 639 patients) T allele with absolute risk decreasing stepwise from 12.2% to 8.6% to 4.7% per each added allele copy, HR 0.64, $p = 0.03$ and HR 0.53, $p = 0.04$ in the TGCC haplotype context. The TGCC ($p = 0.04$) and TCCT ($p = 0.003$) haplotypes exhibited less extensive CAD.
*Conclusions:* On a background of contemporary secondary prevention, variation in the *ABCA1* promoter influences 10-year risk of vascular death and angiographic extent of CAD in men. These insights contribute to identification of patients sharing a specific prognosis, understanding of its etiological basis and development of strategies of risk reduction in CAD.



## 1. Introduction

Effective risk reduction in coronary artery disease (CAD) patients requires among others statin and anti-platelet therapy. Currently however, an unmet medical need exists for additional pharmacological targets of secondary prevention.

Several lines of evidence suggest that the transmembrane ATP-binding Cassette protein A1 (ABCA1) may be a promising target in this context. Recent epidemiological studies in have implicated ABCA1 in cardiovascular risk in the general population [1,2], an effect that currently seems independent of HDL cholesterol [3]. Experimental over-expression of human ABCA1 in mice results in protection from aortic atherosclerosis [4], while ABCA1-deficient mice exhibit mild hemorrhagic diathesis [5]. *ABCA1* deficiency in patients results in familial analphalipoproteinemia (Tangier Disease), a rare and monogenic disorder [6] presenting with diffuse cellular cholesterol ester depositions and thrombocyte dysfunction [7–9]. At the molecular level, ABCA1 is known to facilitate lipid translocation which is determined by transcriptional activity of the *ABCA1* gene and thereby under control of several responsive elements identified at its locus [10–14]. The body of evidence con-

*Abbreviations:* CAD, coronary artery disease; ABCA1, ATP-binding cassette protein A1; REGRESS, Regression Growth Evaluation Statin Study; QCA, quantitative coronary angiography; MSD, mean segment diameter; MOD, minimal obstruction diameter; LD, linkage disequilibrium; ZNF202, zinc finger 202; HR, hazard ratio; HDL, high-density lipoprotein.

* Corresponding author at: Leiden University Medical Center, Department of Cardiology C5-P, PO Box 9600, 2300 RC Leiden, The Netherlands. Tel.: +31 71 5266695; fax: +31 71 5266885.
*E-mail address:* j.w.jukema@lumc.nl (J.W. Jukema).

[1] Dr Jukema is an Established Investigator of the Netherlands' Heart Foundation, 2001D032.

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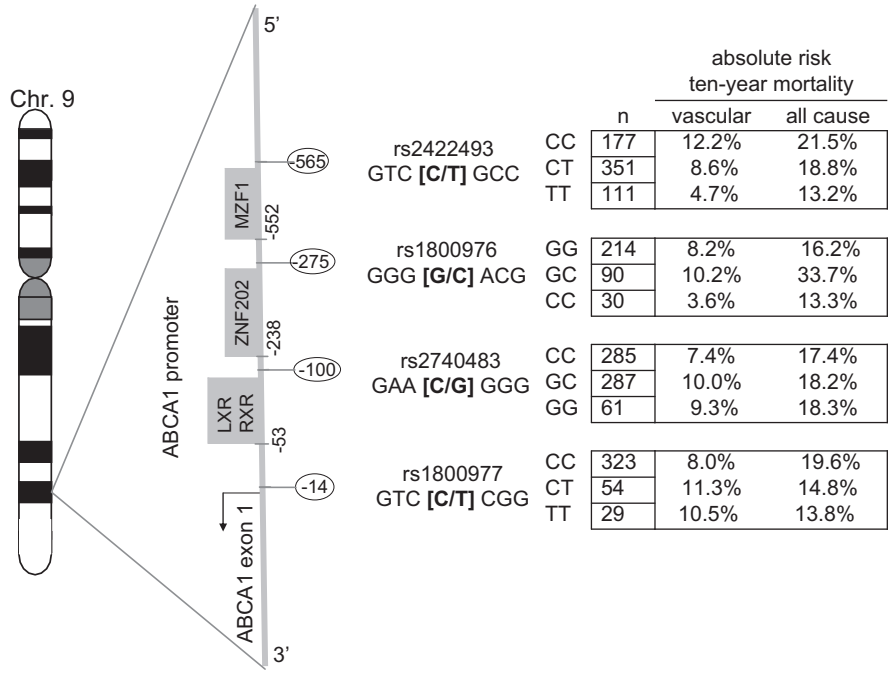


**Fig. 1.** Genomic organization of regulatory sites, common variants and absolute risk at the proximal ABCA1 promoter. The four bi-allelic variants in the current analysis encompass a region comprising regulatory sites such as those for the ZNF202, LXR/RXR and myeloid zinc finger (MZF1) factors displayed (see Refs. [11,13,14], respectively). The distance relative to translation starting point is displayed along its chromosomal position on the long arm of chromosome 9. On the right hand side, frequency of alleles and absolute risk of ten-year morality endpoints in the REGRESS sample stratified for genotype is displayed.

cerning regulation of *ABCA1* transcription has been predominantly focused on the promoter region with a number of common gene polymorphisms situated in close proximity to currently known *ABCA1* regulatory sites (see Fig. 1) [9]. These polymorphisms may thus modulate transcription. In fact, a recent functionality-study reported that carriers of the T allele of the dbSNP rs2422493 polymorphism (C/T, formerly reported as −477), exhibit in vitro lower *ABCA1* mRNA levels in explanted carotid arterial macrophages [15].

Thus, ABCA1 constitutes a potential target to further reduce atherothrombotic risk in a secondary prevention setting. However its engagement in long-term clinical prognosis in CAD patients remains undefined. Prospective investigation of key regulatory sites at the *ABCA1* locus is one approach to confirm and quantify its prognostic contribution in a contemporary secondary prevention setting. We therefore prospectively evaluated the impact of genetic variation in the *ABCA1* promoter region on 10-year mortality outcomes and baseline angiographical outcomes in an established secondary prevention cohort of patients with CAD.

## 2. Methods

### 2.1. Subjects with coronary artery disease

The study participants were derived from the Regression Growth Evaluation Statin Study (REGRESS) study which enrolled 884 males, non-diabetic patients with symptomatic coronary artery disease between 1989 and 1993. The trial design and main findings have been reported previously [16,17]. In brief, the primary objectives of this angiographic trial were to evaluate effects of 24 months of 40 mg pravastatin therapy on the evolution of atherosclerotic lesions in male patients with documented coronary artery disease. Within the framework of the trial, clinical and angiographic follow-up was documented after the initial 2-year trial. A distinct protective effect of pravastatin treatment was observed in the 24 month REGRESS trial and concurrent statin trials, prompting the study coordinators to urge all participants and treating physicians to start (placebo group) or continue (pravastatin group) statin therapy also according to national and international guidelines. A survey in the REGRESS cohort conducted 5 years after completion of the trial showed that 91% of patients were using statin therapy and were therefore on a background of contemporary secondary prevention. All participants gave written informed consent and the clinical trial and subsequent DNA-studies were approved by all seven institutional review boards of the participating centers and by the medical ethics committees of all centers.

### 2.2. Mortality 10-year follow-up

In addition to the 2-year follow-up from the original trial, 10-year follow-up data of the participants were obtained by extracting cause-specific mortality from nation-wide registries. All diagnoses in these registers are coded according to the International Classification of Diseases (ICD9 and ICD10). The research protocol was approved by the institutional review board and ethics committee of the coordinating center (University Medical Center Utrecht). The study database, comprising all 884 REGRESS participants, was linked to the registers on the basis of birth date, sex and postal address code [18]. As is customary due to privacy legislation, patient names were omitted in the linkage process. On a per-patient basis, historical registers of the Dutch inhabitants were searched for this unique combination of characteristics, and once found, this automatically merged migration history over the follow-up time. The vital status of the participants was then obtained through linking municipal administration registries using a 6-charactered postal code. Out of the 884 participants, 861 (97%) could be uniquely traced with the above method. The 23 patients who could not be uniquely traced were right-censored at the end of the 24-month follow-up.

**Table 1**
Study participants' characteristics at baseline according to rs 2422493 genotype of ABCA1.

| | CC $n=177$ | CT $n=351$ | TT $n=111$ | $p$-Value |
|---|---|---|---|---|
| Demographic | | | | |
| Age in years (SD) | 57.0 (8.2) | 55.3 (8.1) | 56.4 (8.0) | 0.07* |
| Body mass index (kg/m$^2$) (SD) | 26.0 (2.9) | 26.1 (2.5) | 26.2 (3.1) | 0.88* |
| Systolic RR (mmHg) (SD) | 134.8 (18.2) | 134.6 (17.7) | 137.0 (19.5) | 0.46* |
| Diastolic RR (mmHg) (SD) | 81.2 (10.8) | 81.3 (9.9) | 82.0 (9.9) | 0.77* |
| Current smoking | 24% | 28% | 29% | 0.62† |
| History of hypertension | 26% | 29% | 35% | 0.25† |
| Familial heart disease | 53% | 48% | 47% | 0.50† |
| Previous MI (%) | 48% | 49% | 49% | 0.96† |
| Angiographical | | | | |
| Mean baseline MOD (mm), median (min–max) | 1.71 (0.8–2.7) | 1.76 (0.8–3.1) | 1.74 (0.6–3.1) | 0.08* |
| Mean delta MOD (mm), median (min–max) | −0.07 (−1.8; −0.5) | −0.06 (−4.0; −0.4) | −0.04 (−0.9; −0.3) | 0.90* |
| Mean delta MSD, mm (SD) | −0.06 (0.22) | −0.09 (0.19) | −0.10 (0.20) | 0.33* |
| Baseline MSD (mm) (SD) | 2.67 (0.36) | 2.75 (0.40) | 2.72 (0.37 | 0.17* |
| No of vessels diseased 1 | 35% | 44% | 41% | 0.31† |
| 2 | 38% | 33% | 35% | |
| 3 | 27% | 22% | 24% | |
| Biochemical | | | | |
| Fasting glucose (mmol/l) | 5.2 (1.2) | 5.4 (1.2) | 5.3 (1.2) | 0.21* |
| Total cholesterol (mmol/l_ | 5.89 (0.92) | 6.05 (0.86) | 6.0 (0.88) | 0.13* |
| HDL-C (mmol/l) | 0.91 (0.23) | 0.92 (0.21) | 0.93 (0.23) | 0.82* |
| LDL-C (mmol/l) | 4.19 (0.82) | 4.31 (0.80) | 4.25 (0.80) | 0.25* |

* One-way ANOVA.
† Pearsons' Chi-square test.

### 2.3. Outcome events

In the outcome events analyses, we considered the primary causes of death. The composite endpoint "death due to vascular disease" consisted of all primary causes of death within the ICD9 codes 410–414 (Ischemic Heart Disease), 430–438 (Cerebrovascular disease), 440–448 (Diseases of arteries, arterioles and capillaries) and ICD10 codes I20–I25 (Ischemic Heart Disease), I60–I69 (Cerebrovascular disease), I70–I79 (Diseases of arteries, arterioles and capillaries) and F01 (Vascular dementia).

### 2.4. DNA analyses and selection of variants in regulatory regions

A previous analysis in REGRESS, examined the effects of ten genetic variants situated in *ABCA1* regulatory regions comprising the proximal promoter, intron 1 and exons 1 and 2 untranslated regions [9]. The pattern of linkage disequilibrium among these ten previously described variants is depicted in online supplemental Fig. 1A, defining a region with minimal evidence of historical recombination situated in the proximal promoter. Furthermore, the body of evidence regarding functionality has been predominantly focused on the promoter region. For these reasons, the current long-term analysis concentrated on the four variants and reconstructed haplotypes encompassing the proximal promoter region. These variants comprised rs2422493 (previously reported as C-477T or C-565T), rs1800976 (previously reported as G-191C), rs2740483 (previously reported as C-17G) and rs1800977 (previously reported as T69C). Notably, these variants are also located in close proximity of known regulatory sites in the promoter (see Fig. 1). Genotypes of the REGRESS participants for whom DNA was available were ascertained by restriction fragment length polymorphism assays using oligonucleotides and endonucleases as described previously [9,19].

### 2.5. Lipoprotein cholesterol content measurements

Total cholesterol was measured with an enzymatic kit (Boehringer Mannheim) and calibrated with a human serum calibrator. HDL cholesterol was measured after precipitation of apolipoprotein B-containing lipoproteins with a 4% tungstate solution and centrifugation, and the triglycerides were analyzed enzymatically (Bayer/Technicon) by a technique that included free glycerol [16]. LDL cholesterol was calculated according to the Friedewald formula (LDL-Cholesterol = (Total Cholesterol) − (HDL-Cholesterol) − 0.45 × Triglycerides).

### 2.6. Angiographic outcomes

Patients underwent coronary arteriography upon enrollment, according to a uniform previously described [16] protocol. After completion of the trial, angiograms were analyzed by quantitative coronary angiography (QCA): the average per patient mean segment diameter (MSD), reflecting the extent of diffuse obstructive coronary artery disease, and per patient average minimal obstruction diameter (MOD), reflecting extent of focal obstructive coronary artery disease were ascertained as described previously [16]. The current analysis included QCA parameters from baseline angiograms, which were available from 99.4% of the study population [16]. Furthermore, the change (delta) reflecting progression of atherosclerosis over the two-year follow-up time, constituting a primary angiographic endpoint of the randomized clinical trial, could be ascertained in 73.8% of the cohort [16] and was also included as an endpoint of the current genetic study.

### 2.7. Data analyses

The absolute risk of mortality outcomes among genotype groups was explored and temporal patterns of risk were visualized by means of the Kaplan–Meier method.

The effects of gene variants on outcomes were estimated using regression analyses. To explore solistic effects of gene variants on time-to-event, univariate proportional hazards (Cox' regression) models were fitted and Hazard Ratios (HR) calculated using SPSS for Windows, release 14.0 (SPSS Inc, Chicago, IL, USA). Given the strong linkage disequilibrium between the variants studied, no formal correction for multiple testing was performed. Interaction between smoking and rs2422493 genotype (as previously suggested [15]) was explored by multivariable Cox' models including smoking, genotype and the interaction term. In order to provide more insight

in relationships found, the role of possible intermediate factors was also explored in multivariate analyses. First, this included an analysis adjusting for HDL-C, LDL-C, triglycerides, hypertension and randomization group. Second, this included an analysis adjusting for baseline MOD.

The mode of inheritance exerting the effects of ABCA1 gene variants remains unknown. Therefore, we compared recessive, dominant and additive models, and selected the optimal model to describe the data based on the lowest Akaike Information Criterion. The additive model appeared to meet these criteria for all mortality outcomes.

To explore the combined effects of multiple linked variants in the ABCA1 promoter region, a haplotype-based approach was undertaken. The haplo.stats package in R for Windows version 2.9.0 (http://www.r-project.org) was used to estimate prevalence of haplotypes within the unphased genotype dataset and the haplo.glm function was used for estimating haplotype-effects on baseline coronary arterial diameters (MOD and MSD). For haplotype-effects on time-to-event, the survival analysis function of the application Thesias [20,21], release 3.1 was used. For all haplotype analyses, additive inheritance effects were assumed. The linkage disequilibrium structure between variants was calculated and visualised using the application Haploview [22], release 4.0.

Demographic, clinical and angiographic characteristics and concentrations of lipids between the three genotypes of rs2422493 were tabulated in Table 1.

Throughout, a two-tailed $p$-value of 0.05 was interpreted as indicating a statistically significant difference. Analyses were performed by two of the authors (J.J.R. and A.H.Z.) and all authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

## 3. Results

### 3.1. Distribution of allele frequencies

Genotype data for the four studied *ABCA1* promoter variants, comprising the numbers of available genotypes and allele frequency distributions are displayed in Fig. 1. For the rs2422493 variant, Table 1 displays baseline characteristics stratified by genotype, which was available from 639 participants due to DNA stock limitations. No significant differences were observed between the genotypes in baseline CAD risk factors including lipoprotein profile, angiographic or lifestyle parameters.

### 3.2. ABCA1 variation, cardiovascular risk and angiographic extent of CAD

For all four polymorphisms, absolute risk of vascular mortality and all cause mortality in genotype groups are listed in Fig. 1. After 10 years of follow-up, carriers of the minor rs2422493 (T) allele had a considerably lower risk of fatal vascular complications, as compared to major (C) allele carriers. A marked allele-dose effect was visible from the survival curves displayed in Fig. 2. For instance, the 10-year absolute risk of death due to vascular disease was as low as 4.7% (standard error 2.1%) in homozygote minor allele (TT) patients, whereas it appeared 8.6% (standard error 1.6%) in heterozygote (CT) patients and reached 12.2% (standard error 2.6%) in homozygote major allele (CC) patients as displayed also in Fig. 1. As displayed in Table 2, the T allele thus marks a state of protection from death due to vascular disease, HR 0.64 (95% confidence interval 0.42–0.97, $p = 0.03$). This effect remained robust upon adjustment for randomization group, HR 0.63 (95% confidence interval 0.42–0.97, $p = 0.03$).

This effect also remained robust upon adjustment for randomization group, hypertension, LDL and HDL (HR 0.61 (0.40–0.95), $p = 0.03$), but lost statistical significance upon correction for baseline MOD (HR 0.68 (0.45–1.1, $p = 0.08$)).

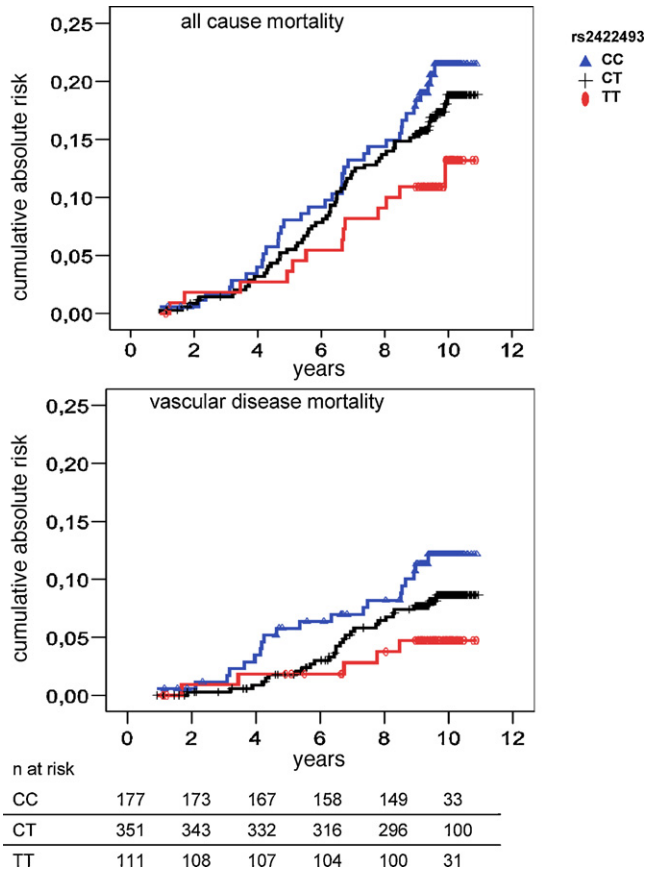


**Fig. 2.** Temporal patterns of 10-year risk by rs2422493 of ABCA1. Survival curves by genotype are displayed for all-cause mortality (upper panel) and mortality from vascular disease (lower panel). Follow-up time in years is displayed horizontally.

All-cause mortality effect did not reach significance, unadjusted HR 0.76 (95% confidence interval 0.57–1.01, $p = 0.06$) nor adjusted for randomization group (HR 0.76 (95% confidence interval 0.57–1.01, $p = 0.06$). Further, no significant effects in univariate analyses were observed for the other three genetic variants (data not displayed). An interaction analysis revealed significant amplification of the rs2422493 genotype effect by smoking status at baseline (see Table 2). Notably, among smokers the T allele marked a higher risk of mortality from all-causes (HR 0.45 (95% confidence interval 0.26–0.78), $p = 0.004$) and from vascular disease (HR 0.28 (95% confidence interval 0.12–0.70), $p = 0.006$), whereas this effect was absent in the non-smoking strata of both endpoints ($p$ for interaction = 0.04 for vascular mortality and p for interaction = 0.02 for all-cause mortality). There was no interaction between rs2422493 and BMI on both endpoints (data not shown).

Five haplotypes meeting the prevalence estimation threshold of 5% were derived, entered in subsequent haplotype-effects analysis, and displayed in the middle of Fig. 3. The effects of the separate haplotypes on coronary arterial diameters at baseline and mortality outcomes are presented in Fig. 3, left and right sides respectively. Haplotype effects analysis identified that the TGCC haplotype was significantly associated with wider mean coronary arterial diameters (MSD) at baseline of the study (regression coefficient 0.092, standard error 0.04, $p = 0.04$), and a protective state from vascular disease mortality at 10 years of follow-up (HR 0.53 (95% CI 0.28–0.98), $p = 0.04$). Moreover the TCCT haplotype had a significant effect (regression coefficient 0.113, standard error 0.04, $p = 0.003$) on focal coronary stenosis (MOD) at baseline of the study, but no

**Table 2**
Effect of rs2422493 genotype of ABCA1 on 10-year survival outcomes. Absolute risk (and SE) are displayed per genotype on the left. Hazard ratios (HR, with corresponding 95% confidence interval), *p*-value per each additional T allele copy are displayed on the right for the overall group and stratified for current smoking.

| Ten-year outcome | rs2422493 genotype | | | | | |
|---|---|---|---|---|---|---|
| | Absolute risk (standard error) | | | Overall | Smokers | Non-smokers |
| | CC (*n* = 177) | CT (*n* = 351) | TT (*n* = 111) | Per-allele HR (95% CI) *p*-Value | Per-allele HR (95% CI) *p*-Value | Per-allele HR (95% CI) *p*-Value |
| Vascular mortality | 1% (2.6%) | 8.6% (1.6%) | 4.7% (2.1%) | 0.64 (0.42–0.97) *p* = 0.03 | 0.28 (0.12–0.69) *p* = 0.006 | 0.84 (0.52–1.36) *p* = 0.47 |
| All-cause mortality | 21.5% (3.2%) | 18.8% (2.3%) | 13.2% (3.7%) | 0.76 (0.57–1.01) *p* = 0.06 | 0.45 (0.26–0.78) *p* = 0.004 | 0.94 (0.67–1.32) *p* = 0.71 |

effect of this haplotype was observed on mortality outcomes. No detectable effects of haplotypes on 2-year progression of CAD were present, and univariate analyses revealed no significant effects of genotypes on extent or two-year progression of atherosclerosis (data not displayed).

## 4. Discussion

In an effort to define new targets of secondary prevention, to date, the genetic determinants of recurrent athero-thrombotic events in patients with established CAD represent an underexposed aspect of cardiovascular genetics. The present findings in such a secondary prevention setting show the significance of common genetic variation at key regulatory sites in the *ABCA1* promoter. Effects of *ABCA* promoter variation on absolute risk of 10-year vascular mortality and angiographic outcomes were observed in 639 men from the REGRESS cohort with known baseline CAD.

First, we found that carriership of the T allele at rs2422493 markedly reduced the absolute risk of vascular mortality in a dose-dependent manner, with an estimated 12.2% 10-year risk in non carriers versus 4.5% in carriers of two T alleles (per-copy HR 0.64, 95% CI 0.42–0.97, $p = 0.03$). Second, a multi-marker analysis, which integrates variation in this regulatory region, identified that one arrangement of linked alleles (the TGCC haplotype, with estimated prevalence of 16.3% in the REGRESS sample) confers significantly lesser extent of diffuse coronary stenosis (MSD) on QCA at study

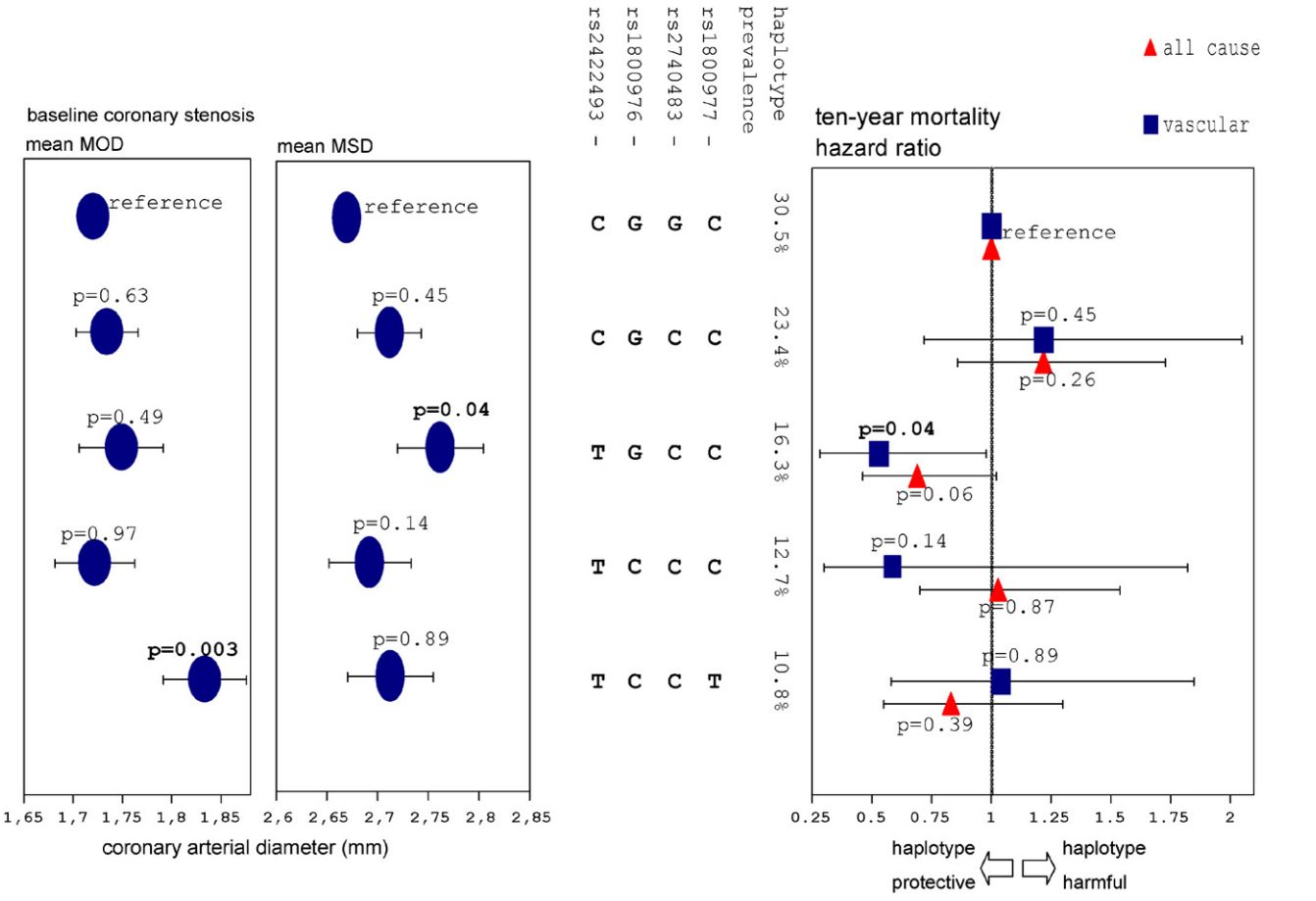


**Fig. 3.** Haplotype effects on angiographic and ten-year survival outcomes. On the left side, quantitative coronary angiographic phenotype estimations (mean MOD, inversely reflecting extent of focal disease and mean MSD, inversely reflecting extent of diffuse disease, both in mm) are displayed for the five common (prevalence >5%) haplotypes derived from the REGRESS sample. On the right hand side, survival effects are displayed in terms of hazard ratio (HR) with 95% confidence intervals. *p*-values pertain to general linear model assessment of effect relative to the most common haplotype serving as reference in all haplotype analyses.

baseline, and this haplotype accordingly also confers a lower relative risk of 10-year vascular mortality (HR 0.53, 95% CI 0.28–0.98, $p = 0.04$). Furthermore the TCCT haplotype (prevalence 10.2%) was identified to confer a lesser extent of focal coronary stenosis (MOD). Of note, both these haplotypes comprise the T allele at rs2422493 and the angiographic findings correspond with the current 10-year mortality effect of that single variant.

### 4.1. Context of the present findings

Noteworthy, thus far there is lack of genome-wide studies to document risk of recurrent events in a secondary prevention setting of patients with CAD. A prospective candidate gene-based study of CAD patients from the LCAS cohort could not detect the effects from the present report [19], albeit in a smaller cohort ($n = 368$) and over a shorter follow up time (2.5 years). Likewise, case–control data suggested no single-variant or haplotype effect in the ECTIM study of 800 myocardial infarction cases and 776 healthy controls [20]. Nevertheless, in synergy with the current findings, a nested case control study in women from the Nurses Health Studies, described a protective effect (OR 0.6 [0.4–0.9]) of the T allele of rs2422493 observing 243 cases of coronary heart disease and 484 healthy controls [23]. Next, a study of 6-year clinical outcomes in 120 young male survivors of a myocardial infarction showed a protective effect of the T allele of rs2422493 on a composite cardiovascular endpoint [24]. Therefore, the current findings on this variant are compatible with and extend available prognostic evidence with 10-year mortality data.

A previous analysis in REGRESS [9] had shown significant effects of rs1800976 and rs1800977 on the composite occurrence of death, myocardial infarction, stroke, transient ischemic attacks and coronary revascularization procedures after 2 years of follow-up. In the current 10-year mortality outcomes, these effects were not found. Some explanations may include the fact that the endpoints were different, the duration of follow-up was longer and the use of statin therapy was present in the vast majority of patients over the last 8 years of the follow-up period.

Regarding extent of vascular disease, to the best of our knowledge, no previous data on *ABCA1* haplotype-effects have been reported. Data on a single-variant effect of rs2422493 have been reported in the LCAS trial cohort, which described that the number of qualifying lesions (30–75% stenosis) was significantly higher in presence of the T allele among the 340 participants [19]. However, T allele carriers in that study exhibited no trend towards higher extent of CAD in terms of other indices such as the QCA derived minimal luminal diameter, and this agrees with the present results. Next, similar to the REGRESS findings, no effect of rs2422493 on number of vessels diseased was observed among 1166 patients with angiographically documented CAD [15]. However, a subgroup analysis from that study suggested that among the 296 non-smokers in that study, the T allele was dose-dependently associated with a higher number of diseased vessels. This prompted us to investigate whether the current 10-year follow-up data show an interaction with smoking in REGRESS. Intriguingly, we observed an inverse interaction with an augmented protective effect of the T allele among smokers (see Table 2). As an explanation for these opposing findings and interactions on different endpoints it might be hypothesized that thrombotic processes such as platelet activation, which has been suggested to depend on ABCA1 function [8], exert divergent effects on respectively angiographic extent of atherosclerosis and eventually development of fatal vascular complications. This could be true in particular in presence of smoking, a known trigger for platelet activation.

Furthermore, the current mortality effects of rs242249 without corresponding differences in HDL over genotype groups (Table 1) as is consistent with other reports [15,19], might indicate that the impact could be tissue specific.

### 4.2. Potential mechanism

The exact mechanism underlying the effects of *ABCA1* promoter variation in cardiovascular disease cannot be brought forward from the current data. In several ways, a possible explanation involves ZNF202, which has a binding site within the studied region and is regarded a prominent down-regulator of *ABCA1* transcription [11,25,26]. In this context, there are limited functional data on the effects of rs2422493 which suggest that carriership of the C allele confers stronger nuclear protein binding in vitro [15]. It is therefore tempting to speculate that C allele carriers in vivo exhibited higher ZNF202 binding affinity, thus more suppression of *ABCA1* transcription and subsequently impaired reverse cholesterol transport with hazardous prognostic effects in vivo.

On the other hand, however, the C allele of rs2422493 was associated with higher *ABCA1* mRNA expression in vitro [15], and at present consistently exhibits hazardous prognostic effects in patients. Furthermore, prospective findings in the Copenhagen City Heart Study suggested an excess cardiovascular risk in presence of variants determining lower ZNF202 expression [27], which is known to parallel high ABCA1 expression [28]. One might therefore question the conventional role attributed to ABCA1 in cardiovascular risk and alternatively reconsider in vivo functions of ABCA1 such as its suspected role in platelet activation [8], which clearly justifies further investigation. Of note, an involvement of ABCA1 in thrombotic processes might provide additional insight in the lesser than expected cardiovascular risk in Tangier disease patients [29] despite lacking HDL.

### 4.3. Study strengths and limitations

To appreciate these findings, some aspects of our study merit consideration. First, the data originate from a randomized trial in which assessment of the benefits of pravastatin treatment were the primary objective. Because the study medication taken during two years of the follow-up time, was allocated at random, i.e., irrespective of genotype, this will not have affected our findings. Second, our follow-up data set was not complete for all patients: 3% of the full cohort could not be uniquely identified in the mortality registries. Since these patients were right-censored at lost-to-follow-up time, again it seems unlikely that this would have affected the primary outcome of the current study. We elected to calculate actuarial survival across genotypes, in contrast to the case–control design. Survival analysis enabled us to efficiently study all available information, including that of censored participants. Third, an important issue is that the results in this study were obtained in a cohort of male Caucasian patients with established CAD. Confirmation of the present result in other trials including female patients and patients of different ethnicity is needed. Nevertheless, these are the first prospective data which describe the long-term effects of ABCA1 promoter variants on fatal vascular complications in a contemporary secondary prevention setting, and strongly affirm a role of these variants in determining cardiovascular risk.

## 5. Conclusion

On a background of contemporary secondary prevention, variation in the ABCA1 promoter determines 10-year risk of vascular death and angiographic extent of CAD in men. The current prospective genetic data confirm and quantify the prognostic contribution of ABCA1 in recurrent cardiovascular events and will help to strategize additional forms of risk reduction in CAD.

## Conflict of interest statement

All authors have no conflicts of interest to disclose.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.07.008.

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