

# Genetic determinants of treatment benefit of the angiotensin-converting enzyme-inhibitor perindopril in patients with stable coronary artery disease

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## Aims

The efficacy of angiotensin-converting enzyme (ACE)-inhibitors in stable coronary artery disease (CAD) may be increased by targeting the therapy to those patients most likely to benefit. However, these patients cannot be identified by clinical characteristics. We developed a genetic profile to predict the treatment benefit of ACE-inhibitors exist and to optimize therapy with ACE-inhibitors.

## Methods and results

In 8907 stable CAD patients participating in the randomized placebo-controlled EUROPA-trial, we analysed 12 candidate genes within the pharmacodynamic pathway of ACE-inhibitors, using 52 haplotype-tagging-single nucleotide polymorphisms (SNPs). The primary outcome was the reduction in cardiovascular mortality, non-fatal myocardial infarction, and resuscitated cardiac arrest during 4.2 years of follow-up. Multivariate Cox regression was performed with multiple testing corrections using permutation analysis. Three polymorphisms, located in the angiotensin-II type I receptor and bradykinin type I receptor genes, were significantly associated with the treatment benefit of perindopril after multivariate adjustment for confounders and correction for multiple testing. A pharmacogenetic score, combining these three SNPs, demonstrated a stepwise reduction of risk in the placebo group and a stepwise decrease in treatment benefit of perindopril with an increasing scores (interaction  $P < 0.0001$ ). A pronounced treatment benefit was observed in a subgroup of 73.5% of the patients [hazard ratio (HR) 0.67; 95% confidence interval (CI) 0.56–0.79], whereas no benefit was apparent in the remaining 26.5% (HR 1.26; 95% CI 0.97–1.67) with a trend towards a harmful effect. In 1051 patients with cerebrovascular disease from the PROGRESS-trial, treated with perindopril or placebo, an interaction effect of similar direction and magnitude, although not statistically significant, was observed.

## Conclusion

The current study is the first to identify genetic determinants of treatment benefit of ACE-inhibitor therapy. We developed a genetic profile which predicts the treatment benefit of ACE-inhibitors and which could be used to optimize therapy.

## Keywords

Pharmacogenetics • Individualized-therapy • Coronary artery disease • ACE-inhibitors • PERGENE • Perindopril • RAAS • Gene

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## Introduction

Angiotensin-converting enzyme (ACE)-inhibitors improve outcome in patients with stable coronary artery disease (CAD) and are recommended in clinical guidelines on secondary prevention of patients with stable CAD.<sup>1–6</sup> Accordingly, ACE-inhibitors are among the most frequently used drugs in these patients. However, in a population of patients with stable CAD the absolute treatment benefits are modest (2% reduction of cardiovascular death or myocardial infarction at follow-up) and, therefore, the number of patients needed to be treated to prevent one event remains relatively high (50 patients treated for 4 years in the EUROPA-trial).<sup>2</sup>

To optimally treat patients and to develop ways to guide ACE-inhibitor treatment, it is necessary to identify those patients who are most likely to benefit from therapy. The obvious first approach would be to use clinical parameters. However, in secondary prevention trials the treatment effect was consistent among all clinical subgroups, and no intermediate parameter could be identified to predict the efficacy of ACE-inhibitor therapy.<sup>1,7–10</sup> Also blood pressure, which guides hypertension treatment, did not predict treatment efficacy in stable CAD.<sup>10</sup> Thus, it is not feasible to base the selection of patients who are likely to respond or not respond to treatment upon clinical characteristics. A new approach is to integrate information on genetic variation among patients. Such an approach could increase the patient's chances to benefit from specific therapies, avoid treatment of patients not likely to benefit and reduce healthcare costs.

The direct pharmacodynamic pathways affected by ACE-inhibitors are the renin–angiotensin–aldosterone system (RAAS), which converts angiotensin-I into angiotensin-II, and the kallikrein–bradykinin (KB) pathway, which degrades bradykinin into inactive peptides.<sup>11–13</sup> We hypothesized that genetic variation in these pathways is associated with the treatment benefit of ACE-inhibitors. The PERGENE substudy of the EUROPA-trial provides the opportunity to evaluate this hypothesis, since this is a large randomized double-blind placebo-controlled clinical trial with complete phenotypic data.<sup>2,14,15</sup> We applied a haplotype-tagging-single nucleotide polymorphism (SNP) procedure in 12 candidate genes to ensure comprehensive coverage of genetic variation in both pathways,<sup>15</sup> and developed a genetic score which predicted risk for future events in the placebo group as well as the treatment benefit with perindopril. Furthermore we performed a preliminary replication of our findings in another clinical trial with the same ACE-inhibitor (PROGRESS).<sup>16</sup>

## Methods

### Study populations and design

The PERindopril GENetic association study (PERGENE) is a substudy of the EUROPA-trial. The designs of both studies were previously described in detail.<sup>14,15</sup> In short, EUROPA randomized 12 218 stable CAD patients to perindopril (8 mg/day) or placebo. Perindopril was associated with a 20% relative reduction [hazard ratio (HR) 0.80; 95% confidence interval (CI) 0.71–0.91] in the rate of the primary endpoint (composite of cardiovascular mortality, non-fatal MI, or resuscitated cardiac arrest) during a mean follow-up of 4.2 years.<sup>2</sup> The PERGENE study investigates whether common genetic variation

is related to the risk of future events and modifies the treatment effect of perindopril.<sup>15</sup> Written informed consent for performing genetic association analyses was obtained from all patients. Unfortunately, no other large database is available to verify our observations. However, we studied our pharmacogenetic score in a substudy of the PROGRESS-trial, a randomized, double-blind, placebo-controlled clinical trial of a perindopril based-regimen (perindopril 4 mg or perindopril 4 mg + indapamide 2.5 mg) vs. placebo in 6105 patients with cerebrovascular disease.<sup>16</sup> The observed relation between the proposed genetic score (see below) and event rates in the placebo group from EUROPA was verified in European patients in the PROGRESS study allocated to receive single (525) or double (666) placebo. As the treatment effect in PROGRESS was contingent on the combination of perindopril with indapamide (duo-therapy), we restricted our analysis to the 1051 European patients who were randomized to perindopril (as single therapy) vs. placebo.

### Data collection

A DNA bio-bank was established within the EUROPA-trial for the PERGENE substudy.<sup>17</sup> Blood samples were received from 10 060 patients and DNA from 9454 patients were successfully isolated using an automated isolation process (Hamilton liquid handler coupled with Magnetic separator for automated DNA extraction; NV, USA). For preliminary replication DNA was isolated from the 1051 samples of Caucasian patients (using perindopril alone vs. placebo) participating in the PROGRESS-trial at the INSERM laboratory in Paris.

### Candidate genes and selection of tagging-single nucleotide polymorphisms

Twelve genes that are known to play an important role in pharmacodynamic pathway of ACE-inhibitors, the RAAS and KB systems, were selected for this analysis (see Supplementary material online, Table S1). The candidate genes were: the renin (REN), prorenin receptor, angiotensinogen, angiotensin-converting enzyme, angiotensin-II receptor type 1 (AT1) and 2, aldosterone synthase, endothelial nitric oxide synthase, kininogen, kallikrein, and bradykinin type 1 (BK1) and 2 receptor genes. To cover common variation in these 12 candidate genes comprehensively, haplotype-tagging SNPs (ht-SNP) were selected based on the linkage disequilibrium (LD) structure as provided by the public databases of HapMap (<http://www.hapmap.org>), PARC, and SeattleSNPs (<http://pga.mbt.washington.edu>).<sup>18</sup> Within these genes, plus their '3 and 5' flanking regions, a total of 52 ht-SNPs were identified. The haplotype-tagging approach was used because within the genes there is a high level of LD, and this approach allowed us to combine minimal genotyping with comprehensive coverage of the genetic variation in the genes.<sup>19</sup> The selection criteria of the ht-SNPs also included: minor allele frequency  $\geq 5\%$ ,  $r^2 < 0.80$ , haplotype frequency  $\geq 1\%$  (HapMap Release 24/Phase II Nov08/on NCBI B36 assembly/DbSNP b126). In the process of selecting tagging SNPs our aim was to include, when available, SNPs for which functionality has previously been described, SNPs that gave an amino acid change or SNPs that were located in regulatory regions or intron–exon boundaries. Further details of this methodology can be found elsewhere.<sup>15</sup> In our population, several SNPs were in stronger LD than suggested by the HapMap database, and we defined our set of tagging SNPs by excluding one of the SNPs if there was a pairwise  $r^2 > 0.95$ .

### Genotyping

Taqman allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) and Sequenom (San Diego, CA, USA) mass-spectrometric

genotyping were used to genotype the selected SNPs, according to the manufacturer's protocols. The assays, primers, and probes for these assays are readily available from the Assay-by-Design service ([www.appliedbio-systems.com](http://www.appliedbio-systems.com)) or can be requested from Sequenom for all mentioned rs-numbers (see Supplementary material online, Table S1). Quality control for the accuracy of genotyping involved testing duplicates from a randomly selected group of samples (5%) for concordance between samples (always >99% replication). Individual SNP call rates ranged between 95 and 98%. To ensure DNA quality, only patients who were successfully genotyped for more than 90% of the 52 SNPs were included in the analyses ( $n = 8907$ ).

## Statistical analysis

We tested whether genotypes and allele frequencies were distributed according to Hardy–Weinberg equilibrium using a  $\chi^2$  test. The treatment effect of perindopril was defined as the reduction in the event rate of the primary endpoint of the EUROPA-trial (composite of cardiovascular mortality, non-fatal MI, and resuscitated cardiac arrest) and compared between genotype strata for each SNP (additive model assumption). Genotype-treatment interactions were assessed with Cox proportional hazards regression models. Two models were fitted: one included genotype, treatment, and treatment  $\times$  genotype interaction, with adjustments for age and gender; the second model additionally included all covariates that were related to the incidence of the primary endpoint in EUROPA.<sup>7</sup> The results for the full model are presented in all analyses, and are concordant with the age/gender model.

Multiple testing corrections of treatment interaction terms, and estimation of empirical  $P$ -values, were implemented using Monte Carlo permutation analysis (10 000 permutations) on a per gene basis.<sup>20</sup> Permutation was chosen as a method of multiple testing correction, because due to the LD between the SNPs and the fact that the genes are located within a common pathway, Bonferroni adjustment would be too conservative. As we corrected for the number of tagging SNPs within each of the 12 candidate genes, the expected number of 'chance' findings is correctly calculated as  $12 \times 0.05 = 0.6$  SNPs. Permuted  $P$ -values below 0.05 were considered to be statistically significant.

Haplotypes were inferred using the estimation–maximization algorithm implemented in haplo.stats.<sup>21</sup> The associations between the estimated haplotypes and risk of the primary endpoint, taking into account the posterior probabilities of the haplotype estimates, were assessed with the GLM function in haplo.stats. The haplotype analysis used the same models as the Cox analysis. Global  $P$ -values for treatment  $\times$  haplotype interaction were estimated with a likelihood ratio test, comparing models with and without the interaction terms.

A pharmacogenetic score was constructed based on SNPs that modified the treatment effect by counting the number of alleles that were associated with a decreased benefit of treatment with perindopril. Since each allele can be present as heterozygote or homozygote the score for three SNPs ranges from 0 to 6. For each category of the score, the relative and absolute risks of events were calculated for patients allocated to perindopril or placebo as well as the treatment benefit. Baseline clinical characteristics and intermediate phenotypes, such as blood pressure at baseline were compared between the categories of the pharmacogenetic score. In an additional analysis, we assessed the relation between patients with scores  $<3$  and  $\geq 3$  and the incidence of the primary endpoint during 4 years of follow-up using multivariate Cox regression analysis (full model).

The pharmacogenetic score was verified in the PROGRESS-trial. Because of the relatively small group sizes, the score was categorized as  $\leq 1$ , 2, and  $\geq 3$  to study the relation between the score and event

rate in patients allocated to placebo and the treatment effect of perindopril.

All genetic polymorphisms which modified the treatment effect of perindopril (permuted  $P$ -value of  $<0.10$ ) in the EUROPA-trial were tested on the same corresponding endpoint (cardiovascular mortality, MI) in the European subjects from the PROGRESS-trial.<sup>16</sup> The interaction effects on treatment of the three individual SNPs were further verified in a combined meta-analysis of the two studies. Results from the two studies were combined using an inverse variance method in a random effects model.<sup>22,23</sup> Additionally, an analysis of treatment effect relative to the overall study effect (as a per cent change in treatment effect according to genotype) was performed to study the modification of treatment benefit in both studies.

All analyses were conducted using R software. Meta-analyses were conducted using RevMan 5.0 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2008). Analyses are based on intention-to-treat principle. In statistical analyses, a  $P$ -value of  $<0.05$  (two-sided) was considered significant.

## Results

Baseline characteristics of the PERGENE study population were similar to those of the total EUROPA-trial and are shown in Table 1. All genetic variants were in Hardy–Weinberg equilibrium. With our approach, we covered all common genetic variation (MIF  $> 5\%$ ) and  $\geq 90\%$  of the total genetic variation in the individual candidate genes. Complete data on follow-up and covariates were obtained for 8746 patients from the EUROPA-trial. The mean age was 59.9 (9.3) years and 85.7% were male. Median follow-up was 4.2 years.

### Genetic determinants of treatment benefit of perindopril in EUROPA (PERGENE)

In the study population, 785 events (9.0%) occurred, of which 342 in patients with perindopril (8.0%) and 443 in patients receiving placebo (10.2%). The HR for the overall treatment effect was 0.80 (95% CI 0.68–0.92). In the unadjusted analysis (without adjustment for confounders or correction for multiple testing), seven SNPs in four genes significantly modified the treatment effect of perindopril: AT1 rs275651 and rs5182; REN rs2887284, rs10900555, and rs11571082; BK1 rs12050217; AGT rs4762). In the multivariate model with correction for multiple testing, three SNPs in two genes remained significant (AT1 rs275651 and rs5182; BK1 rs12050217).

In the bradykinin type I (BK1) receptor gene; rs12050217 was a strong modifier of the treatment benefit of perindopril. Patients with the AA genotype (62%) had a 7.3% risk of cardiovascular death or myocardial infarction when using perindopril whereas patients with this genotype in the placebo group had a 10.8% risk, which is a 36% event reduction with perindopril [HR 0.64 (95% CI 0.55–0.78)]. For the AG (33.2%) and the GG genotypes (4.7%) the HR for reduction of treatment benefit were 1.02 (0.79–1.29 and 1.10 (0.56–2.19), respectively (Table 2). The  $P$ -values for interaction were 0.004 (empirical) and 0.012 (permuted). In the angiotensin-II type I (AT1) receptor gene, rs275651 and rs5182 ( $r^2 = 0.03$ ) significantly modified the treatment benefit of perindopril, with empirical  $P$ -values of 0.008 and 0.011, and permuted

**Table 1** Baseline characteristics of the PERGENE study population (n = 8907)

Characteristics	Total group	Score <3	Score ≥3
Age, years	59.9 (9.3)	59.8 (9.3)	60.0 (9.3)
Gender, % female	14.5	14.5	14.5
Hypertension, %	28.5	28.2	29.1
Diabetes, %	12.7	12.9	12.4
Hypercholesterolaemia, %	62.8	63.2	62.2
Smoking, %	14.8	14.4	15.4
Body mass index (>30 kg/m <sup>2</sup> ), %	21.3	21.4	21.3
Symptomatic CAD, %	25.3	25.4	25.3
Family history of CAD, %	27.2	27.3	27.1
Prior myocardial infarction, %	65.0	65.1	65.0
Prior revascularization, %	54.6	54.9	53.8
Prior CVA or PVD, %	8.9	8.7	9.4
Medication use			
Platelet-inhibitors, %	92.2	92.3	92.0
Beta-blockers, %	63.2	63.2	63.4
Lipid-lowering agents, %	55.3	55.9	54.4
Calcium antagonists, %	31.7	31.3	32.5
Total cholesterol, mg/dL	5.4 (1.1)	5.4 (1.0)	5.4 (1.1)
Creatinine clearance, μmol/L	86.5 (25.7)	86.7 (26.0)	86.1 (25.1)
Randomization, allocated perindopril, %	49.9	49.7	50.3
Systolic blood pressure, mmHg	136.9 (15.2)	136.9 (15.3)	136.8 (15.1)
Diastolic blood pressure, mmHg	81.8 (8.1)	81.8 (8.2)	81.8 (8.1)
Blood pressure reduction by perindopril, mmHg <sup>a</sup>	8.6/4.0	8.6/4.0	8.6/4.0

Summary statistics for continuous variables are presented as mean (standard deviation (sd)). Categorical data are summarized as percentages.

<sup>a</sup>Blood pressure reduction was calculated as the mean difference in blood pressure from screening visit 1 to randomization after the 4 week run-in period of the EUROPA-trial in which all patients were treated with the ACE-inhibitor perindopril.

*P*-values of 0.049 and 0.054 (borderline), respectively. No further associations of treatment interaction were observed for the other genes (see Supplementary material online, *Table S1*). Also no associations were observed between these individual SNPs and the rates of the primary endpoint in either the placebo- or the perindopril-treated groups.

The haplotype analysis confirmed the association between the identified SNPs and treatment effect modification observed in single SNP analysis, as presented in Supplement material online, *Tables S2a* and *b*. In both genes, the haplotype analysis identified similar alleles to be associated with a decreased treatment benefit of perindopril as the single SNP analysis.

## Pharmacogenetic score

When we combined the three SNPs in a pharmacogenetic score (composed of rs12050217, rs5182, and rs275651) the event rate decreased with an increasing score in patients allocated to placebo (from 12.2 to 8.1%), although the event rate increased in patients allocated to perindopril (from 6.3 to 10.4%; *Figure 1*). Accordingly a stepwise decrease in treatment benefit of perindopril was observed with increasing score (*P*-value for interaction <0.0001). We identified 73.5% of the population with a consistent (score = 2) or improved (score = 0 or 1) treatment effect (score

<3; HR 0.67; 95% CI 0.56–0.79) and 26.5% of the population without benefit from treatment with perindopril (score ≥3; HR 1.26; 95% CI 0.97–1.67) as presented in *Figure 2*. The *P*-value of interaction between treatment effect and risk score <3 or ≥3 is <0.0001.

In patients with scores ≥3, we observed a harmful trend with perindopril, although this was not statistically significant (*P*-value 0.10). These patients are considered as 'adverse responders' to perindopril.

In the overall study population, patients with a score ≥3 had a slightly lower risk compared with patients with a score <3, although this difference was not statistically significant: HR 0.88 (95% CI 0.76–1.04). Patients allocated to placebo with score ≥3 had a significantly reduced risk (HR 0.68; 95% CI 0.53–0.84) when compared with those with a score <3. In contrast patients with a score ≥3 allocated to perindopril had a higher risk (HR 1.18; 95% CI 0.94–1.49), demonstrating the interaction of the pharmacogenetic score and treatment benefit.

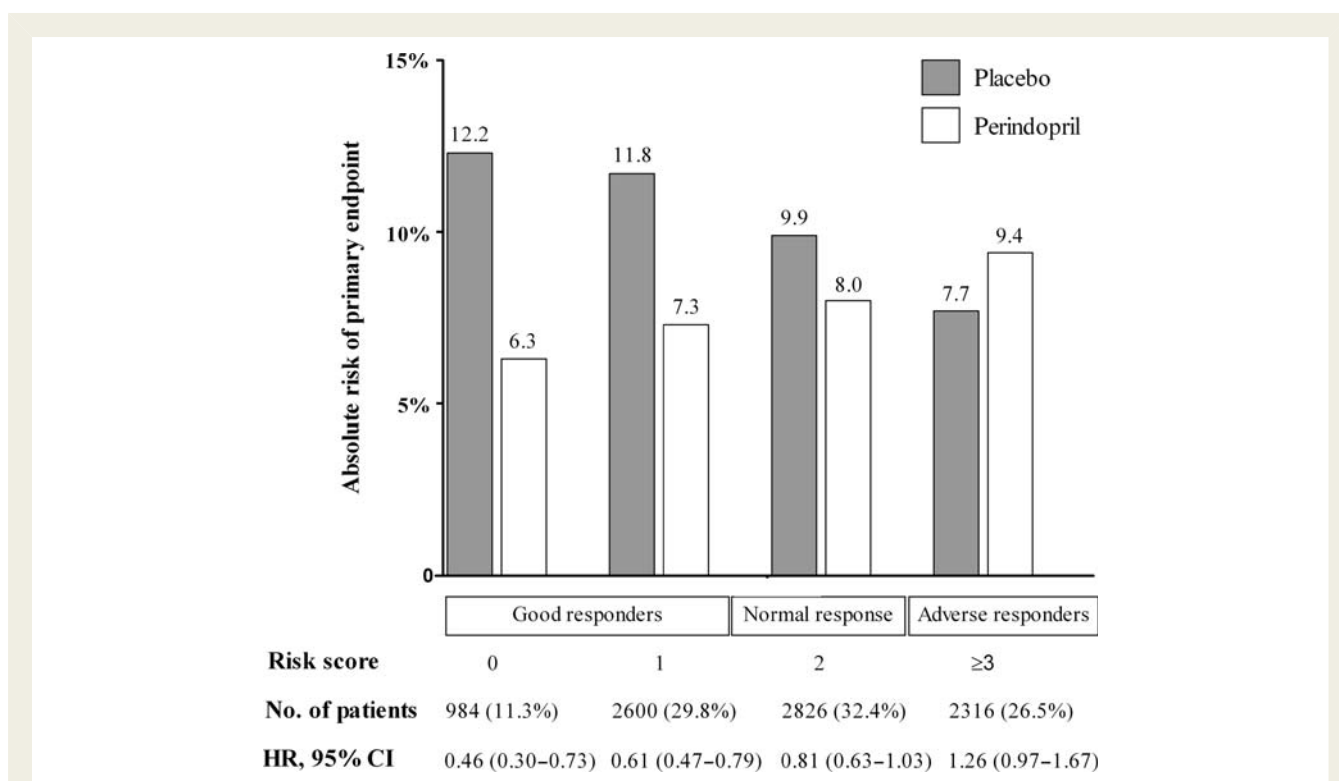
No differences in clinical characteristics, including blood pressure, were observed between patients with scores ≥3 and <3 (*Table 1*; all *P*-values = ns). Furthermore, no differences in intermediate phenotypes were observed in terms of blood pressure and blood pressure reduction during the run-in period

**Table 2** Modification of the treatment benefit of angiotensin-converting enzyme-inhibitor therapy in renin–angiotensin–aldosterone and kallikrein–bradykinin system genes

	SNP	Allele	Genotype frequencies			Location	Treatment benefit (perindopril vs. placebo)			Interaction perindopril–placebo, HR 95% CI	Empirical P-value*	Permuted P-value*
			%	%	%		Homozygous common allele, HR 95% CI	Heterozygous, HR 95% CI	Homozygous minor allele, HR 95% CI			
Angiotensin-II type I receptor	rs275651	A>T	67.4	29.4	3.3	Promoter	0.65 (0.53–0.81)	1.07 (0.81–1.41)	0.97 (0.46–1.92)	1.42 (1.09–1.85)	0.008	0.049
	rs10935724	A>C	44.3	44.2	11.5	Intron	0.83 (0.68–1.03)	0.73 (0.59–0.91)	0.71 (0.47–1.07)	0.88 (0.71–1.09)	0.23	0.80
	rs931490	A>G	66.6	30.0	3.4	Intron	0.73 (0.61–0.86)	1.03 (0.78–1.35)	0.84 (0.39–1.82)	1.29 (0.99–1.68)	0.05	0.29
	rs4681440	C>T	68.6	28.3	3.2	Intron	0.79 (0.67–0.94)	0.79 (0.60–1.04)	0.62 (0.26–1.42)	0.93 (0.71–1.21)	0.56	0.99
	rs5182	C>T	27.3	49.9	22.8	Exon	0.99 (0.74–1.27)	0.84 (0.67–1.02)	0.59 (0.44–0.80)	0.77 (0.63–0.94)	0.011	0.054
	rs5186	A>C	51.9	40.6	7.5	Exon	0.70 (0.58–0.85)	0.88 (0.70–1.11)	0.93 (0.55–1.57)	1.23 (0.98–1.54)	0.068	0.35
Bradykinin type I receptor	rs4905475	G>C	81.2	17.7	1.1	Promoter	0.77 (0.66–0.90)	0.90 (0.63–1.25)	0.94 (0.23–4.90)	1.13 (0.81–1.58)	0.46	0.92
	rs12050217	A>G	62.1	33.2	4.7	Intron	0.64 (0.55–0.78)	1.02 (0.79–1.29)	1.10 (0.56–2.19)	1.44 (1.13–1.83)	0.004	0.012
	rs885845	C>T	41.7	45.2	13.1	Intron	0.66 (0.53–0.82)	0.95 (0.76–1.15)	0.80 (0.51–1.18)	1.16 (0.94–1.43)	0.16	0.50
	rs2071084	G>A	68.4	28.3	3.2	Exon	0.83 (0.70–0.99)	0.71 (0.53–0.90)	0.82 (0.41–1.63)	0.89 (0.69–1.15)	0.37	0.85

The treatment effect refers to the reduction in risk of the primary endpoint (cardiovascular mortality, MI, or resuscitated cardiac arrest) by perindopril as compared with placebo during 4 years of follow-up. The treatment effect of perindopril in the main EUROPA-trial was HR 0.80 (95% CI 0.71–0.91). Cox proportional hazard regression analysis was used to estimate treatment effects adjusted for age, gender, systolic blood pressure, diabetes mellitus, smoking, body mass index >30, creatinine clearance, prior myocardial infarction, prior stroke or peripheral vascular disease, symptomatic coronary artery disease, and family history of coronary artery disease. Complete data on follow-up and covariates in 8746 patients. LD between rs275651 and rs5182,  $r^2 = 0.03$ . P-values were corrected for multiple testing by gene-based permutation analysis. Empirical and permutation P-values based on 10 000 permutations.

\*P-values for interaction.



**Figure 1** Pharmacogenetic profile of identified single nucleotide polymorphisms and the treatment effect of perindopril in stable coronary artery disease patients from the EUROPA-trial. Multivariate Cox proportional hazard regression analysis was used to calculate treatment effects adjusted for age, gender, systolic blood pressure, total cholesterol, diabetes mellitus, smoking, body mass index, creatinine clearance, history of myocardial infarction, history of stroke or peripheral vascular disease, prior revascularization, symptomatic coronary artery disease, and family history of coronary artery disease. 8726 out of 8746 patients have complete genotype data on rs275651, rs5182, and rs12050217. The group with score <3 consisted of 6410 patients (73.5%) who experienced a pronounced treatment effect [HR 0.67 (95% CI 0.56–0.79)]. The group with score  $\geq 3$  consisted of 2316 patients (26.5%) who experienced a diminished treatment benefit (HR 1.26; 95% CI 0.97–1.67). The *P*-value of interaction between treatment effect and risk score <3 or  $\geq 3$  is <0.0001. The individual HR for each score in the  $\geq 3$  group are the following: risk score 4 (HR 1.49; 95% CI 0.84–2.87; *n* = 531), risk score 5 (HR 1.10; 95% CI 0.32–3.74; *n* = 101), risk score 6, *n* = 7 patients, HR not calculable, combined risk score 5 + 6 (HR 1.12; 95% CI 0.33–3.69). HR, hazard ratio; CI, confidence interval.

of the EUROPA-trial (Table 1). Thus, the observed treatment interaction cannot be explained by clinical differences between the genotypes, but only by the existing genetic differences.

### Pharmacogenetic score in progress

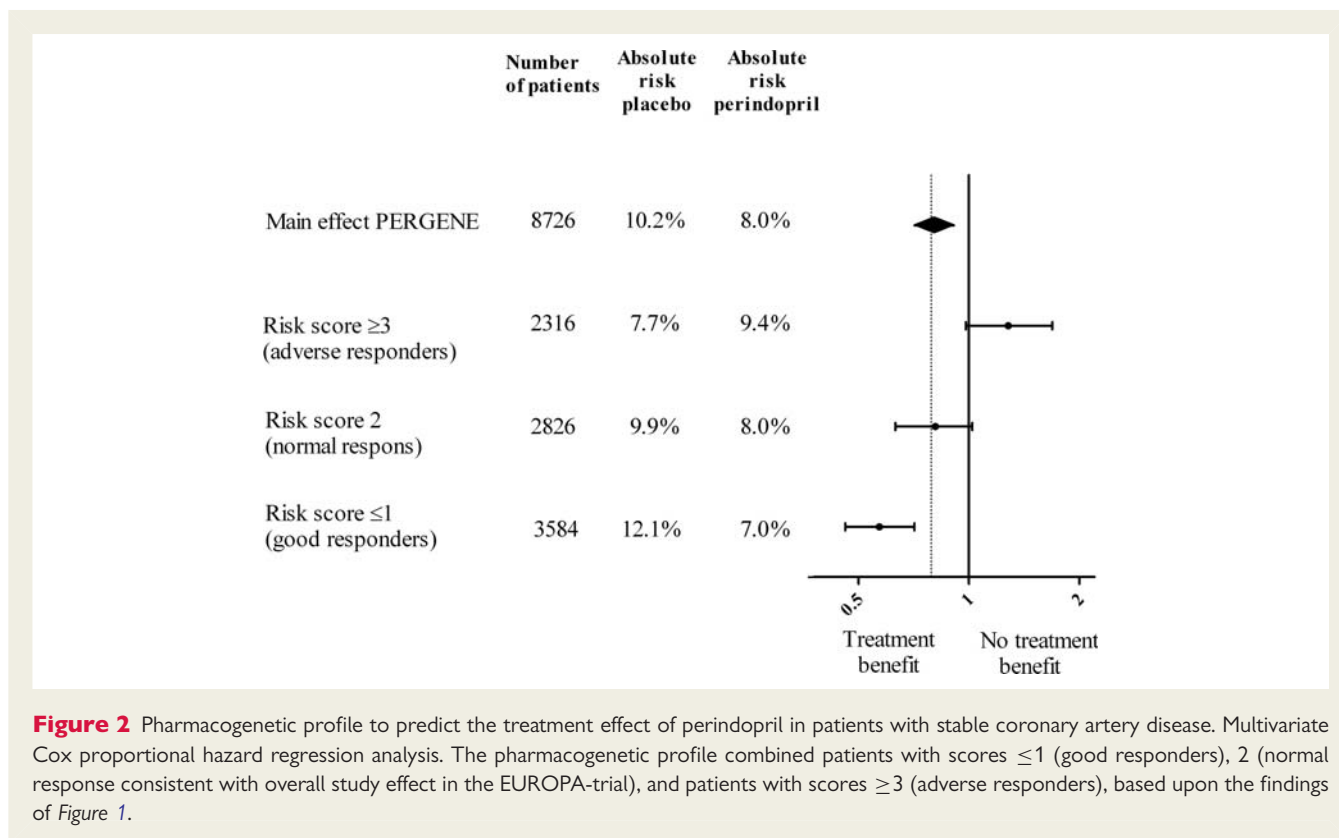
In the subgroup of 1191 European patients from the PROGRESS-trial who were allocated to placebo, either single (perindopril) placebo or double (perindopril and indapamide) placebo, the absolute risk of the composite endpoint decreased from 10.0%, 8.9–8.0% with increasing score  $\leq 1$ , 2,  $\geq 3$  which was consistent with the observations in PERGENE.

In the subgroup of European patients from the PROGRESS-trial who were allocated to perindopril as single therapy (*n* = 526) or placebo (*n* = 525), the minor allele frequencies of the three SNPs were similar to those in the PERGENE population. The composite endpoint occurred in 103/1051 patients (9.5%). In these patients from PROGRESS, no overall treatment benefit of perindopril was apparent (HR 1.19; 95% CI 0.81–1.79) as compared with placebo during follow-up. However, the estimates of interaction effect on treatment in PROGRESS were of similar direction and magnitude

as observed in the PERGENE study for each of the three individual SNPs (Table 3), although CIs were wide and statistical interaction terms were not significant in this relatively small replication cohort. In a combined analysis of the interaction effects on treatment, the initially observed *P*-values from EUROPA improved by adding PROGRESS (*n* = 1051) for each of the three individual SNPs. The combined interaction effects were HR 1.41; 95% CI 1.12–1.78 (*P* = 0.003); HR 0.78; 95% CI 0.65–0.94 (*P* = 0.010); and HR 1.42; 95% CI 1.13–1.78 (*P* = 0.003) for the SNPs rs275651, rs5182, and rs12050217, respectively (Figure 3).

The results of the genetic risk score in PROGRESS are presented in Table 4. In patients with a risk score  $\leq 1$ , perindopril treatment was associated with a 32% reduction in the composite endpoint [HR 0.68 (0.35–1.27)], although in the patients with a risk score of 2 or  $\geq 3$  HR's were 1.58 (0.75–3.71) and 1.74 (0.81–3.91), respectively. As in PERGENE, the absolute risk decreased in the placebo group and increased in the perindopril group with increasing genetic risk score in PROGRESS.

Figure 4 shows the concordance in the association between genetic score and treatment benefit in the PERGENE and



**Figure 2** Pharmacogenetic profile to predict the treatment effect of perindopril in patients with stable coronary artery disease. Multivariate Cox proportional hazard regression analysis. The pharmacogenetic profile combined patients with scores  $\leq 1$  (good responders), 2 (normal response consistent with overall study effect in the EUROPA-trial), and patients with scores  $\geq 3$  (adverse responders), based upon the findings of Figure 1.

**Table 3** Treatment effect of perindopril in caucasian subjects of the PROGRESS-trial ( $n = 1051$ )

Gene	SNP	Allele	Genotype frequencies (%)			Single therapy (perindopril only)	
			1/1	1/2	2/2	Common allele	Minor allele carriers <sup>a</sup>
AT1 receptor	Rs275651	A>T	66.2	29.6	4.2	1.04 (0.64–1.70)	1.46 (0.70–3.30)
	Rs5182	C>T	29.5	50.8	19.7	1.57 (0.71–3.56)	1.03 (0.64–1.67)
BK1 receptor	Rs12050217	A>G	62.6	33.5	3.8	0.95 (0.57–1.61)	1.55 (0.78–3.04)

Overall study effect in PROGRESS ( $n = 1051$ ) was HR 1.19 (0.78–1.79), 526 patients allocated perindopril and 525 placebo.

Treatment effect analyses are adjusted for age, gender, systolic blood pressure, diabetes mellitus, smoking, body mass index, history of MI, history of stroke, prior revascularization, peripheral vascular disease, symptomatic CAD, serum creatinin.

<sup>a</sup>Heterozygous and homozygous minor allele groups combined due to low sample size in the homozygous minor allele group. As treatment effect could not be estimated for rs275651 and rs12050217 in multivariate model ( $n < 40$ ), they were combined with the heterozygous minor allele group as 'minor allele carriers' for risk estimates for all three SNPs.

PROGRESS studies, presenting the change in treatment benefit relative to the overall treatment effect in each study, although this was positive in PERGENE and neutral in PROGRESS.

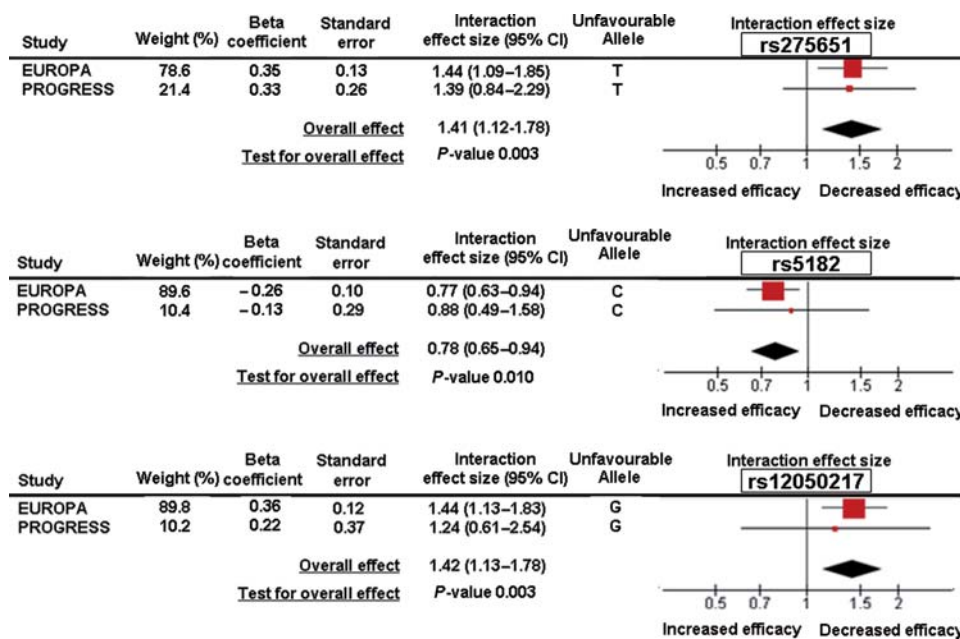
## Discussion

The current study demonstrates that the treatment benefit of ACE-inhibitor therapy by perindopril may be modified by variation in two genes in the RAAS and KB systems: the AT1 receptor gene, and the BK1 receptor gene. With the proposed pharmacogenetic score, composed of these variants, patients with a consistent or enhanced treatment benefit could be identified (73.5% of the PERGENE population), and distinguished from patients with no

benefit or a possibly harmful treatment effect (26.5% of the PERGENE population).

The concept of pharmacogenetics to individualize medicine is emerging rapidly and clinically highly relevant as it has the potential to revolutionize clinical practice. Several successes of this approach have recently been demonstrated for different cardiovascular agents, such as the activation of clopidogrel,<sup>24</sup> the risk of rhabdomyolysis associated with statin therapy,<sup>25</sup> and anticoagulation therapy by warfarin.<sup>26</sup> Our study is the first large-scale pharmacogenetic analysis of patients with stable CAD randomized to ACE-inhibitor therapy vs. placebo.

The pharmacogenetic score predicted the presence or absence of treatment benefit with the ACE-inhibitor perindopril in the



**Figure 3** Replication of findings: meta-analysis of treatment interaction effects. The interaction effect sizes, standard errors and 95% CI were estimated using Cox proportional hazard regression analysis. Results from the two studies were combined in meta-analyses using an inverse variance method in a random effects model. Available sample sizes for EUROPA and PROGRESS were 8746 and 1051, respectively. Heterogeneity tests for the meta-analysis for all three single nucleotide polymorphisms were non-significant (all  $I^2 = 0$  for all three single nucleotide polymorphisms). The initial  $P$ -values of interaction in EUROPA for rs275651, rs5182, and rs12050217 were 0.008, 0.011, and 0.004, respectively. Meta-analysis of the interaction effects from the two populations improved the initially observed  $P$ -values for all three single nucleotide polymorphisms ( $P$ -values 0.003; 0.010; 0.003, respectively).

**Table 4** Verification of risk score in EUROPA and PROGRESS-trials

	Number of patients <sup>a</sup>	Risk of events place, ACEi (%)	HR	95% CI
<b>EUROPA-trial</b>				
Overall study	$n = 8726$	10.2	8.0	0.80 (0.71–0.91)
Risk score $\leq 1$	$n = 3584$	12.1	7.0	0.58 (0.46–0.72)
Risk score 2	$n = 2826$	9.9	8.0	0.81 (0.63–1.03)
Risk score $\geq 3$	$n = 2316$	7.7	9.4	1.26 (0.97–1.67)
<b>PROGRESS-trial</b>				
Overall study	$n = 1051$	8.2	10.8	1.19 (0.81–1.79)
Risk score $\leq 1$	$n = 394$	10.9	10.1	0.68 (0.35–1.27)
Risk score 2	$n = 338$	6.4	10.9	1.58 (0.75–3.71)
Risk score $\geq 3$	$n = 288$	7.1	11.9	1.74 (0.81–3.91)

Multivariate Cox regression analysis adjusted for age, gender, systolic blood pressure, body mass index (BMI > 30), serum creatinin, peripheral vascular disease, family history of CAD, diabetes mellitus, current smoking, prior MI, prior CVA.

<sup>a</sup>Risk score on three SNP's available in 1020 patients.

PERGENE study. This association was independent of baseline clinical characteristics and blood pressure, in accordance with previous studies in which clinical patient characteristics or subgroup

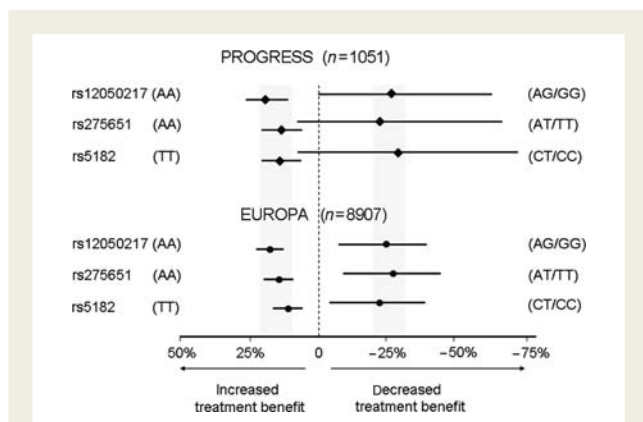
analyses did not reveal any heterogeneity in the effect of treatment with perindopril.<sup>1,2,8–10,27</sup>

The interaction of genetic variation with the treatment response of ACE-inhibitors has previously been investigated in a few small studies, which were not randomized or lacked a placebo-control group and, therefore, the reported relations were largely inconclusive.<sup>12,15,27,28</sup> A few large randomized clinical trials with ACE-inhibitors have been conducted with systematic collection of DNA. Two of these large studies found no association of the ACE insertion/deletion (I/D) polymorphism with treatment response for different anti-hypertensive drugs.<sup>29,30</sup> Similarly, in our study, the proxy (high LD;  $D' = 1.0$ ;  $r^2 = 0.9$ ) of the ACE I/D polymorphism, rs4343, was not related with the treatment benefit of perindopril.<sup>31</sup>

A limitation of the previous studies was that they focused on one single polymorphism, which does not account for the complexity of the RAAS and KB systems. To allow truly meaningful conclusions, it is necessary to comprehensive coverage of all RAAS and KB system genes, with multiple tagging SNPs within multiple candidate genes in a common pathway, as we did in this study.

In the main analysis of the EUROPA-trial, treatment with perindopril resulted in a relative risk reduction of 20% for the primary endpoint, which was consistent across all clinical subgroups.<sup>7</sup> In contrast, the subgroups based on the proposed genetic score have a wide range of treatment effects, from patients with a score of 0 (11.3% of all patients) with a 54% reduction in the primary endpoint during follow-up, via patients with a score of 1 (29.8%) who experienced a 39% relative risk reduction and





**Figure 4** Relative treatment effects of perindopril in EUROPA and PROGRESS (% change in treatment effect).

patients with a score of 2 (32.4%) with a 19% relative risk reduction. All patients with a score  $<3$  experienced a reduction of the risk for cardiovascular death or myocardial infarction with perindopril. In contrast, patients with a score  $\geq 3$  (26.5%) experienced no benefit at all and tended towards an adverse response to perindopril treatment during 4 years of follow-up. Refraining from treatment with perindopril in these patients, who were relatively insensitive or resistant to ACE-inhibitor therapy, may considerably reduce healthcare cost, avoid unnecessary side-effects and increase overall efficacy of the drug.

Our findings suggest that the genetic variants modifying the treatment effect of perindopril are particularly located in the AT1 and BK1 receptor genes. The SNPs in the AT1 receptor were located in the promoter (rs275651) and exon (rs5182), the SNP in the BK1 receptor was located in an intron. These three SNPs were tagging SNPs and may either be functional themselves or in linkage equilibrium with other functional SNPs. So far, the effect of these SNPs on the functional nature of the protein is not known and we can only speculate on the underlying mechanisms. The AT1 receptor does mediate all the well-known effects of angiotensin-II, including vasoconstriction, water and salt retention, aldosterone synthesis, and hypertrophy. The role of the B<sub>1</sub> receptor is less well established. Bradykinin is a potent vasodilator that also induces anti-atherosclerotic and anti-thrombotic effects, which are mediated by bradykinin type II (B<sub>2</sub>) receptors. Previous studies indicated that the clinical benefit of ACE-inhibitors depends, at least in part, on B<sub>2</sub> receptor activation.<sup>32</sup> B<sub>1</sub> receptors are weakly expressed under physiological conditions, but are strongly induced in response to pathological conditions and/or RAAS blockade.<sup>33,34</sup> Interestingly, it has been suggested that B<sub>1</sub> receptors are directly activated by ACE-inhibitors (thus resulting in an increase in endothelial NO release, for instance in the heart<sup>35,36</sup>), by which they contribute to the cardioprotective effects of ACE-inhibitors, but this has not been uniformly confirmed by others.<sup>37</sup> Another possibility is that the up-regulated B<sub>1</sub> receptors are activated by their endogenous ligand during ACE-inhibition. Given the hypotensive,<sup>38</sup> cardioprotective<sup>33</sup> and cerebro-protective<sup>39</sup> effects of such activation, as observed in animal models, one might speculate that patients with genetic defects in their B<sub>1</sub> receptor display a diminished response

to ACE-inhibition. Clearly, more basic research is needed to investigate this novel concept.

In our study, an increasing genetic score was associated with a decrease in absolute risk of CVD events in placebo-treated patients and an increase in absolute risk in perindopril-treated patients in both studies (PERGENE and PROGRESS), independent of clinical characteristics. It may be suggested that the absolute risk of events in these patients was rather low, preventing any benefit of the addition of an ACE-inhibitor. However, the absolute risk for cardiovascular death or myocardial infarction in these patients was 7.7% at 4.2 years follow-up. In an earlier analysis of the EUROPA-trial a consistent treatment benefit was observed in the lower risk tertile (based on assessment of clinical characteristics) with a risk of only 5.3% in the placebo group as well as in the higher risk tertiles.<sup>7</sup> We observed no significant differences in clinical characteristics and intermediate phenotypes (blood pressure and blood pressure reduction during the run-in period) between patients with scores  $<3$  and  $\geq 3$ . Thus, the mechanism underlying the association between the proposed genetic score and treatment response is not explained by clinical characteristics. Unfortunately, no serum or plasma was available to measure levels of RAAS factors in our patients. Future studies will have to be designed to address this issue.

The current study has several limitations that should be noted. The EUROPA-trial consisted of predominantly male Caucasian subjects with stable CAD, who were treated with the ACE-inhibitor perindopril, which limits the generalizability of the results regarding type of patients and type of agent. New pharmacogenetic studies in different patient populations and with different ACE-inhibitors as well as angiotensin-II receptor blockers are warranted. In EUROPA, we studied the ACE-inhibitor perindopril at a dose of 8 mg/day. One could argue that patients not benefiting from treatment were undertreated; however, 8 mg/day is a relatively high dose, and the effect on blood pressure was similar among patients with scores  $<3$  and  $\geq 3$ . The generalizability of our results to other ACE-inhibitors is unknown. Although differences in pharmacological properties do exist between ACE-inhibitors,<sup>40</sup> the clinical relevance of these differences is uncertain and different ACE-inhibitors consistently improve outcome in trials of patients with CAD or heart failure.<sup>1</sup> Although we analysed a large group of patients, it should be appreciated that testing of multiple genes and SNPs might result in chance findings. We corrected for multiple testing using permutation and by preliminary confirmation in another cohort. We choose for permutation and not Bonferonni correction, since Bonferonni is an overly conservative method with the strength of our a priori study hypothesis and the LD between the SNPs located within genes in a common pathway. We would have liked to perform more extensive replication, but unfortunately, a replication cohort of similar size and design as EUROPA is not available. For an initial replication of our findings, we had the opportunity to use data of 1051 European patients of PROGRESS studying the same ACE-inhibitor, perindopril, albeit in lower dose of 4 mg. The PROGRESS study was designed to optimize BP treatment in stroke patients rather than assess the effect of the ACE-inhibitor on cardiovascular endpoints in stable CAD. Because the treatment benefit in PROGRESS was known to be contingent on the combination with indapamide (2.5 mg),<sup>16</sup> we studied patients randomized to single therapy with perindopril or placebo,

which ensures comparability with the EUROPA-trial subjects. In PROGRESS a similar direction and magnitude of the pharmacogenetic interaction was observed and the combined *P*-values improved for all three SNP's by adding PROGRESS to the EUROPA data set, which lends support to our findings. Despite the similar trend in interaction effect, the individual interaction terms of the three SNP's in PROGRESS did not reach statistical significance. This lack of significance is most likely related to limited power because of the relatively small number of patients which could be analysed in PROGRESS (*n* = 1051, 526 perindopril, 525 placebo). Furthermore, the genetic risk score showed concordance in the treatment interaction effect in PROGRESS and PERGENE, which lends additional weight to the findings. The relative change in treatment benefit associated with the genetic variants was identical in both trials (Figure 4).

The current study also has several strengths to be appreciated. It is unique because of its sample size, design (randomized placebo-controlled), accurate phenotypic data and comprehensive coverage of multiple genes in a common pathway. In addition, the genetic variants appeared to have incremental value over and above the clinical risk factors included in the multivariate model. It is also unique since some preliminary replication of the findings in PERGENE was possible in the PROGRESS-trial, even though this was underpowered.

In conclusion, our findings show that three out of four patients had an enhanced benefit of ACE-inhibitor therapy (33% reduction of cardiovascular death or MI, up to 54% in patients with pharmacogenetic score = 0). In contrast, one out of four patients experienced no treatment benefit and a possible adverse outcome with perindopril. The latter patients, with the highest score, had the lowest risk in the placebo group. By developing a pharmacogenetic score related to treatment response, patients can be selected who are most likely to benefit from such treatment and can be distinguished from those without benefit, or even with an adverse response to preventive therapy. This concept of tailored-therapy by pharmacogenetics may have large impact on future clinical practice. Yet the current findings do need further replication by other randomized cohorts, as well as by basic research into the underlying biological plausibility and functional consequences of BK1 receptor variation. Nevertheless, the heterogeneity in event rates and treatment effect as observed in both studies indicates the strong potential of tailored-therapy as clearly not all patients benefit equally from preventive therapy.

Through pharmacogenetic profiling, physicians may be able to predict the response to preventive treatment and distinguish 'positive responders' and 'negative responders' before the start of drug therapy. Taken together, such pharmacogenetic analyses open up a perspective to individualize preventive therapy<sup>24–26</sup> which may avoid unnecessary treatment, and considerably reduce health care costs. To further explore this concept, we suggest that future randomized clinical trials should integrate similar pharmacogenetic approaches in the study design.

## Supplementary material

Supplementary material is available at *European Heart Journal* online.

## Author contributions

Study design: M.B., J.J.B., R.F., K.F., M.P.M.M., W.R., M.L.S.; Data collection: J.J.B., M.P.M.M., T.N., M.L.S., A.U.; Statistical assessment: E.B., J.J.B., C.M.D., A.I., J.C.M.W.; Outcome assessment: E.B.; J.J.B., C.M.D., A.H.J.D., R.F., M.L.S.; Writing: all authors contributed in writing the manuscript.

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