Individualized ACE-inhibitor Therapy in Stable Coronary Artery Disease Based on Clinical and Pharmacogenetic Determinants; The PERGENE risk model

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ABSTRACT

Background: Patients with stable CAD constitute a heterogeneous group in which the treatment benefits by ACE-inhibitor therapy vary between individuals. Our objective was to integrate clinical and pharmacogenetic determinants in an ultimate combined risk prediction model.

Methods and results: Clinical, genetic and outcomes data were used from 8726 stable CAD patients participating in the EUROPA/PERGENE trial of perindopril versus placebo. Multivariable analysis of phenotype data resulted in a clinical risk score (range: 0-21 points). Three SNPs (rs275651 and rs5182 in the angiotensin-II type I-receptor gene and rs12050217 in the bradykinin type I-receptor gene) were used to construct a pharmacogenetic risk score (PGXscore, range: 0-6 points). 785 patients (9.0%) experienced the primary endpoint of cardiovascular mortality, non-fatal MI or resuscitated cardiac arrest during 4.2 years of follow-up. Absolute risk reductions ranged from 1.2% to 7.5% in the 73.5% of patients with PGXscore of 0-2. As a consequence, estimated annual numbers needed to treat ranged from as low as 29 (clinical risk score ≥10 and PGXscore of 0) to 521 (clinical risk score ≤6 and PGXscore of 2). Furthermore, our data suggest that long-term perindopril prescription in patients with a PGXscore of 0-2 is cost-effective.

Conclusions: Both baseline clinical phenotype, as well as genotype determine the efficacy of widely prescribed ACE-inhibition in stable CAD. Integration of clinical and pharmacogenetic determinants in a combined risk prediction model demonstrated a very wide range of gradients of absolute treatment benefit.

The European Trial on Reduction of Cardiac Events with Perindopril in Stable Coronary Artery Disease (EUROPA) and the Heart Outcomes Prevention Evaluation (HOPE) have demonstrated the effectiveness of angiotensin-converting enzyme (ACE)-inhibitors perindopril and ramipril respectively, by reduction of mortality and morbidity from cardiovascular events among patients with stable coronary artery disease (CAD)[1,2]. Consequently, ACE-inhibitors are recommended in clinical guidelines on secondary prevention in patients with stable CAD and hence widely used in this population.[3–5]

However, patients with stable CAD constitute a heterogeneous group in which the absolute risk of cardiovascular complications varies between individuals.[6,7]

Several approaches towards the identification of those patients that are most likely to benefit from ACE-inhibitor therapy have previously been reported. A previously published post-hoc analysis of the EUROPA trial studied baseline clinical risk factors such as age, gender, smoking, cholesterol and blood pressure levels.[6] A risk score founded on such baseline clinical risk factors was able to identify patients at high, medium and relatively low absolute risk (>3%, 1-3% and 1% per annum respectively) of experiencing cardiovascular death, non-fatal myocardial infarction (MI) and resuscitated cardiac arrest.[6] In contrast to the *absolute* treatment benefit, the *relative* treatment effect of perindopril, however, was not modified by the baseline level of risk.[6] Similar conclusions were drawn after investigation of the relation between treatment benefit by perindopril and baseline renal function or the degree of blood pressure reduction.[8–10]

A novel approach towards selection of those that are likely to respond (or not) to ACE-inhibitor therapy is to identify information on genetic variation among patients.[11] A recent publication by our group demonstrated that genetic variation in the renin-angiotensin-aldosterone system (RAAS) and the kallikrein-bradykinin (KB) pathway is associated with the treatment benefit of perindopril.[12] Three single nucleotide polymorphisms (SNPs), two of which in the angiotensin-II type I (AT1) receptor gene and one in the bradykinin type I (BK1) receptor gene, were used to construct an integer-based pharmacogenetic risk score (PGXscore), ranging from 0 to 6 points.[12] We were able to identify two distinct subgroups within the overall study population of 8726 patients on the basis of this PGXscore.[12] One subgroup (73.5% of the patients) was characterized by a more pronounced treatment benefit, whereas no treatment benefit was apparent in the remaining 26.5% of patients.

This current analysis is an ultimate extension of both the previously published clinical risk model[6] and pharmacogenetic risk profile.[12] Its purpose is two-fold: 1) to investigate the relation between identified genetic determinants of treatment benefit and different levels of baseline clinical risk; 2) to integrate clinical and pharmacogenetic determinants in an ultimate combined risk prediction model.

METHODS

Study population and design

The PERindopril GENEtic association study (PERGENE) is a substudy of the EUROPA trial. The designs of both studies have been reported previously.[1,12] In brief, the EUROPA trial was a randomized, double-blinded, placebo-controlled study designed to assess the effect of perindopril (8 mg daily) on the combined primary endpoint of cardiovascular mortality, non-fatal MI and resuscitated cardiac arrest in 12218 patients with stable CAD, but without overt heart failure or uncontrolled hypertension. The use of perindopril resulted in a 20% relative risk reduction (adjusted HR 0.80, 95% CI: 0.71-0.91) in the rate of the primary endpoint during a mean follow-up of 4.2 years.[1]

A DNA bio-bank was established within the EUROPA trial for the purpose of the PERGENE substudy, which investigates whether genetic variation is a determinant of the risk of future adverse cardiovascular outcome and/or treatment benefit by the use of perindopril.[11] DNA was successfully isolated in 9454 patients, using an automated isolation process.[11] Comprehensive coverage of genetic variation in both the RAAS and KB pathways was ensured by a haplotype-tagging-single nucleotide polymorphism (ht-SNP) procedure in 12 candidate genes, as described in detail previously.[11,12]

Our study was approved by the Institutional Review Board of every participating center and written informed consent for genetic association analyses was obtained from all patients.

Clinical risk score

Univariable and multivariable Cox' proportional hazard regression analyses were performed to study the relation between the primary endpoint (cardiovascular mortality, non-fatal MI and resuscitated cardiac arrest) and baseline clinical patient characteristics, such as demographic and clinical variables, medical history, laboratory tests and concomitant medication. Interaction by treatment was investigated for each clinical characteristic. A final multivariable clinical risk model was constructed using a backward stepwise elimination procedure in which removal testing was based on the probability of the likelihood-ratio statistic based on the maximum partial likelihood estimates. In order to develop a clinical risk-scoring system, the log HRs from the final multivariable model were converted to an estimated risk score.[6,13] Clinical risk scores were calculated for each of the patients of the currently described population (only those trial participants of whom both baseline clinical characteristics and (pharmaco)genetic profile were complete). The study population was divided into tertiles in order to distinguish low, medium and high clinical risk profiles.

Pharmacogenetic risk profile and replication

The PERGENE substudy assessed 52 SNPs with the use of Taqman allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) and Sequenom (San Diego, CA, USA) mass-spectrometric genotyping. 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 selected 52 SNPs were included in the PERGENE analyses (n = 8907).[12]

Seven SNPs have previously been reported to significantly modify the treatment effect of perindopril in univariate analyses.[12] After multivariate adjustment and correction for multiple testing, three SNPs remained significant modifiers of the perindopril treatment effect: rs275651 and rs5182 in the angiotensin-II type I (AT1) receptor gene and rs12050217 in the bradykinin type I (BK1) receptor gene. These three SNPs formed the foundation of a previously published PGXscore, ranging from 0 to 6 points, which was constructed by counting the number of alleles that were associated with a decreased benefit of perindopril treatment.[12] The association between the PGXscore and treatment benefit by perindopril, as found in PERGENE,[12] was replicated in the PROGRESS study, which investigated the treatment effect of perindopril in patients with cerebrovascular disease.[14]

Statistical analysis

Differences in baseline clinical characteristics between low, medium and high clinical risk groups were assessed by chi-square tests in case of categorical data or one-way analysis of variance in case of continuous data. A multivariate Cox proportional hazards regression model was fitted with the following covariates: clinical risk score, PGXscore, treatment and treatment*PGXscore interaction (full model). The baseline hazard function $H_0(t)$ was estimated by dividing the cumulative hazard at the end of follow-up through the exponential function of the mean of the covariates. The cumulative survival under perindopril treatment versus placebo at the median follow-up of 4.2 years was calculated for each clinical risk score within the separate pharmacogenetic risk strata as follows: S (4.2years) = 0.033975 * exp(0.196*clinical risk score – 0.203*PGXscore – 0.793*treatment – 0.318*interaction term) With respect to "treatment" placebo was defined as 0 and perindopril treatment as 1. The "interaction term" was the multiplication of the PGXscore * treatment. Absolute and relative risks, as well as crude and adjusted hazard ratios (HR) are presented with 95% confidence intervals (CI). Numbers needed to treat (NNT) in order to prevent one event per annum were calculated as the inverse of the absolute risk reduction at the mean clinical risk scores per stratum.

The performance of the model consisting of clinical risk score only was compared by two different methods with the full model with respect to discrimination. First, the c-index and areas under the two receiver operating characteristic curves were compared by a nonparametric method, as previously described by de Long et al.[15] Secondly, the difference in model-based discrimination slopes was evaluated through integrated discrimination improvement (IDI).[16] Calibration of both the model consisting of clinical risk score only and the full model was tested with the Hosmer-Lemeshow [H-L] goodness-of-fit test. All statistical tests were two-sided with a type I error level of 0.05, except for the IDI for which a conservative significance level of 0.01 was maintainted.[16]

We performed a cross-validation within our own dataset by bootstrap methods as suggested by Harrell et al.[17] We constructed 300 bootstrap samples (training) from the full original sample with the same size as the original (test). Models were built in the training sets. C-indices were then obtained in these training sets ($C_{training}$) and compared with the c-indices of the models when applied to the test set (C_{test}). The optimism in the fit from bootstrap sample i is defined as $O_i = C_{i,training} - C_{i,test}$. We report the mean O of these optimism estimates. Analyses were performed with IBM SPSS statistics version 23.0 and STATA version 12.

Cost-effectiveness analysis

We examined the potential cost-effectiveness of the combined clinical risk score and PGXscore. The time horizon was restricted to the duration of the EUROPA trial/PERGENE study (mean follow-up of 4.2 years). Costs were set at 15 euros for the analysis of the three SNPs of the PGXscore, 50 euros for perindopril (based on the current price of perindopril 8 mg tablets in the Netherlands), and 3,000 euros for a clinical event (a weighted average of the costs of treating myocardial infarction and the costs of cardiac death). The health loss of a clinical event within the trial duration was set at 0.6 years (a weighted average of the relative frequency and life-years lost from myocardial infarction (0 years) and cardiac death (2 years).

The following patient management strategies were examined, against the strategy of no perindopril treatment (as the comparator):

1) Pharmacogenetic testing only in patients with a high clinical risk score and perindopril treatment only if PGXscore=0-2
2) Pharmacogenetic testing only in patients with a medium or high clinical risk score and perindopril treatment only if PGXscore=0-2
3) Pharmacogenetic testing in all patients and perindopril treatment only if PGXscore=0-2
4) Perindopril treatment in all patients irrespective of PGX score.

RESULTS

Complete data on baseline clinical patient characteristics and (pharmaco)genetic profile were obtained for 8726 patients (of which 4338 were allocated to perindopril and 4388 to placebo). Median follow-up was 4.2 years (interquartile range 4.0-4.5 years), during which 785 patients (9.0%) experienced the primary endpoint of cardiovascular mortality, non-fatal MI or resuscitated cardiac arrest. Treatment with perindopril was protective in the overall study population; the number of patients on perindopril treatment that experienced the primary endpoint was 346 (8.0%) versus 439 (10.0%) on placebo (adjusted HR 0.80, 95% CI: 0.68-0.92) Baseline characteristics of the overall study population and various subgroups according to the clinical risk level are provided in table 1. Interaction between study treatment and clinical characteristics (including concomitant medication) was not found.

Table 1. Baseline study population characteristics

	Total population	CLINICAL RISK LEVEL			p-value *
		Low	Medium	High	
N (%)	8726	3167 (36.3)	3474 (39.8)	2085 (23.9)	
Age, years	59.8 (9.3)	57.7 (8.0)	59.4 (9.2)	63.8 (10.0)	<0.001
Male gender (%)	85.5	81.5	87.6	88.2	<0.001
Hypertension (%) †	29.0	23.0	28.0	39.0	<0.001
Diabetes mellitus (%)	13.0	4.0	11.0	30.0	<0.001
Hypercholesterolemia (%) ‡	63.0	69.0	60.0	58.0	<0.001
Current smoking (%) §	15.0	6.0	16.0	25.0	<0.001
Obesity (BMI>30 kg/m^2) (%)	21.3	8.2	24.0	36.7	<0.001
Symptomatic CAD (%) ||	25.4	9.5	25.9	48.9	<0.001
Family history of CAD (%)	27.0	22.0	29.0	32.0	<0.001
Previous MI (%)	65.0	44.0	73.0	84.0	<0.001
Previous revascularisation (%)	55.0	75.0	49.0	33.0	<0.001
Previous stroke or PAD (%)	8.9	0.8	5.2	27.5	<0.001
CONCOMMITANT MEDICATION					
Platelet-inhibitors (%)	92.0	94.0	92.0	89.0	<0.001
Beta-blockers (%)	63.0	62.0	65.0	63.0	0.104
Lipid-lowering agents (%)	55.0	64.0	53.0	46.0	<0.001
Calcium-antagonists (%)	32.0	29.0	31.0	37.0	<0.001
Systolic blood pressure (mmHg)	136.9 (15.2)	132.7 (13.9)	137.7 (15.1)	142.1 (15.5)	<0.001
Diastolic blood pressure (mmHg)	81.8 (8.1)	80.6 (7.9)	82.4 (8.1)	82.7 (8.3)	<0.001
Creatinine clearance (µmol/L) #	86.5 (25.7)	88.9 (22.2)	87.7 (26.6)	80.9 (28.3)	<0.001
Total cholesterol (mmol/L)	5.4 (1.0)	5.1 (0.9)	5.5 (1.0)	5.7 (1.1)	<0.001

Table 1. *(continued)*

	Total population	CLINICAL RISK LEVEL			p-value *
		Low	Medium	High	
OUTCOME					
Randomization, allocation to perindopril (%)	49.7	51.0	48.9	49.5	0.296
Primary endpoint	**9.0**	**4.6**	**8.8**	**16.2**	**<0.001**
Systolic / diastolic blood pressure reduction by perindopril (mmHg)**	8.6 / 4.0	7.3 / 3.9	9.2 / 4.1	9.6 / 4.1	<0.001 / 0.416
RISK SCORE		**0-6**	**7-9**	**10-21**	
Mean clinical risk score	**7.67 (2.83)**	**4.84 (1.20)**	**7.93 (0.80)**	**11.53 (1.74)**	**N.A.**
Mean pharmacogenetic risk score	**1.82 (1.13)**	**1.82 (1.12)**	**1.82 (1.12)**	**1.86 (1.11)**	**0.435**

Summary statistics for continuous variables are presented as mean (standard deviation). Categorical data are summarized as percentages. CAD=coronary artery disease MI=myocardial infarction N.A.=not applicable PAD=peripheral artery disease

* For differences between low, medium and high clinical risk levels.

† Blood pressure >140/95 mm Hg or receiving antihypertensive treatment.

‡ Previously known total cholesterol >6·5 mmol/L or receiving lipid-lowering treatment.

§ Use of tobacco within the last month

|| Stable angina pectoris or history of congestive heart failure

Estimation by Cockroft-Gault equation

** 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 perindopril.

Significant baseline clinical risk predictors and the point-scoring system, which derived from backward elimination, are presented in table 2. The log HRs from the final multivariable model that were converted to the clinical risk score are provided in the Online data supplement. The clinical risk score could theoretically range from 0-32, yet calculated individual scores within our study population ranged from 0-21 with a mean value of 7.67 +/- 2.83 (figure 1). The 33rd and 67th percentiles were at 6.00 and 9.00 points respectively, and used as cut-offs in order to distinguish low, medium and high clinical risk levels. The skewness of the distribution (figure 1) prevented formation of three groups of similar size. It should be noted that the high risk group consists of 23.9% of the overall study population (tables 1 and 3). Incidences of all known baseline cardiovascular risk factors were highest in the higher clinical risk groups (table 1), with exception of previously diagnosed hypercholesterolemia, which actually was lowest in the high-risk subgroup. In accordance, high-risk patients also presented with the lowest rate of statin use. These findings, however, were counterbalanced by the fact that patients in the high-risk subgroup did have the highest total cholesterol levels (table 1).

The primary endpoint rates in the low, medium and high clinical risk groups were 4.6%, 8.8% and 16.2% respectively (p<0.001, table 1). These differences in event rate

Table 2. Clinical risk scores of baseline risk parameters

Continuous clinical risk parameters				**Clinical Risk Score points**
Age	Systolic blood pressure	Creatinine clearance	Total cholesterol mmol/L (mg/dL)	
<67	≤130	>70	≤3.5 (≤135)	**0**
67-69	>130-≤160	>55-≤70	>3.5-≤5.0 (>135-≤193)	**1**
70-72	>160	>35-≤55	>5.0-≤6.5 (>193-≤251)	**2**
73-76		≤35	>6.5-≤8.0 (>251-≤309)	**3**
77-79			>8.0 (>309)	**4**
80-82				**5**
83-85				**6**
>85				**7**
Dichotomous clinical risk parameters				
Previous stroke or PAD				**3**
Male gender				**2**
Obesity (BMI>30 kg/m^2)				**2**
Current smoking				**2**
Symptomatic CAD				**2**
Diabetes mellitus				**2**
Previous MI				**2**
Family history of CAD				**1**
Previous revascularisation				**–1**

The range of clinical risk scores = 0-32 and points for each of applicable variables need to be added to each other. CAD=coronary artery disease MI=myocardial infarction PAD= peripheral artery disease.

Table 3 . Distribution of patients over clinical and pharmacogenetic risk strata

Clinical risk level	**Low**	**Medium**	**High**
Clinical risk score	**0-6**	**7-9**	**10-21**
Pharmacogenetic risk score N(%)			
0	362 (4.1)	390 (4.5)	232 (2.7)
1	945 (10.8)	1037 (11.9)	618 (7.1)
2	1027 (11.8)	1144 (13.1)	655 (7.5)
≥3	833 (9.5)	903 (10.3)	580 (6.6)

Treatment benefit of perindopril was only demonstrated within the group of patients with pharmacogenetic risk scores < 3 (N=6410, 73.5% of the total study population). The Linear-by-Linear Association p-value for the entire table is 0.43.

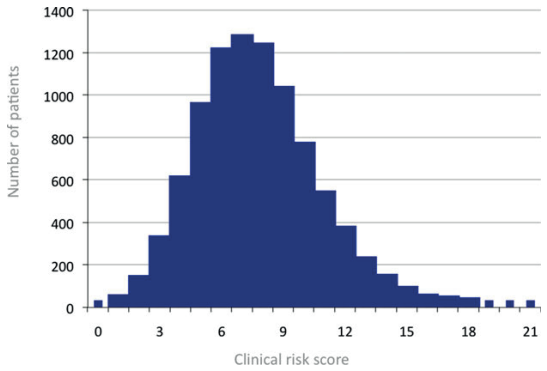

Figure 1. Clinical Risk Score distribution
The mean value of the clinical risk score (N=8726) was of 7.67 +/- 2.83

can be explained by the observed differences in baseline clinical risk factors, but not by confounding due to study drug allocation, since the latter was similar over the three clinical risk strata (p=0.296, table 1).

Adjusted HRs for the treatment effect of perindopril were 0.72, 0.70 and 0.91 for the lowest to highest clinical risk tertiles respectively. Heterogeneity of treatment effect was tested and ruled out (p=0.31). Thus, the relative treatment benefit was not modified by the baseline clinical risk level. However, baseline clinical risk level did modify *absolute* risk reductions. The use of perindopril in the overall study population (n=8726) resulted in a 2.23% risk reduction of the primary endpoint (95% CI: 1.03 – 3.44, annual NNT 189, 95% CI:122-401). However, absolute risk reductions varied from 1.24% to 2.17% and 3.97% in the lowest, medium and highest clinical risk tertiles. As a consequence, NNTs were inversely related to increasing clinical risk scores (table 4).

Table 4. Numbers needed to treat (per annum)

Clinical risk level	**Low**	**Medium**	**High**
Clinical risk score	**0-6**	**7-9**	**10-21**
NNT per clinical risk stratum	382	218	119
NNT per pharmacogenetic risk stratum			
0	93	54	29
1	164	92	50
2	521	298	164
≥3*	*–529*	*–302*	*–164*

*Stratum with non-significant risk increase due to use of perindopril.

The pharmacogenetic risk scoring system, based on the previously identified 3 SNPs, is presented the Online data supplement. Risk alleles for lack of treatment benefit were T, C, and G for Rs275651, Rs5182 and Rs12050217 respectively. The individual PGXscores range from 0-6 points with a mean value of 1.82 +/- 1.13 (table 1). Significant heterogeneity of treatment effect across pharmacogenetic profiles was observed. A pronounced treatment benefit was observed in 6410 patients (73.5%) with PGXscore <3 (adjusted HR 0.67, 95% CI: 0.56-0.79), whereas no benefit was observed in the remaining subgroup of 2316 patients (26.5%) with PGXscore ≥3 (adjusted HR 1.26, 95% CI: 0.97-1.67) (table 3 for patient distribution).

The use of perindopril in patients with PGXscores of 0 and 1 point resulted in absolute risk reductions of 7.50% (95% CI: 3.69 – 11.73) and 4.30% (95% CI: 2.00 – 6.53) respectively. Consequently, annual numbers needed to treat were 55 (95% CI: 113 – 38) for patients with a PGXscore of 0 and 97 (95% CI: 210 – 63) for patients with a PGXscore of 1. The point estimate of the absolute risk reduction associated with the use of perindopril in the subgroup of PGXscore of 2 was in the same positive direction, yet non-significant (1.34%, 95% CI: −0.77 to 3.47 and NNT (per annum)= 311, 95% CI: −546 to 122).

In contrast, a non-significant estimated absolute risk increase of 1.32% was observed in patients with a PGXscore ≥3 using perindopril (95% CI for risk increase −0.97 to 3.67 and NNT (per annum)= −315, 95% CI: −118 to 433).

Combined baseline clinical and pharmacogenetic risk profiles

Mean pharmacogenetic risk scores were identical over all three clinical risk strata (p=0.435, table 1) and formal testing did not trace interaction between the clinical and PGXscores. The distribution of patients over the various clinical and pharmacogenetic risk strata is given in table 3. Figures 2a-2d describe the relation between absolute risks of the primary endpoint, clinical risk profile and treatment for each of the separate pharmacogenetic risk strata. Lack of treatment benefit was observed across the entire spectrum of clinical risk in patients with a PGXscore ≥3 (figure 2d and figure 3).

Increasing clinical risk scores led to increasingly pronounced risk differences between perindopril and placebo in all pharmacogenetic strata. Hence, extremes of treatment effect were found in patients with high clinical risk profiles. For example, the use of perindopril in patients with a clinical risk score of 19 resulted in an estimated absolute risk reduction of 28.42% (95% CI: 22.46 – 34.09) in case of a PGXscore of 0 versus an estimated risk increase of 5.82% (95% CI: 1.78 – 9.83) in case of a PGXscore ≥3 (figure 3). Concordantly, NNTs decreased in subgroups with higher clinical risk profiles and lower PGXscores, both of which were associated with more pronounced treatment effects (table 4). Estimated numbers needed to treat were as low as 29 (95% CI: 17 – 113) in patients with a high clinical risk profile and a PGXscore of 0, whereas those with a low

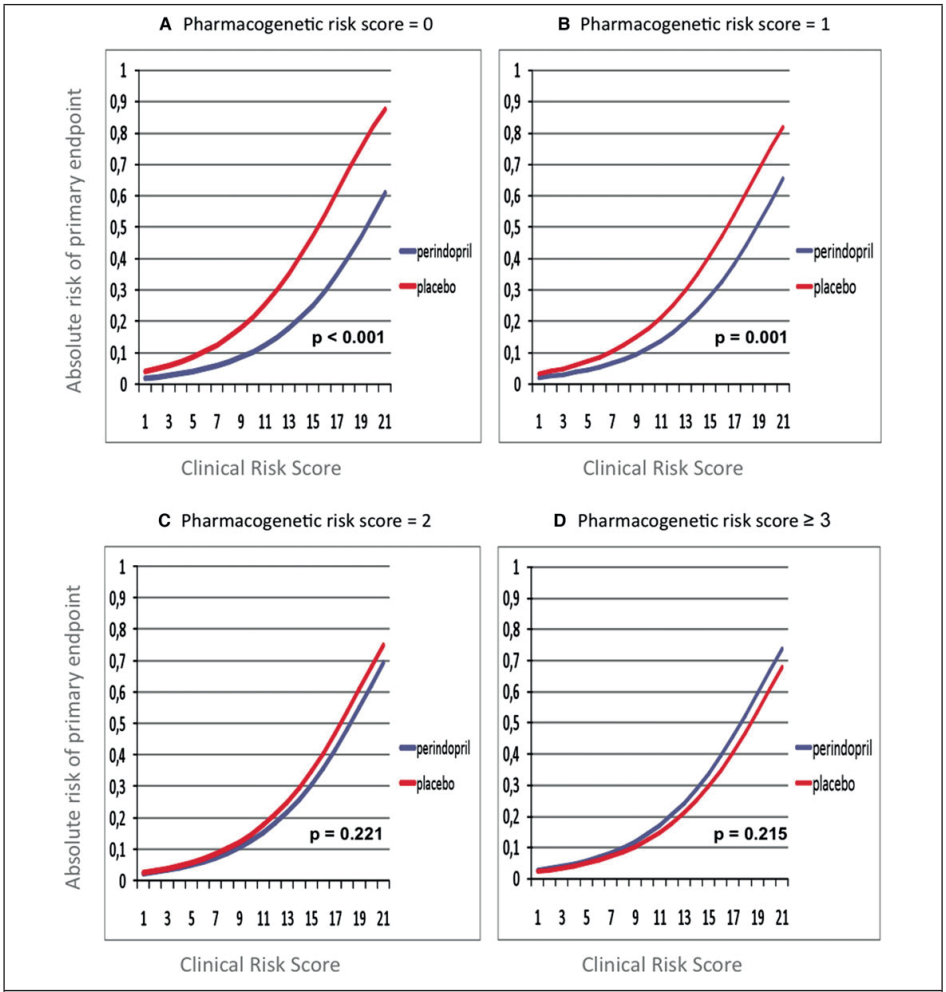

Figure 2. Absolute risks of the primary endpoint across different clinical and pharmocogenetic risk strata

P-values were derived from multivariate Cox proportional hazards regression models fitted with the following covariates: clinical risk score, PGXscore, treatment and treatment*PGXscore interaction. PGX-score= pharmacogenetic risk score

clinical risk profile and a PGXscore of ≥3 did not experience any benefit (NNT= −529, 95% CI: −105 to 189).

In separate analyses with only cardiovascular mortality or non-fatal MI as sole endpoint, we observed directional concordance, compared to the presented analysis of the combined primary endpoint, with respect to NNTs over the various clinical and pharmacogenetic risk strata.

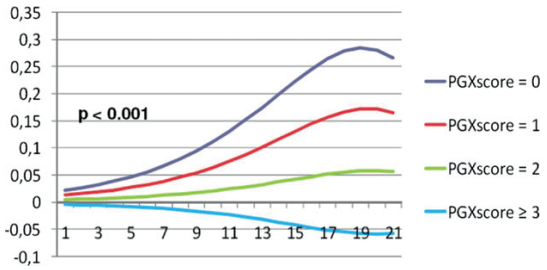

Figure 3. Absolute risk reduction (Y-axis on a 0 to 1 scale) by perindopril across different levels of clinical (X-Axis) and pharmacogenetic risk
PGXscore = pharmacogenetic risk score

Discrimination and calibration of the clinical and combined risk models

Calibration and discrimination were assessed for two models: A) the model consisting of clinical risk score only, and B) the full model consisting of clinical risk score, PGXscore and treatment*PGXscore interaction.

Addition of pharmacogenetic information on top of clinical risk profile resulted in better discrimination. The c-index for the full model (0.68, 95% CI: 0.66-0.70) was significantly higher than the c-index for the model consisting of the clinical risk score only (0.66, 95% CI: 0.64-0.68) (p=0.0015). The full model also resulted in a significantly better discrimination when assessed with integrated discrimination improvement (magnitude of increase in IDI: 0.00472, p=0.0002). Validation of both models by bootstrap methods showed that the bias in the estimated discrimination performance (c-index) is likely to be small, since the mean optimism estimates were only 0.006 and 0.007 for the clinical

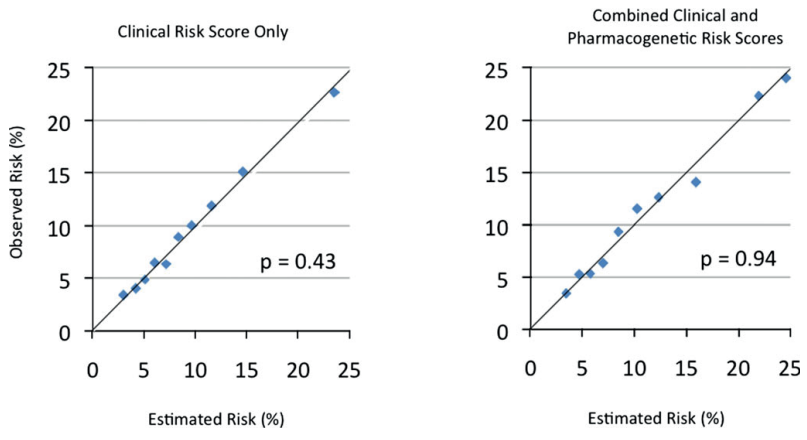

Figure 4. Observed versus estimated risks according to the clinical and combined (full) risk prediction models
P-values were derived from Hosmer-Lemeshow goodness-of-fit tests

risk score and the full model, respectively. Finally, The H-L goodness-of-fit tests were non-significant (p=0.43 for the model with the clinical risk score only and p=0.94 for the full model), indicating adequate calibration for both models (figure 4).

Cost-effectiveness of tailored perindopril treatment on the basis of pharmacogenetic testing

The results of the cost-effectiveness analysis, against the strategy of no treatment with perindopril as comparator, are displayed in table 5.

The highest number of gained life-years is observed in strategies 3 and 4. Strategy 3 implies that all patients are genetically tested and only those with a PGXscore of 0-2 are treated with perindopril. Strategy 4 implies that none of the patients are genetically tested and all are treated with perindopril. Strategy 4 however is dominated by strategy 3. The lower incremental cost-effectiveness ratio (ICER) of strategy 3 indicates that tailored perindopril therapy on the basis of the PGXscore will ultimately reduce costs, with a similar effectiveness in terms of gained life-years.

Strategy one results in the least life-years gained, but also in the least costs and the lowest ICER and therefore may be an option when strictly reasoning from the cost perspective alone.

Table 5. Costs, gained life-years and incremental cost-effectiveness ratio of various treatment strategies, against the strategy of no treatment with perindopril as comparator.

Strategy	Number of patients treated with perindopril, N (%)	Incremental costs (weighted)	Life-years gained (weighted)	ICER
1. Pharmacogenetic testing only in patients with a **high clinical risk score** (≥10) and perindopril treatment only if **PGXscore=0-2**	1505 / 8726 (17.2)	30.38	0.0017	18,139
2. Pharmacogenetic testing only in patients with a **medium or high clinical risk score** (≥7) and perindopril treatment only if **PGXscore=0-2**	4076 / 8726 (46.7)	90	0.0032	27,987
3. Pharmacogenetic testing in **all patients** and perindopril treatment only if **PGXscore=0-2**	6410 / 8726 (73.5)	147	0.0040	36,743
4. Perindopril treatment in **all patients irrespective of PGXscore**	8726 (100)	232	0.0035	67,230

The time horizon was restricted to the duration of the EUROPA trial/PERGENE study (mean follow-up of 4.2 years). Costs are in euros. PGXscore = pharmacogenetic risk score. ICER = incremental cost-effectiveness ratio

DISCUSSION

The present study highlights that clinical as well as pharmacogenetic determinants independently modify *absolute* treatment benefit by ACE-inhibitor perindopril in a population of patients with stable CAD. Moreover, both clinical and pharmacogenetic profiles could be expressed in risk scores that are fairly simple to use for clinical decision-making. We propose the use of a PGXscore on top of known clinical risk factors for better risk stratification and more concrete estimation of absolute treatment benefits of ACE-inhibitor therapy in daily clinical practise. Increasing clinical risk scores and decreasing PGXscores were consistently and positively related to the absolute treatment benefit by ACE-inhibitor perindopril. Impressive risk gradients and, as a consequence, important differences in NNTs were found across various subgroups. The annual NNT in the overall study population was 189, whereas estimates as low as 106 for the entire clinical high-risk subgroup and even 29, in case of a combined high clinical risk profile and a PGXscore of 0, were observed. On the other hand, the entire subgroup of patients with a PGXscore ≥3 (26.5% of the overall study cohort) was characterized by a lack of treatment benefit, which was consistent across all three clinical risk levels.

The clinical risk score in our study was based upon easily obtainable traditional risk factors that have repeatedly proven to be valuable predictors.[18–20] The full model predicted the highest *absolute* risk reductions in patients with higher clinical risk profiles. In this regard, it remains important to emphasize that formally no heterogeneity of relative treatment effect was found across the various clinical risk levels. Furthermore, the mean clinical risk score in the high-risk level was 11.53. Scores of e.g. 19 can therefore be regarded as extremely high. Such extreme risk scores were underrepresented in our RCT data, but nevertheless such patients do present themselves in clinical practise. It is plausible that in such extremely high-risk individuals, the risk is largely determined by the aforementioned risk factors, and that an ACE-inhibitor alone will have *relatively* less effect on survival. In other words, the magnitude of both controllable and uncontrollable clinical risk factors in such a patient could have a *relatively* more profound effect on the risk of reaching the primary endpoint, than the potential *relative* treatment benefit by an ACE-inhibitor alone. Obviously, the absolute risk benefit will remain high in such patients and treatment with an ACE-inhibitor should therefore be warranted. This finding, however, once again emphasises the necessity of proper management of all controllable risk factors in patients with stable CAD.

With this in mind, it is remarkable that a history of coronary revascularization was associated with a modestly reduced risk for the primary endpoint (−1 point) in the presented risk model. This particular observation should be interpreted with some reservation, since several specifically designed trials, such as RITA-2,[21] COURAGE[22] and BARI 2D[23] failed to demonstrate survival benefit of coronary revascularisation over optimal medical therapy.

The pharmacogenetic risk score in our study was based upon three SNPs that have previously emerged after comprehensive coverage of the RAAS and KB systems and subsequent correction for multiple testing.[12] Furthermore, the pharmacogentic risk score has previously been replicated in participants of the PROGRESS-trial.[12] Clinical risk factors,[6] renal function,[8] degree of blood pressure reduction[9] and a number of biomarkers[24,25] have been explored within the EUROPA trial, yet only pharmacogenetic information has permitted to distinguish responders to perindopril from non-responders (26.5% of all patients). Furthermore, the PGXscore accentuated striking differences in absolute treatment benefits of ACE-inhibitor therapy within each of the separate clinical risk strata.

The data that are presented here are unique. Although it is widely recognized that both phenotype as well as genotype play a fundamental role in health and disease outcome, very few reports exist that actually combine both for prognostication. To our best knowledge, this is the first and only manuscript that combines clinical and genetic information in patients with CAD. Pharmacogenetic information is successfully translated into a potential clinical utility to study the gradients of treatment effect by an ACE-inhibitor. The sample size is large and various additional qualities of a well-designed placebo-controlled double-blinded RCT, such as high quality phenotypical data and independent event adjudication are apparent. Previous studies that have investigated the relation between genetic variation and treatment benefit by ACE-inhibitor therapy usually were characterized by small sample sizes and non-randomized designs without placebo controls.[26] Only two studies with large sample sizes have been reported. Harrap and colleagues studied macrovascular events, dementia and cognitive decline in 5688 patients with a history of cerebrovascular disease in the PROGRESS study and found no interaction between genetic variation and treatment benefit by perindopril.[27]

Negative findings were also published by the GenHAT investigators, who studied cardiovascular mortality and non-fatal MI in 7528 patients on a lisinopril based regimen in the setting of an active-controlled RCT.[28] These two studies obviously differ from our present study in the type of study population, endpoints and study drug. The most remarkable difference with our study, however, is the fact that both studies solely focussed on a single ACE insertion/deletion polymorphism, thus not taking account of the full complexity of the RAAS and KB systems. Furthermore, our PGXscore was replicated in the PROGRESS study, in which a similar direction and magnitude of pharmacogenetic interaction was observed.[12]

Our findings also have some limitations. This study describes differences in treatment benefit across a range of clinical and genetic subgroups. The constituents of the clinical scoring system are all well established cardiovascular risk factors and the PGXscore has been replicated. Still, it is important to realize that, in general, any post-hoc analysis based on subgroups should primarily be regarded as hypothesis-generating. Confirmation of

our findings in other large datasets would invigorate the presented conclusions and derived clinical implications. The EUROPA trial was powered for detection of treatment benefit for the entire study population irrespective of clinical or pharmacogenetic risk categories. Thus lack of power cannot be excluded as an explanation for the observed non-significant treatment benefit in the higher PGX scores. On the other hand it must be noted the absolute numbers of study participants in PGXscores ≥ 2 are higher than those below (table 3).

Patients enrolled in the EUROPA trial primarily consisted of Caucasian males without overt heart failure, who were randomized to placebo or perindopril 8 mg daily. The generalizability of the presented results towards other patient groups, e.g. those with a higher proportion of women, heart failure, patients of other ethnicities, or those using other ACE-inhibitors or lower dosages of perindopril, may therefore be limited. Testing of these particular genetic variants in a large randomized heart failure trial would be required before suggesting the same phenomenon exists in that very different patient group.

Our combined primary endpoint consisted of cardiovascular mortality, non-fatal MI and resuscitated cardiac arrest. Resuscitated cardiac arrest however only occurred in very few instances. Therefore our results with respect to clinical and pharmacogenetic determinants of treatment benefit are primarily associated with the incidence of cardiovascular mortality and non-fatal MI.

In order to facilitate clinical utility and ease of use, we specifically chose to develop an integer-based risk score. Disadvantages of integer-based risk scores in general include the fact that not all variables have exactly the same contribution to the model. Furthermore, certain combinations of risk factors may act synergistically to increase risk in a manner that is more than additive. Such synergy may be underestimated in a purely additive integer-based risk score.[29]

Replication of the three SNPs that formed the PGXscore in the PROGRESS trial[14] demonstrated concordant associations between the risk score and treatment benefit by perindopril.[12] The individual interaction terms of the three SNPs, however, did not reach statistical significance in that particular trial due to limited statistical power (replication could take place in 1051 patients only). Unfortunately larger replication cohorts are not available.

Although the clinical risk model consists of established cardiovascular risk factors, formally the combined clinical and pharmacogenetic risk score has not been independently validated on a separate dataset.

Finally, our risk model does not contain data on circulating serum biomarkers other than total cholesterol and creatinine levels. A prespecified substudy of the EUROPA trial, called PERTINENT, actually did study bradykinin, angiotensin II, but also markers of endothelial function (nitric oxide synthase) and inflammation (C-reactive protein, tumour

necrosis factor-α and von Willebrand factor). The use of perindopril was reflected in various circulating biomarker levels which were interpreted as a biochemical indication of normalization of the angiotensin II/bradykinin balance, reduction of inflammation and prevention of endothelial apoptosis.[24,25] Unfortunately, the cohort in which these biomarkers were assessed was too small in order to properly study the interaction between the various serum biomarkers and treatment effect by perindopril on clinical endpoints.

In conclusion, our results show that a combination of phenotypical and genetic information can be used to demonstrate a range of gradients of absolute treatment benefit by ACE-inhibitor therapy in an otherwise seemingly homogeneous population of patients with stable CAD. Clinical and pharmacogenetic profiling in individual patients may both clarify their distinct level of absolute risk of adverse events and furthermore also the degree of risk reduction by an ACE-inhibitor regimen. Refraining from ACE-inhibitor therapy in those patients that are expected to lack any treatment benefit may avoid unnecessary side-effects, reduce healthcare costs and increase overall efficacy of the drug. Future randomized clinical trials could advance the field of individualized medicine by incorporation of a similar combined clinical and pharmacogenetic approach in their study design.

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Conflict of interest disclosures

There is no commercial association that might pose a conflict of interest in connection with this manuscript. The sponsor of the EUROPA trial, Servier, had no role in the design, conduct, analysis or interpretation of this substudy, nor in the preparation, review or approval of this manuscript.

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ONLINE DATA SUPPLEMENT

Individualized ACE-inhibitor Therapy in Stable Coronary Artery Disease Based on Clinical and Pharmacogenetic Determinants;
The PERGENE risk model

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Online Table 1. The regression coefficients from the final multivariable model that were converted to the clinical risk score.

Variable	Regression coeeficients (ß)
Age (years)	0.023
Systolic bloodpressure (mmHg)	0.005
Creatinine clearance (ml/min)	–0.004
Total cholesterol (mmol/L)	0.125
Previous stroke or PAD	0.552
Male gender	0.447
Obesity (BMI>30 kg/m^2)	0.377
Current smoking	0.462
Symptomatic CAD	0.430
Diabetes mellitus	0.449
Previous MI	0.422
Family history of CAD	0.086
Previous revascularisation	–0.168

For the continuous variables (age, systolic bloodpressure, creatinin clearance and total cholesterol) the regression coefficients are described per unit of increase of that particular independent variable.

Online Table 2. Pharmacogenetic risk score on the basis of allele distribution

Angiotensin-II type I-receptor		Bradykinin type I receptor	**Pharmacogenetic Risk Score points**
SNP: Rs275651	Rs5182	Rs12050217	
AA	TT	AA	**0**
AT	CT	AG	**1**
TT	CC	GG	**2**

The range of pharmacogenetic risk scores = 0-6. Risk alleles for decreased benefit of perindopril treatment were T, C, and G for Rs275651, Rs5182 and Rs12050217 respectively. Points for each of the three separate SNPs need to be added to each other. E.g. a patient with AA, CC, AG (for Rs275651, Rs5182 and Rs12050217 respectively) has an individual pharmacogenetic risk score of 0+2+1 =3

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