Research Article
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Pharmacogenomics

Genome-wide association study identifies CAMKID variants involved in blood pressure response to losartan: the SOPHIA study

Background: Essential hypertension arises from the combined effect of genetic and environmental factors. A pharmacogenomics approach could help to identify additional molecular mechanisms involved in its pathogenesis. **Aim:** The aim of SOPHIA study was to identify genetic polymorphisms regulating blood pressure response to the angiotensin II receptor blocker, losartan, with a whole-genome approach. **Materials & methods:** We performed a genome-wide association study on blood pressure response in 372 hypertensives treated with losartan and we looked for replication in two independent samples. **Results:** We identified a peak of association in *CAMK1D* gene (rs10752271, effect size -5.5 ± 0.94 mmHg, $p = 1.2 \times 10^{-8}$). *CAMK1D* encodes a protein that belongs to the regulatory pathway involved in aldosterone synthesis. We tested the specificity of rs10752271 for losartan in hypertensives treated with hydrochlorothiazide and we validated it *in silico* in the GENRES cohort. **Conclusion:** Using a genome-wide approach, we identified the *CAMK1D* gene as a novel locus associated with blood pressure response to losartan. *CAMK1D* gene characterization may represent a useful tool to personalize the treatment of essential hypertension.

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Keywords: angiotensin II receptor blockers • genome-wide association analysis • genomics • hypertension • losartan • pharmacogenomics

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Background

Different classes of effective antihypertensive drugs that act on a variety of blood pressure (BP) regulating mechanisms are currently available. In spite of this, BP is adequately controlled in less than 40% of treated hypertensive patients. Moreover, individual responses to a given antihypertensive therapy display considerable heterogeneity [1], which is most likely due to diversity in the physiopathological and pathogenetic mechanisms involved in essential hypertension (EH). At present the choice of an antihypertensive drug for a patient remains empirical and cannot be based on precise clinical, laboratory and instrumental *a priori* criteria. Although there have been attempts to identify which patients could respond to a given antihypertensive drug on the basis of anthropometric (e.g., age, sex and BMI) or biochemical (e.g., renin profile and insulin sensitivity) parameters, these methods have only been of marginal clinical use [2]. Hypertension arises from the combined effect of genetic and environmental factors, and BP variance explained by genetic factors is at least 30% [3]. Since an efficient therapy may interact with some of the pathways controlling BP, a pharmacogenomics approach using drugs with a known mechanism of action could help to detect some new steps involved in the pathogenesis of EH.

The aim of the SOPHIA study was to identify polymorphisms regulating BP response to the angiotensin II receptor blocker, losartan (LOS). To address this issue we performed a genome-wide association study (GWAS) by genotyping on the Illumina 1M

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array [4] a sample of 494 essential hypertensive patients from the SOPHIA study [5] treated with LOS. We then verified the specificity for LOS, by testing the SNPs significantly associated with response to LOS in a sample of EH patients treated with hydrochlorothiazide (HCTZ). Moreover, we looked for replication of association of the BP response to LOS in two independent samples of hypertensives from the GERA2 [6] and GENRES [7] studies.

Materials & methods

Sample description

The study was approved by the ethics committee of the University of Sassari (Sassari, Italy) and supported by the local ethics committees and all participants provided written informed consent to the study [5]. All study procedures were in accordance with the Declaration of Helsinki and the institutional/international guidelines on high blood pressure management: thus, our research was conducted according to the principles of ethics.

Supplementary Figure 1 (see online at: www.future-medicine.com/doi/suppl/10.2217/pgs.14.119) shows the entire patients' flow from the screening start (week -8) to the end of the study (week +4) and all preanalysis quality control steps. In particular, we enrolled 722 subjects with mild-to-moderate EH aged from 18 to 59 years without comorbidities. Twelve clinical research centers all over Italy participated in collecting phenotypic data and DNA samples for the SOPHIA study between 2005 and 2009. At the screening visit (week -8), patients had to display systolic BP from 140 to 179 mmHg and diastolic blood pressure from 90 to 109 mmHg. At each visit, office BP was measured in the sitting position after 5 min rest, three times and with a 30–60 s interval between readings, using a certified electronic device (OMRON 705IT). The same range had to be confirmed 4 weeks later (week -4), as well as at the end of the 8 week run-in period (week 0). To avoid or minimize carryover effects, 616 of the patients had never been treated for hypertension before, whereas the 106 who had been previously treated, had been in pharmacological washout for at least 6 months before starting LOS. During a run-in period of 8 weeks, the patients followed a diet program that provided 100–140 mEq of sodium and 50–70 mEq of daily potassium to minimize the lifestyle differences, since sodium and potassium intake are known to influence intermediate phenotypes such as plasma renin activity and plasma aldosterone. At the end of this period, 50 mg/day of LOS in open label was prescribed orally for 4 weeks to 539 patients; the remaining 183 patients out of the starting cohort (i.e., 25.3%) had their BPs normalized during the 8 week run-in period.

During the 4 weeks of treatment with LOS, 21 patients left the study for poor compliance and 24 for side effects (cough n = 15, sexual dysfunction n = 9), leaving 494 patients for genotyping and downstream analysis.

Additional information is available in Supplementary Methods 1 and in [5].

We also compared the results of the pharmacogenomics of LOS to those of HCTZ in 558 EH patients (see Supplementary Methods 2 & Supplementary Figure 1) [8]. We tested the best findings for replication of association in two independent samples of hypertensives of European ancestry from the GERA2 [6] and GENRES studies [7] (see Supplementary Methods 3). For all the samples, we analyzed the office BP response.

Genotyping & imputing

Genotyping details are provided in Supplementary Methods 4. DNA was extracted from peripheral blood with standard procedures. SOPHIA and HCTZ samples were genotyped using the Illumina Human1M-Duo array (Illumina Inc., CA, USA) within the HYPERGENES project [9] or the Illumina HumanOmniExpress array within the InterOmics project [10].

Genome-wide imputation was performed using Markov Chain based haplotyper (MACH) software [11] and the Caucasian (CEU) HapMap haplotypes (release 22), as reference. We also imputed the region surrounding the *CAMK1D* gene using MaCH with the 1000 Genomes haplotypes (release March 2012) as reference, to further enrich this locus with the highest number of genotypes.

Statistical analysis

The primary end point (i.e., the phenotype under investigation) was the change in systolic BP after 4 weeks of treatment (ΔSBP4), which is the difference between systolic BP (SBP) at the end of treatment and SBP at the end of run-in, the day before the first pill of LOS.

All quality control analyses were performed in accordance with the protocol proposed by Anderson *et al.* [12]. Patients' call rate threshold was set at 0.95. For each patient, data from the X chromosome were used to check for discordance with ascertained sex. After imputation, SNPs with a call rate >99% and a minor allele frequency ≥3% were included in the analysis (Supplementary Methods 5 & Supplementary Figure 1).

We assessed population stratification using principal components analysis as implemented in EIGENSOFT [13,14] to infer continuous axes of genetic variation (Supplementary Methods 5 & Supplementary Figure 2). To assess the genotype to phenotype association we per-

formed a linear regression on ΔSBP4 under an additive model with adjustment for sex, age, basal SBP and for ancestry principal components (PCs), as implemented in PLINK [15].

We tested the best findings of SBP response to LOS ($p \leq 10^{-5}$) for association with the change in diastolic BP response after 4 weeks of treatment (ΔDBP4) by performing a linear regression analysis under an additive model using sex, age, basal DBP and for the ancestry PCs as covariates.

To verify that the identified markers were specific for LOS, we evaluated their predictive role on ΔSBP4 using an independent sample of 558 naive hypertensives treated with 25 mg of HCTZ for 4 weeks as their first antihypertensive treatment (Supplementary Methods 2). We performed a linear regression analysis under an additive model using sex, age, basal SBP and for the ancestry PCs as covariates. SNP x treatment interaction analyses were conducted under an additive genetic model using the quantitative trait interaction analysis as implemented in PLINK [15]. Analyses on ΔSBP4 residuals were adjusted for sex, age, basal SBP and PCs.

To replicate our findings we tested the association in two independent samples of European ancestry hypertensives from the GERA2 [6] and GENRES studies [7] (Supplementary Methods 3).

Results

Table 1 reports the characteristics of the study sample, composed of 372 patients.

SOPHIA participants were white Caucasians from continental Italy and Sardinia and included 92 (24.7%) women. Age averaged 45.7 years (standard deviation [SD]: ±7.4), average basal SBP and DBP were 148.9 mmHg (SD: ±7.1) and 96.5 mmHg (SD: ±3.7), respectively.

After quality control of the 494 samples genotyped, 372 individuals and 1,705,664 SNPs were available for the analyses (see Supplementary Methods 5).

A quantitative trait analysis, with ΔSBP4 (adjusted for ancestry PCs, sex, age and basal SBP) as the dependent variable (Supplementary Figures 3 & 4), identified one SNP (rs10752271) achieving the genome-wide significant threshold. Moreover, the q–q plot showed some SNPs ($p \leq 10^{-5}$) deviating above the diagonal (the distribution reference line). The number of SNPs with $p \leq 10^{-5}$ was 130 (Supplementary Table 1 & Supplementary Figure 4).

In particular, four SNPs (rs10752271, rs10906202, rs4747995 and rs10737061) in the *CAMK1D* gene were significantly associated with SBP response to LOS (Supplementary Table 1). Specifically, the imputed SNP rs10752271 in intron 2 reached the Bonferroni's threshold ($p = 1.2 \times 10^{-8}$), with an effect size of -5.5 ± 0.94 mmHg (G risk allele frequency = 0.10; Figure 1). Rs10906202 and rs4747995 (imputed and genotyped, respectively) in intron 3, are in linkage disequilibrium (LD) with rs10752271 whereas the genotyped rs10737061 is not in LD with rs10752271, although maps to approximately 600 bp upstream. All these SNPs showed only slightly lower p-values and effect sizes (Supplementary Table 1).

Owing to its highly significant association with ΔSBP4, we focused on *CAMK1D*. We fine mapped the gene region by imputing the 1000 Genomes haplotypes and tested the additional genotypes for association with LOS response. We identified another poly-

Table 1. Characteristics of participants by treatment.

Characteristic	Losartan (n = 372)	HCTZ (n = 558)	p-value
Men/women (n)	280/92	401/157	NS
Age (years)	45.7 ± 7.4	48.3 ± 8.8	<0.0001
BMI baseline (kg/m²)	26.9 ± 2.9	27.2 ± 3.8	NS
Pretreatment SBP (mmHg)	148.9 ± 7.1	157.1 ± 12.7	<0.0001
Pretreatment DBP (mmHg)	96.5 ± 3.7	102.3 ± 7.7	<0.0001
ΔSBP (mmHg)	-11.8 ± 9.1	-14.6 ± 13.1	0.0004
ΔDBP (mmHg)	-8.8 ± 6.2	-9.4 ± 8.9	NS
Serum potassium (mmol/l)	4.2 ± 0.4	4.2 ± 0.4	0.05
Urine sodium (mEq/24 h)	147.5 ± 53.9	152.8 ± 53.6	NS
Urine potassium (mEq/24 h)	62.5 ± 25.5	57.2 ± 18.4	0.0003

The table shows the characteristics of drug-treatment groups (mean ± standard deviation).
p-value of the comparison among treatment groups. Between-group comparisons of means and frequencies relied on ANOVA or χ^2, as needed.
ΔDBP: Difference between DBP at the end of treatment and SBP at the end of run-in; ΔSBP: Difference between SBP at the end of treatment and SBP at the end of run-in; DBP: Diastolic blood pressure; HCTZ: Hydrochlorothiazide; NS: Not significant; SBP: Systolic blood pressure.

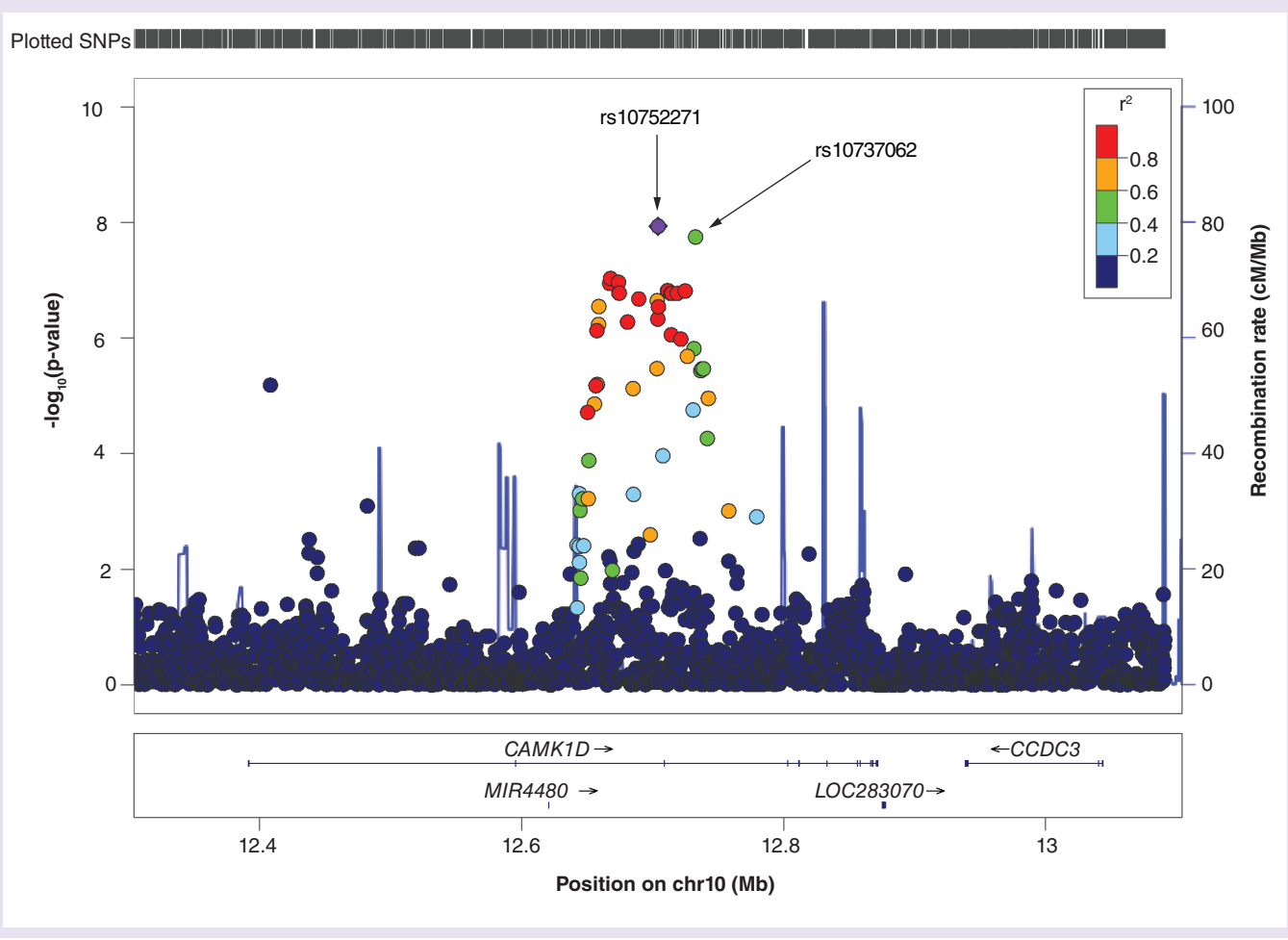

Figure 1. Local Manhattan plot for the *CAMK1D* region. Each circle represents a SNP, the y-axis is the -$\log_{10}$ association p-value for losartan response, and the x-axis represents the physical position on the chromosome (build 37, hg19). The circles are filled with colors according to the linkage disequilibrium (r^2) between the given SNP and the lead SNP rs1075271.

morphism, rs10737062 not in LD with rs10752271, in intron 3, associated with ΔSBP4 with an effect size of -5.54 ± 0.96, p = 1.8 × 10^{-8} (G risk allele frequency = 0.11, Figure 1). This SNP was not present in the HapMap reference panel used in the previous imputation.

rs10752271 was also associated with the individual ΔDBP4 (p = 2 × 10^{-5}; effect size = -3.05 ± 0.71). Similar results were observed for the other SNPs in *CAMK1D* (Supplementary Table 2).

Among 130 SNPs with a p-value ≤10^{-5} in ΔSBP4, 121 were also associated with ΔDBP4 (Supplementary Table 2).

To confirm the specificity of these markers for LOS, we tested their association with ΔSBP4 in patients treated with HCTZ.

Table 1 reports the characteristics of HCTZ sample. Within the HCTZ cohort we analyzed 558 hypertensives (28.1% women). All participants were white Caucasians from continental Italy and Sardinia.

LOS and HCTZ samples were similar for BMI and urinary sodium. Urinary potassium was significantly higher in LOS samples compared with HCTZ; pretreatment BP was higher in HCTZ, whereas age was lower in LOS.

Among the 131 best SNPs for LOS, only ten were marginally associated with BP response to HCTZ (Supplementary Table 3). rs10752271 and rs10737062, did not show significant association to HCTZ BP response (p = 0.15, effect = -1.6 ± 1.1; and p = 0.48, effect = -0.8 ± 1.1, respectively; Figure 2 & Supplementary Table 3). We also confirmed the specificity of BP response to LOS by performing a SNP x treatment interaction analyses that was significant for both SNPs (rs10752271, p = 0.01; and rs10737062, p = 0.005; Figure 2).

Replication of findings in independent samples

We tested for replication of SNP rs10752271 in two independent EH samples from the GERA2 [6] and

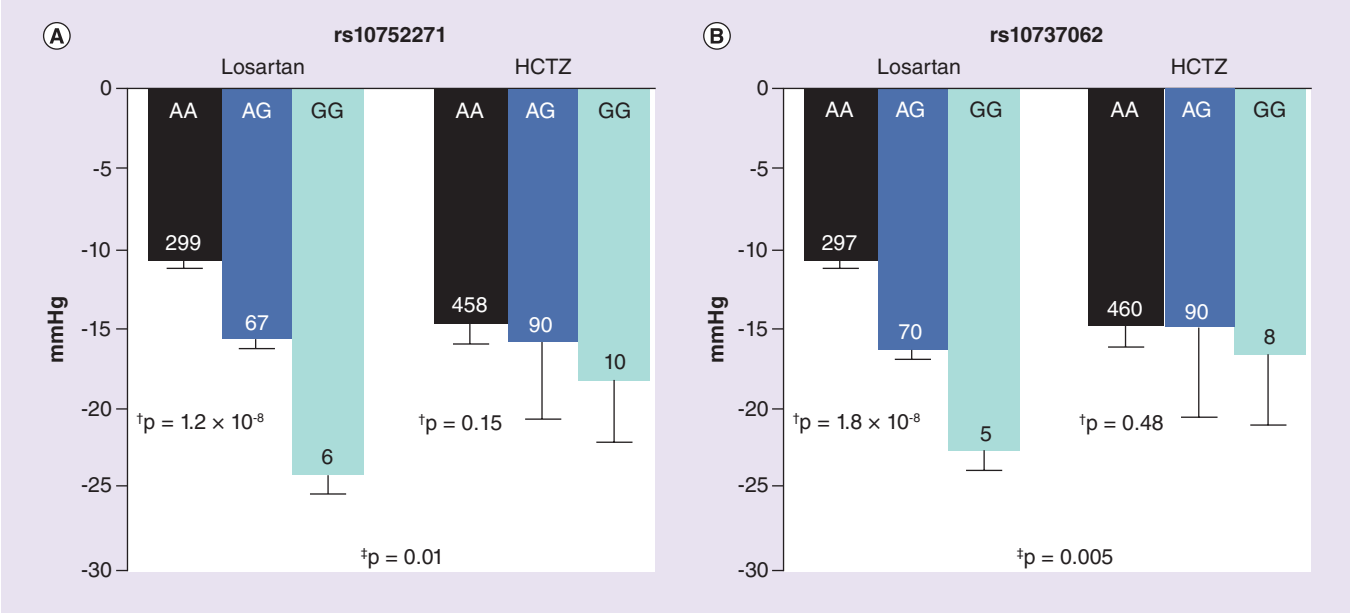

Figure 2. Mean systolic blood pressure response to losartan and hydrochlorothiazide relative to rs10752271 and rs10737062.
(A) rs10752271 and **(B)** rs10737062. In each column, the numbers of individuals per genotype are indicated.
†Linear regression analysis comparing genotypes in each treatment.
‡SNP x treatment interaction analysis comparing losartan and HCTZ.
HCTZ: Hydrochlorothiazide.

GENRES [7] studies. GERA2 studied the effect of candesartan on 198 patients using a linear regression additive model adjusting for age and sex; GENRES studied the effect of LOS on 216 males using a similar linear regression model obviously omitting sex as covariate. In GERA2, rs10752271 was not associated with BP response to candesartan (p = 0.9 and effect size = -0.3 ± 2.55), whereas in GENRES the association was confirmed with a similar effect size (p = 0.04, effect size = -5.3 ± 2.5; Table 2 & Figure 3).

Genotypes for the imputed rs10737062 were not available for replication in both studies since imputation had been performed with HapMap as reference.

Discussion

The SOPHIA study is a genome-wide pharmacogenomics assessment of the BP response to LOS.

Using a linear regression analysis, we identified 130 SNPs associated with ΔSBP4 with $p \leq 10^{-5}$ as a dependent variable. Furthermore, 121 of these 130 SNPs were also associated with ΔDBP4. The specificity of the SNPs was verified using an independent sample treated with HCTZ. The top hit, rs10752271 in the *CAMK1D* gene, shows an effect size of -5.5 ± 0.94 and a p-value of 1.2×10^{-8}. This finding is supported by a cluster of SNPs in weak LD with each other (Figure 1).

The *in silico* replication in the GENRES [7] study, also utilizing LOS as the ARB, confirmed the association of rs10752271 with similar effect size of the G risk allele (Figure 3).

The protein encodes by *CAMK1D* is expressed in the glomerular cortex where angiotensin II and potassium determine increases in cytosolic calcium that activate CaMK1D protein. This in turn increases *CYP11B2* gene transcription and aldosterone production by modulating the activities of target transcription factors, such as NURR1, ATF1, ATF2 and CREB [16].

The importance of calcium in determining acute and chronic aldosterone secretion in the adrenal zona glomerulosa, acting largely through CaMK1D activation, has been consistently demonstrated [17,18]. CaMK1D can also regulate gene transcription by phosphorylating various substrates [19]. Interestingly, increases in intracellular calcium in the zona glomerulosa of the adrenal cortex, has been shown recently to be a common pathway in the activation of *CYP11B2* transcription in sporadic and familial hyperaldosteronism [20,21]. The central role of increased aldosterone production in the pathogenesis of EH, has been unraveled in the Framingham Offspring Study, where aldosterone levels were directly associated with

Table 2. *In silico* replication results.

Studies	Beta	Standard error	p-value
SOPHIA	-5.5	0.94	1.20×10^{-8}
GENRES	-5.3	2.5	0.04
GERA2	-0.35	1.97	0.9

The table shows rs10752271 association results (Beta, standard error and p-values) for the SOPHIA, GERA2 and GENRES studies.

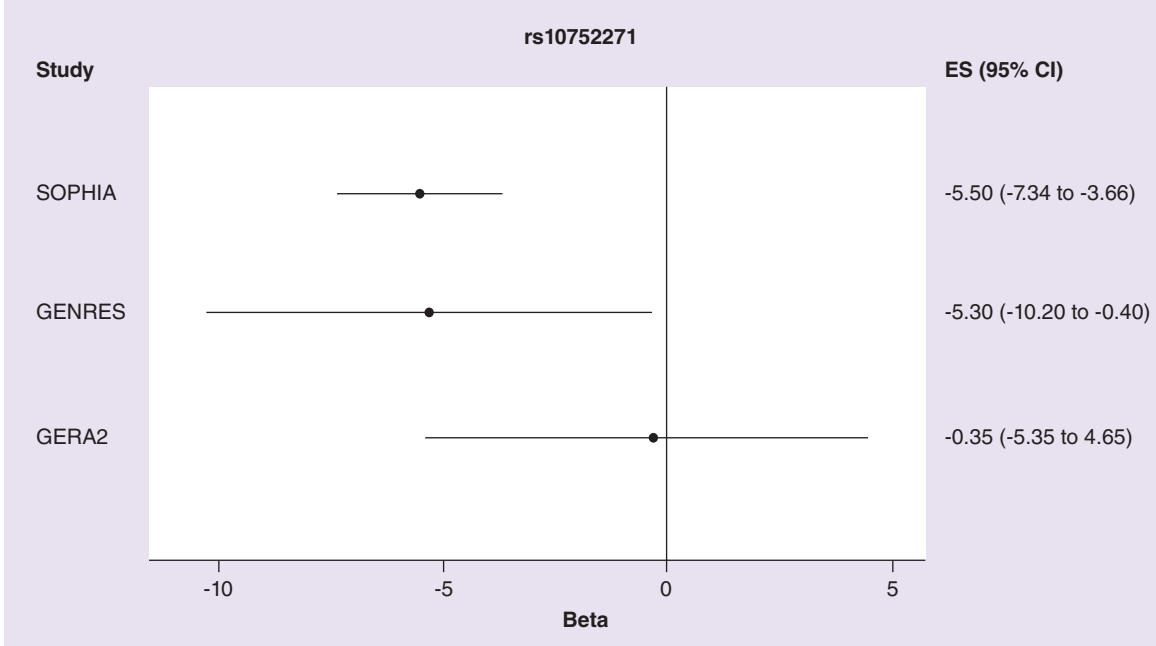

Figure 3. Effect size of blood pressure response in SOPHIA, GENRES [7] and GERA2 [6] studies. The circles and the horizontal lines correspond with the effects (beta) and 95% CI of each study.
ES: Effect size.

an increase in blood pressure and the development of hypertension, independent of sodium intake and other potential confounding factors [22].

A major characteristic of the SOPHIA cohort is that it includes newly recruited never treated essential hypertensives. A complete clinical, laboratory and instrumental workup was made to define the diagnosis of 'EH' over a run-in period of 8 weeks with a controlled diet: only those patients whose BP remained ≥140/90 mmHg at the end of this period were included in the study. This should minimize the inclusion of 'false hypertensives' in the study cohort. The condition of 'never treated hypertensive' was considered mandatory: as already well ascertained years ago [23]; up to 12 months after treatment withdrawal may be required to restore BP to pretreatment levels. Moreover, the effects of previous drugs continue to act at the different levels of the biological organization thus making the findings of a pharmacogenomics study questionable in the case of a cohort composed by previously treated patients. We think these considerations are indeed a key point in the study design and conduction of a pharmacogenomics study: never treated hypertensives are rather difficult to find and it took several years to assemble our cohort. These considerations are expressed in detail elsewhere [24].

To test for replication we used the GERA2 and GENRES studies. Different inclusion criteria were applied for patient's recruitment (run-in period, basal BP, previous antihypertensive treatment). In spite of these differences we could replicate the effect size of our best finding in GENRES.

Previous studies used a candidate gene strategy to identify SNPs that predict the BP response to ARBs. Conflicting results were obtained for the insertion/deletion polymorphism within the *ACE* gene. Some studies found the insertion allele to be associated with a greater decrease in BP [25], whereas others have not been able to confirm the finding [26,27].

Essential hypertensive patients carrying the C allele of the -344 T/C polymorphism in the *CYP11B2* gene were reported to respond to ARB with a larger BP decrease [28], whereas this was not confirmed in the present study (p = 0.67).

The GENRES study evaluated the impact on ambulatory BP response to LOS and other antihypertensive drugs of 19 loci identified in previous GWAS in EH. An intronic SNP in *STK39* (rs6749447) was found to be associated with 24 h ambulatory BP response to LOS (p = 0.0005/0.0002 for SBP and DBP, respectively) [29]. We could not confirm this SNP (p = 0.86) and did not find additional associated SNPs in *STK39*. In a GWAS, where good responders to candesartan have been compared with poor responders, Turner *et al.* described rs11649420, in the gamma subunit of the *SCNN1G* gene, as strongly associated with drug response [6]. The odds of good BP response to candesartan, for rs11649420 GG genotype, were threefold greater than for the combined AA+AG group, and the odds of good BP response to

HCTZ were twofold less than the combined AA+AG group. We could not validate this finding either contrasting good and poor responders [30] or performing a linear regression analysis with ΔSBP as a dependent variable. We did not find any other associated SNP in *SCNN1G*. Recently, a GWAS on a Japanese cohort, performed with a design similar to that applied in the GENRES study, reported a marginal association with *ABCC9* [31]. We did not replicate this finding.

Conclusion

Using a genome-wide approach, we identified in SOPHIA and confirmed in GENRES the *CAMK1D* gene as a novel locus associated with BP response to LOS (rs10752271). Because of its specific association with LOS, this locus could represent a novel tool for a genetic characterization of hypertensive patient's responsiveness to an antihypertensive treatment.

Our study presents some limitations. First, we are aware of the limited number of SNPs at genome-wide association level, probably due to the small sample size not comparable to more powerful GWAS but in line with other pharmacogenomics studies. Second, we identified rs10752271 associated with BP response to LOS in two Caucasian populations; however, further investigation in different ethnic groups is needed.

Future perspective

There is a wide variability in antihypertensive drug response and data available for treatment choice are limited. Our study identified a novel plausible genetic marker associated with LOS treatment. This result requires further investigation to clarify the mechanism through which the identified gene influences LOS BP response.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Pharmacogenomics of hypertension

- A pharmacogenomics approach could help to identify additional molecular mechanisms involved in the pathogenesis of essential hypertension.
- In this study a genome-wide association between polymorphisms and blood pressure response to losartan was evaluated in essential hypertensives.

Results: significant association between rs10752271 & blood pressure response to losartan

- A peak of association in the *CAMK1D* gene was identified. In particular rs10752271 showed an effect size of -5.5 ± 0.94 and a p-value of 1.2×10^{-8}.
- We validated rs10752271 *in silico* in the GENRES cohort. The specificity for losartan was confirmed in a cohort of essential hypertensives treated with hydrochlorothiazide.
- CAMK1D belongs to the regulatory pathway involved in aldosterone synthesis.

Conclusion

- The rs10752271 polymorphism in the *CAMK1D* gene could represent a novel tool for individualized antihypertensive treatment.

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