Genetic variation in the KCNMA1 potassium channel α subunit as risk factor for severe essential hypertension and myocardial infarction

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Objective **The large conductance Ca^{2+}-dependent potassium channel plays a critical role in the control of vascular tone, coupling local increases in intracellular Ca^{2+} to membrane hyperpolarization and vascular relaxation. It also impacts blood pressure by modulating the renin–angiotensin–aldosterone system. Previous studies have shown that a polymorphism in the β_1 regulatory subunit of the Ca^{2+}-dependent potassium channel modulates the risk of diastolic hypertension in humans.**

Methods **We have studied polymorphisms in the pore-forming α subunit gene (*KCNMA1*) and their association to hypertension and myocardial infarction.**

Results **Sequencing of the *KCNMA1* gene revealed two genetic variants (polymorphisms C864T and IVS17) in population-based epidemiological studies (4786 participants). We detected a significant increase in the frequency of the IVS17+37T>C polymorphism with severe systolic hypertension (48.3% for normotensive vs. 69% for severe systolic hypertension, $P = 0.03$) and with severe general hypertension (48.7 vs. 65.8%, $P = 0.04$), although the adjusted odd ratios did not reach statistical significance. Four C864T/IVS17 haplotypes were identified. Haplotype 4 (encompassing the C allele of the IVS17 polymorphism and the T allele of the C864T polymorphism) was related with increased severity of systolic and general hypertension as well as increased risk of myocardial infarction.**

Conclusion **Our study provides genetic evidence that highlights the relevance of the Ca^{2+}-dependent potassium channel in the control of human blood pressure and its impact on cardiovascular disease. *J Hypertens* 26:2147–2153 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Abbreviations: KCNMA1, Ca++-dependent potassium channel alpha1 subunit; KCNMB1, Ca++-dependent potassium channel beta1 subunit; PAR, Population attributable risk; REGICOR study, Registre Gironí del Cor

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Introduction

Hypertension is a major public health problem and a multifactorial disorder presenting abnormalities at various levels, particularly vascular volume homeostasis and vascular tone [1,2]. Several studies have found an association between high blood pressure (BP) and variations in different genes involved in these homeostasis systems, particularly those affecting sodium handling in the kidney [3,4]. However, recent research is revealing nonrenal mechanisms that affect the contractility of the vascular smooth muscle cells, and are therefore relevant to the pathogenesis of hypertension [5–11].

One of the nonrenal factors associated with the control of human BP is the large conductance Ca^{2+}-dependent K^+ channel [7,12]. In vascular smooth muscle, the Ca^{2+}-dependent potassium channel is formed by four ion-conducting α subunits and regulatory β_1 subunits and is a key element in the control of vascular tone by coupling local increases in intracellular Ca^{2+} to augmented channel activity [13–16] and vascular relaxation [17,18]. We have previously characterized a gain-of-function polymorphism (E65K) in the *KCNMB1* gene, coding for the human Ca^{2+}-dependent potassium channel β_1 subunit, which is associated with low prevalence of moderate-to-severe diastolic hypertension [7,12]. Downregulation of the β_1 subunit has also been reported in hypertensive rat models [6], and polymorphisms in the *KCNMB1* gene are associated with the baroreflex function in humans [19]. More recently, an α subunit knockout mouse model revealed, in addition to the control of vascular smooth muscle tone, a novel and interesting role of Ca^{2+}-dependent potassium channel in

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the control of BP: the regulation of the rennin–angiotensin–aldosterone system [20].

Altogether, these findings confirm the view that the Ca^{2+}-dependent potassium channel is an interesting element to study in relation to the pathogenesis of hypertension. In this respect, human genome-wide linkage studies of preeclampsia [21], dilated cardiomyopathy [22], and systolic and diastolic BP variation [23] have found an association with the *KCNMA1* locus in the cytogenetic band 10q22.3 that codes for the Ca^{2+}-dependent potassium channel α subunit [24].

The aim of the present study was to search for human essential hypertension-related genetic variants in the *KCNMA1* gene in a population-based genetic epidemiological study. Association studies are probably the most common, and at times are the only means to identify single polymorphisms in candidate genes that are relevant to human complex diseases such as hypertension. More comprehensive association studies involve the analysis of haplotypes, defined as a set of closely linked alleles inherited as a unit or the combination of multiple alleles on one chromosome. Our screening of the exons and exon–intron boundaries of *KCNMA1* revealed two polymorphisms, which were analysed in respect of their association with systolic and diastolic hypertension and myocardial infarction (MI). From these polymorphisms, haplotypes were also constructed and analysed for associations.

Methods

Population-based genetic epidemiological study

The representative population sample was composed of 4786 participants, aged 25–74 years (mean age 48.7 years), randomly selected in two cross-sectional studies carried out in the province of Girona, Spain, in 1995 and 2000 (Registre Gironí del Cor; REGICOR study) to establish the prevalence of cardiovascular risk factors in this region. Full details have been previously provided [25]. Of the study population, 4165 participants were not under antihypertensive drug therapy. Participants were classified into various normotensive or hypertensive groups according to systolic blood pressure (SBP), diastolic blood pressure (DBP), or SBP and DBP values together. With respect to SBP, participants were defined as normotensive SBP (SBP < 140 mmHg), mild systolic hypertensive (SBP = 140–159 mmHg), moderate systolic hypertensive (SBP = 160–179 mmHg), and severe systolic hypertensive (SBP ≥ 180 mmHg). Considering only DBP, participants were normotensive (DBP < 90 mmHg), mild diastolic hypertensive (DBP = 90–99 mmHg), moderate diastolic hypertensive (DBP = 100–109 mmHg), and severe diastolic hypertensive (DBP ≥ 110 mmHg). Finally, hypertension categories were defined following the WHO hypertension criteria [26] as: normotensive (SBP < 140 mmHg and DBP < 90 mmHg), mild hypertensive (SBP = 140–159 or DBP = 90–99), moderate (SBP = 160–179 or DBP = 100–109), and severe (SBP ≥ 180 or DBP ≥ 110). When SBP and DBP fell into different categories, the higher category was selected for the purposes of classification. Normotensive participants were always the reference group. The clinical characteristics of the study population are shown in Table 1. As expected, patients with hypertension were older, more overweight, and more likely to have diabetes than normotensive participants. All participants provided their informed consent. The study was approved by the Institutional Ethics Committee of the Institut Municipal d'Investigació Mèdica, and individual results were provided to all participants.

Table 1 Characteristics of overall population presented by general hypertensive status

	Normotensive ($n = 2623$)	Hypertensive patients ($n = 2163$)[a]	*P*
Age, years	44.6 (12.2)	57.7 (11.6)	<0.001
Sex, men/women	1165/1458	1155/1008	<0.001
BMI, kg/m^2	25.6 (3.9)	28.8 (4.5)	<0.001
SBP, mmHg	116.6 (11.8)	146.3 (17.9)	<0.001
DBP, mmHg	74.5 (8.4)	87.5 (9.8)	<0.001
Diabetes mellitus, %	4.5	23.7	<0.001
Antihypertensive treatment, %	–	28.7	
ISV17+37 T>C, %			
TT	51.6	50.5	
CT	40.1	40.9	0.782
CC	8.4	8.6	
C864T,%			
CC	42.1	42.5	
CT	44.9	44.4	0.956
TT	13.1	13.1	

Continuous variables are expressed as mean (SD). BMI, body mass index; DBP, diastolic blood pressure; SBP, diastolic blood pressure. [a]Normotensive participants under antihypertensive therapy are included in this group.

Measured variables

BMI was determined as weight divided by squared height (kg/m^2). The mean BMI of the participants in the study was 27.3 kg/m^2. BP measurements were obtained with a periodically calibrated mercury sphygmomanometer. The operator had completed a certification process in the standardized measurement technique at a central laboratory, and determinations were always made by the same person. A cuff adapted to upper-arm perimeter (young, adult, and obese) was selected for each participant. BP measures were determined in the seated position. The first measurements were performed after a 5-min rest, and the second measurements were taken at least 20 min later. The value used was the arithmetic mean of both determinations.

Case–control study

We used a nested case–control design. One thousand four hundred and nineteen patients (1107 men and 312 women) with a first MI admitted to the only reference coronary unit in the catchment's area were recruited between January

1996 and December 1998 in Gerona, Spain. The 4786 participants recruited in the two cross-sectional studies served as controls and were judged free of angina or MI by history, physical examination, electrocardiography, and routine laboratory data.

Screening of *KCNMA1* gene

Genomic DNA was extracted with the ABI Prism 6700 workstation (Applied Biosystems, Foster City, California, USA) following manufacturer's instructions. Exons and exon–intron boundaries of the *KCNMA1* gene were amplified from genomic DNA of 11 participants with severe hypertension (SBP > 160 mmHg or DBP > 90 mmHg with antihypertensive therapy) and 12 strictly normotensive (SBP < 120 mmHg and DBP < 80 nmmHg) participants, both groups of similar age. PCR products were analysed by direct sequencing. Among several genetic variants, two of them, F229 (rs1131824; C864T GeneBank NM_001014797) and IVS17+37T>C (rs16934182) (IVS17), were selected and identified respectively in the fourth exon and the 17th intron of the *KCNMA1* gene [Ensemble vertebrate genome annotation (VEGA) gene OTTHUMT00000048877] by the dideoxynucleotide-sequencing method (ABI PRISM BigDye Terminator 3.0; Applied Biosystems) and were confirmed by sequencing of the reverse strand, using the forward and reverse primers 5′- TTGAATGGAAAGACCCCTTG -3′ and 5′- TCTTGACTGCGAGAGCAGAG -3′ for the C864T variant and 5′-AGTGGACATTGGACTCTGGACA-3′ and 5′-ACATGAACTGGAGTCCGTGGAA-3′ for the IVS17 variant. These two polymorphisms were selected because they were the only common exonic and exon–intronic variants with the rare allele present in more than one participant in our sequencing population.

Following the identification of polymorphisms C864T and IVS17 of the *KCNMA1* gene by sequencing, these polymorphisms were analysed in every one of the participants of the study by real time-PCR (ABI PRISM 7900HT; Applied Biosystems), using primers 5′-CCTGCCAGAATTTCTACAAAGATTTCAC-3′ and 5′-AAACACCCCATTGTGATACTGAACA-3′, and TaqMan probes FAM-CCGCAAGCCAAAGT-MGB and VIC-CCCGCAAGCCGAAGT- MGB for the T and C alleles of the C864T variant, respectively, and primers 5′-AGGTAAGAGTTTATTTTCAACTGGCTTTCT-3′ and 5′-CTACTTCCGTGGGTCAAGGT-3′, and TaqMan probes FAM-TCTACAGCCAGGCCAG-MGB and VIC-CTACAGCCGGGCCAG-MGB for the T and C alleles of the IVS17 variant, respectively. The genotype success rate was 91.2% and 96.1% for the C864T and the IVS17 polymorphisms, respectively.

PCR detection of potential KCNMA1 splice variants

Uterine arteries were obtained from patients undergoing hysterectomy at the Clinical Hospital of Barcelona, with protocols approved by the human Investigation Ethics Committee of the Hospital. Samples were placed in RNA later (Ambion, Applied Biosystems) and handling was carried out as previously described [27]. Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, California, USA), derived from the single-step RNA isolation method developed by Chomczynski and Sacchi [28], for subsequent alternative splicing and gene expression analyses. One-step reverse transcription (RT)-PCRs (QIAGEN, Hilden, Germany) was performed on 1 μg of total RNA using *KCNMA1* specific primers from exon 14–20 (primer forward, 5′-CGCAGAGTTGAAGTTGGGCTTCAT-3′; primer reverse, 5′-CTTGGGTGCACACCAGTGAAACAT-3′) from the Ensemble VEGA exon description (OTTHUMT00000048877). RT-PCR products were separated on a 2.0% agarose gel containing 0.05% ethidium bromide as described previously [29]. Quantitative real-time RT-PCR of KCNMA1 gene was carried out on an ABI Prism 7900HT (Applied Biosystems) using the TaqMan Gene expression Assay Hs00266938 m1 (Applied Biosystems). The amplification of cDNA was performed in 20 μl final volume and each reaction consisted of 10 μl TaqMan Universal PCR Master Mix (Applied Biosystems), 1 μl TaqMan Gene Expression Assays, and 3 μl cDNA, following manufacturer instructions (10 min at 95°C, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min). All samples were run in triplicate and results were analysed with SDS Software TM (Applied Biosystems).

Statistical analysis

Deviation from the Hardy–Weinberg equilibrium was assessed using a chi-square test with one degree of freedom to compare the observed and expected genotype frequencies among the participants. A chi-square or Fisher's exact test, as appropriate, was used to compare categorical variables between groups. Continuous variables were compared between groups with the Student's *t*-test. The Bonferroni correction was used as a multiple-comparison correction. The odds ratio (OR) and 95% confidence intervals (CIs) for the effect of categorized age groups on MI risk were estimated using logistic regression analyses adjusted for the effects of other cardiovascular risk factors.

Pairwise linkage disequilibrium between the polymorphisms was quantified using the Shi's standardized coefficient D' ($|D'|$). Haplotype frequencies and haplotype-based analysis for confounding variables were examined based on a Bayesian algorithm, the R software, freely available at http://www.R-project.org. The dominant model was fitted by the function 'haplo.glm' in the R package 'haplo.stats'. To deal with haplotype phase ambiguity, this function uses a method that performs an iterative two-step Expectation–Maximization, with the posterior probabilities of pairs of haplotypes per participant used as weights to update the regression coefficients, and the regression coefficients used to update the posterior probabilities. Statistical significance was assumed at $P < 0.05$ level. The statistical package for

the social sciences (SPSS Inc., Chicago, Illinois, USA) version 13.0 was used for statistical analysis.

Given the cross-sectional nature of our study, population-attributable risk (PAR) was calculated by the case–control approach, for which the estimation of OR is similar to that obtained by using incidence rates in populations:

$$PAR\,(\%) = Pe\,(OR\text{-}1) \times 100 / [1 + Pe\,(OR\text{-}1)],$$

in which Pe is the proportion of a determined haplotype among the nonhypertensive participants and OR is the OR of severe systolic hypertension or severe general hypertension for this haplotype adjusted by age, sex, BMI, and diabetes mellitus.

Results

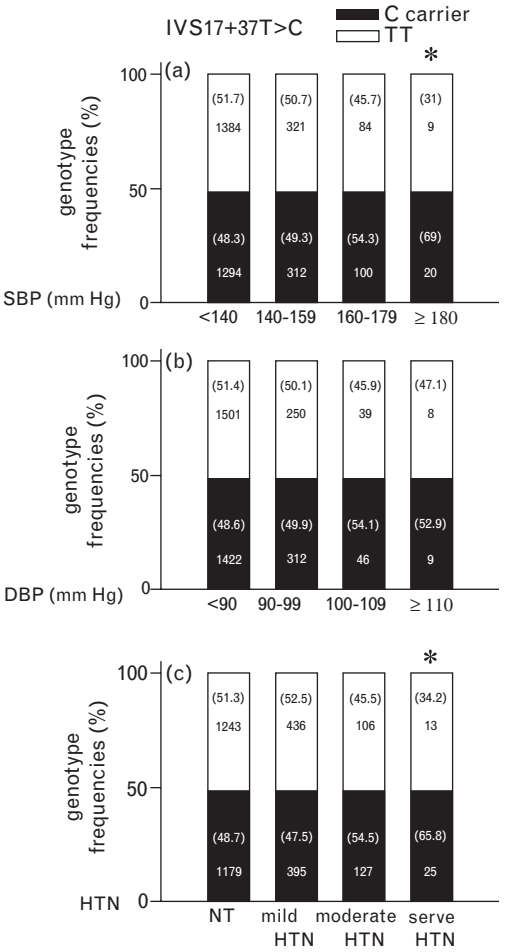
KCNMA1 polymorphisms

The 27 exons and the corresponding exon–intron boundaries of the human *KCNMA1* gene were amplified by PCR and analysed by direct sequencing. The polymorphisms C864T and IVS17+37T>C were identified in the 229th codon within the fourth exon and 37 base pairs from the 17th exon within the 17th intron, respectively. The distribution of genotypes for each polymorphism followed the Hardy–Weinberg equilibrium. Furthermore, there is linkage disequilibrium among the polymorphisms tested ($D' = 0.143$, $r^2 = 0.004$, $P < 0.01$). There were no differences either in genotype frequencies between normotensive and patients with hypertension or in allelic frequencies between treated and untreated patients with hypertension or between men and women. In order to avoid interferences of therapy on BP levels and to properly classify participants, analyses were performed only in untreated participants (except when the contrary is specified).

We analysed the association of individual polymorphisms and hypertension. No association between the C864T polymorphism and either SBP, DBP or general hypertension categories was detected. We detected a significant increase in the frequency of the IVS17+37T>C polymorphism with severe systolic hypertension (48.3% for normotensive vs. 69% for severe systolic hypertension, $P = 0.03$) (Fig. 1a). A similar significant association was found with severe general hypertension (48.7 vs. 65.8%, $P = 0.04$) (Fig. 1c) but no association was seen with diastolic hypertension (Fig. 1b). Logistic regression analysis adjusted for age, sex, BMI, and diabetes mellitus revealed a trend towards an association of the IVS17+37T>C polymorphism and severe systolic hypertension in the overall population (OR = 2.10, 95% CI = 0.934–4.74), though did not reach statistical significance ($P = 0.07$). Mean SBP was found to be significantly higher in carriers of the IVS17+37T>C polymorphism compared with TT homozygotes (127 ± 68 mmHg; $n = 1726$ vs. 126 ± 22 mmHg; $n = 1798$, $P = 0.02$). Moreover, there was a linear increasing trend of SBP values among TT (126 mmHg), CT (127 mmHg), and CC (129 mmHg) genotypes ($n = 1798$, 1428 and 298, respectively, $P < 0.01$). As there is a lineal relationship of age with BP, we specifically assess the effect of age on the association of IVS17 genotypes with SBP by classifying the study population in age quartiles. As expected, there was a significant increasing trend of SBP with advanced age, but this trend was similar for both IVS17 genotypes (data not shown).

C864T/IVS17 haplotype probabilities were inferred for each participant of the overall population. Haplotype frequencies are shown in Table 2. Age, sex, BMI, and diabetes mellitus adjusted ORs of the different hypertension categories for haplotypes 2, 3 and 4 versus haplotype 1 were estimated. The risks of haplotype 4 (encompassing the C allele of the IVS17 polymorphism and the T allele of

Fig. 1



IVS17 genotype frequencies. (a) IVS17 polymorphism distribution at each level of systolic blood pressure, (b) diastolic blood pressure, and (c) general blood pressure categories with respect to the normotensive group. The number of participants for each genotype and blood pressure level is shown with the percentage in brackets. *P-value <0.05. DBP, diastolic blood pressure; HTN, hypertension; SBP, systolic blood pressure.

Table 2 Population haplotype frequency inferred from C864T and IVS17 polymorphisms

Haplotype	C864T allele	IVS17 allele	Haplotype frequency (%)
1	C	T	44.6
2	T	T	26.7
3	C	C	20.0
4	T	C	8.7

the C864T polymorphism) were 2.48 (1.07–5.78, $P = 0.03$) for severe systolic hypertension and 2.27 (1.02–5.07, $P = 0.04$) for severe general hypertension. Haplotypes 2 and 3 showed no association with an increased risk of severe systolic hypertension and severe general hypertension. No statistically significant association was observed for any haplotype when participants were classified according to DBP. Inclusion of treated patients in the antihypertensive therapy-adjusted analyses did not modify the association of haplotype 4 with severe systolic and severe general hypertension (OR = 2.42, 95% CI = 1.29–4.51, $P < 0.01$ and 2.28, 95% CI = 1.28–4.03, $P < 0.01$, respectively). Altogether, the magnitude and the direction of the associations were consistent with a progressive deleterious effect of *KCNMA1* haplotype 4 for the severity of systolic hypertension.

Case–control study

The association between haplotype 4 and severity of hypertension prompted us to study the possible relationship of the *KCNMA1* genetic variation with MI in a case–control study. Separately considered, we observed a trend to increased myocardial risk of both *KCNMA1* polymorphisms, although associations did not reach statistical significance after adjusting for age, sex, BMI, hypertension, and diabetes mellitus. (OR = 1.21, 95% CI = 0.96–1.31, $P = 0.14$ for the IVS17+37T>C, and OR = 1.12, 95% CI = 0.99–1.38, $P = 0.06$ for the C864T polymorphism). Next, T-carriers of the IVS17+37T>C polymorphism were pooled together with C-carriers of the C864T polymorphism and compared with TT and CC homozygotes, respectively. The combined presence of IVS17+37C allele and 864T allele ($n = 1402$) was found to be associated with a significantly increased risk of MI compared with reference genotypes ($n = 1010$) (OR = 1.33, 95% CI = 1.04–1.69, $P = 0.02$) in a model also adjusted for age, sex, BMI, hypertension, and diabetes mellitus. Haplotype 4 frequency increased from 8% in controls to 12% in patients with MI. In concordance with this observation, haplotype 4 (encompassing the C allele of the IVS17 polymorphism and the T allele of the C864T polymorphism) was significantly associated with MI after adjusting for age, sex, BMI, hypertension, and diabetes mellitus (OR = 1.26, 95% CI = 1.01–1.56, $P = 0.04$), confirming the independent association of this haplotype with increased MI risk.

Population-attributable risk

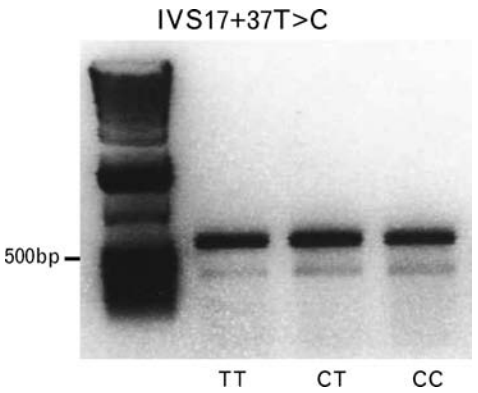
The PAR for the haplotype 4 (encompassing the C allele of the IVS17 polymorphism and the T allele of the C864T polymorphism) (i.e., the reduction in hypertension prevalence that could be expected from eliminating the effect of the haplotype in the entire population) was 11.4% for severe systolic hypertension and 9.9% for severe general hypertension.

Polymerase chain reaction detection of potential KCNMA1 splice variants and KCNMA1 expression

Considering that the association of KCNMA1 haplotypes with hypertension and MI and that C864T haplotype represents a synonymous single nucleotide polymorphism (Phe229Phe), we evaluated whether the IVS17 polymorphism may generate functional alterations by creating new spliced variants. Transcript scanning of cDNA generated from human uterine artery mRNA was performed using primers designed to amplify across the IVS17 polymorphism (Fig. 2). The analyses of exons 14–20 containing amplicons showed the expected band of 637 bp, indicating no modification in the splicing of *KCNMA1* (Fig. 2). An additional lower band of 379 bp was observed in all three different genotypes for IVS17 polymorphism. Sequencing of the lower band demonstrated that it corresponded to a new splice variant skipping exons 17–19, which was not associated with any genotype of the polymorphisms described here. Therefore, from these analyses of uterine artery samples, we believe that no alternative splice variants were induced by the presence of the IVS17+37T>C polymorphism, either in homozygosis (CC) or heterozygosis (CT).

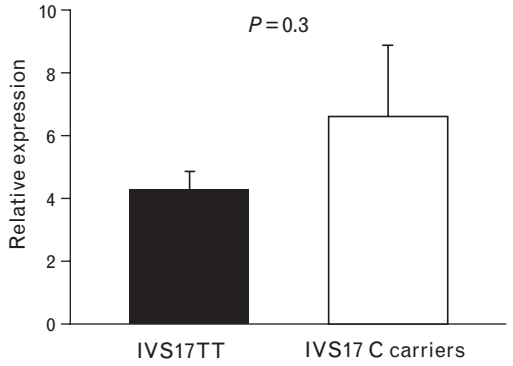
KCNMA1 expression at the transcriptional level was also tested in uterine arteries by real-time RT-PCR

Fig. 2



Large-conductance K^+ channel (BK) from uterine arteries shows alternative splicing that is unrelated to the IVS17 variant. BK_{Ca} (*KCNMA1*) reverse transcription-polymerase chain reaction from arteries with different genotypes for IVS17 show two bands: the upper is the full-length amplicon (637 bp) and the lower band lacks exons 17–19 (379 bp). Replicates were obtained for all genotypes TT ($n = 6$), CT ($n = 2$), and CC ($n = 3$).

Fig. 3



Levels of KCNMA1 in human uterine arteries. Bar plot of the transcript abundance in uterine arteries determined by real-time reverse transcription-polymerase chain reaction in uterine arteries from IVS17+37T homozygous participants (black; $n = 7$; age $= 46.2 \pm 2.7$) and IVS17+37T>C carriers (white; $n = 6$; age $= 46.5 \pm 1.4$).

(normalized to 18S RNA). Figure 3 shows that that there was no statistically significant ($P = 0.3$) change in the relative abundance of KCNMA1 between uterine arteries obtained from IVS17+37T homozygous participants ($n = 7$; age $= 46.2 \pm 2.7$) and IVS17+37T>C carriers ($n = 6$; age $= 46.5 \pm 1.4$).

Discussion

Large conductance, Ca^{2+}-dependent potassium channels (Maxi K) are of paramount relevance in the control of vascular tone [30]. Therefore, we have carried out the first large, population-based genetic epidemiological study to evaluate genetic variants of the Ca^{2+}-dependent potassium channel α subunit (*KCNMA1*) that may be associated with hypertension and MI.

Two gene polymorphisms, C864T and IVS17+37T>C, were identified as leading to different haplotypes. An increasing trend in the frequency of the IVS17+37T>C polymorphism with the severity of systolic and combined hypertension was observed, which reached statistical significance when haplotype 4 analysis was performed. Haplotype analyses also revealed that haplotype 4 is independently associated with an increased risk of MI. Although our data fit well with the existing evidence pointing to Ca^{2+}-dependent potassium channels as key elements in the control of vascular tone, we have found no functional channel deficiency that might explain the association of *KCNMA1* genetic variation with an increased risk of hypertension and MI. In this respect, one responsible polymorphism resides in an intronic region and does not seem to affect alternative splicing. Another possibility is that the IVS17 polymorphism, which breaks a tandem of three CUGs that could be a binding site for proteins regulating translation (CUG binding proteins) [31], may somehow modulate *KCNMA1* mRNA levels.

Our expression data show no differences in KCNMA1 expression levels between carriers of the IVS17 polymorphism and those without this polymorphism. However, we cannot completely exclude that the expression of KCNMA1 is modified under homozygotic conditions for the IVS17 polymorphism because only two participants were homozygotes for the IVS17 polymorphism. Altogether, the most likely interpretation is that the identified polymorphisms are relevant to the pathophysiology of hypertension via linkage disequilibrium with a truly functional, but at present unknown, sequence variation elsewhere.

Polymorphism in the Ca^{2+}-dependent potassium channel β_1-regulatory subunit (*KCNMB1*) leading to a channel gain-of-function is associated with diastolic hypertension, whereas genetic variation in the Ca^{2+}-dependent potassium channel α subunit (*KCNMA1*) is associated with systolic hypertension. We can offer two possible explanations for this apparent paradox. If the identified polymorphisms in the *KCNMA1* gene are truly responsible for the increased risk of hypertension, due to a still unidentified mechanism, it could be possible that systolic hypertension is the result of both increased vascular tone and hyperaldosteronism, a recently proposed mechanism based on the study of *KCNMA1* knockout mice [20].

Hyperaldosteronism, a salt-retaining condition that results in increased systolic volume, might therefore have a more direct impact on systolic pressure than alterations in the compliance of the arterial wall (the vascular tone) that are related to changes in DBP, such as the condition generated by the E65K polymorphism in the β_1 subunit [7,12].

Variants in Ca^{2+}-dependent potassium channel may be responsible for variability in heart rate and baroreflex function [19]. In this respect, it seems plausible that increased vascular stiffness or changes in cardiac stroke volume, secondary to genetic variants in the *KCNMA1* gene, may have a role in the selective increase of SBP found in association with the IVS17 polymorphism. As we have no data on heart rate, it was not possible to investigate the stroke volume, which is certainly a limitation of the study.

Alternatively, the fact that genetic variation in the *KCNMA1* gene is associated with systolic hypertension (present study) and polymorphisms in the *KCNMB1* are associated with diastolic hypertension [7,12] may reflect that the former are just genetic markers in linkage disequilibrium with a truly functional defect present somewhere else.

Overall, the present findings are compatible with an association of *KCNMA1* genetic variations with an increase in the risk of systolic severe hypertension and MI. Further

analysis in other populations will be required to confirm the role of *KCNMA1* genetic variation in BP variation, severity of hypertension, and MI risk.

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There are no conflicts of interest.

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