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A common *NOS1AP* genetic polymorphism is associated with increased cardiovascular mortality in users of dihydropyridine calcium channel blockers

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Neuronal nitric oxide synthase (nNOS) regulates intracellular calcium handling
- A polymorphism in the *NOS1AP* gene (rs10494366), a regulator of nNOS, is associated with QTc prolongation
- Calcium channel blockers also affect calcium handling by blocking the L-type calcium channel

WHAT THIS STUDY ADDS

- We assessed the association between the polymorphism rs10494366 in the *NOS1AP* gene and mortality in calcium channel blocker users
- The TG and GG genotype are associated with increased cardiovascular mortality in dihydropyridine calcium channel blocker users

AIM

Recently, a polymorphism in the *NOS1AP* gene (rs10494366), a regulator of neuronal nitric oxide synthase (nNOS), was associated with QTc prolongation. Both nNOS and calcium channel blockers (CCBs) regulate intracellular calcium levels and have an important role in cardiovascular homeostasis. The aim was to investigate whether this polymorphism is associated with cardiovascular mortality in users of CCBs.

METHODS

The data from the Rotterdam study, a population-based closed cohort study of Caucasian individuals of ≥55 years of age, were used. We identified 1113 participants in the Rotterdam Study who were prescribed CCBs for the first time between 1991 and 2005. All-cause and cardiovascular mortality was assessed in participants who were prescribed CCBs with different *NOS1AP* rs10494366 genotypes using Cox proportional hazard models.

RESULTS

In participants starting on dihydropyridine CCBs (amlodipine, nifedipine and others) all-cause mortality ($n = 79$) risks were higher in participants with the TG [hazard ratio (HR) 2.57, 95% confidence interval (CI) 1.24, 5.34] or the GG genotype (HR 3.18, 95% CI 1.18, 8.58) than in participants with the referent TT genotype. Cardiovascular mortality ($n = 54$) risks were 3.51 (95% CI 1.41, 8.78) for the TG genotype and 6.00 (95% CI 1.80, 20.0) for the GG genotype. No differences in all-cause mortality or cardiovascular mortality were seen in participants starting with the nondihydropyridine CCBs verapamil or diltiazem.

CONCLUSION

The minor G allele of rs10494366 in the *NOS1AP* gene is associated with increased all-cause and cardiovascular mortality in Caucasian users of dihydropyridine CCBs. The mechanism underlying the observed association is unknown.

Introduction

Nitric oxide (NO) is an important regulator of intracellular calcium handling, and controls many processes in cardiovascular homeostasis, such as myocardial contraction [1, 2]. Nitric oxide synthase (NOS) produces endogenous NO from the amino acid L-arginine. Recently, the single nucleotide polymorphism (SNP) rs10494366 in the *NOS1 adaptor protein* (*NOS1AP*) gene was associated with a prolonged QTc interval in five independent populations [3–5]. NOS1AP is a regulator of neuronal NOS (nNOS, encoded by NOS1), one of the isoforms of NOS. Contraction of the cardiomyocyte is triggered by a short calcium influx through the voltage gated L-type calcium channels on the cell membrane [6]. Intracellular calcium is stored in the sarcoplasmic reticulum (SR) and two calcium channels control the release of calcium to and reuptake from the cytosol [1]. First, the ryanodine receptor releases calcium from the SR into the cytosol, which causes contraction of the cardiomyocyte. Second, the sarcoplasmic reticulum Ca^{2+}-ATPase regulates reuptake of calcium in the SR and stops the contraction of the cardiomyocyte. In the cardiomyocyte nNOS is localized on the SR, and it is hypothesized that nNOS has an effect on one or more of these calcium channels and transporter [7–11].

Calcium channel blockers (CCB) bind to a receptor on the voltage gated L-type calcium channel, promoting the closed position of the calcium channel and reducing calcium influx into the cell. Dihydropyridine CCBs, such as amlodipine and nifedipine, preferentially affect the blood vessels, causing vasodilation, whereas the nondihydropyridine CCBs verapamil and diltiazem have a higher affinity for the heart and have a negative chronotropic and inotropic effect [12].

Since nNOS affects the intracellular calcium levels and either directly or indirectly the calcium currents through the L-type calcium channel, the target of the CCBs, we hypothesized that the *NOS1AP* polymorphism rs10494366 might be associated with mortality in users of CCBs.

Methods

Setting

Data were obtained from the Rotterdam Study, a population-based prospective cohort study of cardiovascular, neurodegenerative, locomotor and ophthalmological diseases. All inhabitants of the suburb Ommoord in Rotterdam, who were ≥55 years old and had lived in the suburb for ≥1 year, were invited to participate in the study between 1990 and 1993. Of the 10 275 eligible persons, 7983 participated (78%) and have been followed since then [13, 14]. All participants were Caucasian. The study was approved by the Medical Ethical Committee of the Erasmus MC and all participants gave written informed consent.

At baseline, trained interviewers administered a questionnaire during a home interview, covering socioeconomic background and medical history, among other topics. During subsequent visits to the study centre, additional interviewing, laboratory assessments and clinical examinations were performed, including recording of electrocardiograms (ECGs). Follow-up examinations were carried out periodically (every 4–5 years). The seven pharmacies in Ommoord dispense the prescriptions of >99% of all participants. Information on all filled prescriptions from 1 January 1991 until 1 January 2005 was available and included the product name of the drug, the amount dispensed, the prescribed dosage regimen and the date of dispensing. The duration of each prescription was calculated by dividing the total number of tablets or capsules dispensed by the daily prescribed number of tablets or capsules.

Cohort definition

We identified all participants in the Rotterdam Study, who received a first prescription for a CCB between the baseline interview and 1 January 2005. Participants who were prescribed CCBs between 1 January 1991 and 1 July 1991 were excluded from the analysis, because they might have been using CCBs before 1 January 1991 in a period for which we had no pharmacy data. Participants who did not receive CCBs in the period of ≥6 months before the first prescription in the database were regarded as incident users. The time of the first prescription for a CCB was regarded as the date of entry into the study cohort. Participants were followed until one of the following events led to censoring: the end of the last prescription for a CCB, a period of no CCB use of ≥90 days calculated from the prescription data, switch to another CCB than the one on which the participant started, the occurrence of one of the study outcomes, or the end of the study period, whichever came first.

Outcomes

All mortality cases in the study cohort were identified, by obtaining at regular intervals the vital status of the participants from the municipal population registry. After notification of death, cause and circumstances were established by information from the general practitioner, letters and, in case of hospitalization, discharge reports from medical specialists. Two research physicians coded all mortality independently according to the International Classification of Diseases (ICD), 10th edn [15]. In case of disagreement, consensus was sought. Participants who died within 14 days after the end of the last prescription for a CCB were included in the analysis as current users.

In a subsequent analysis, cases of mortality that were coded as cardiovascular (ICD codes I00–I99) were selected and cardiovascular mortality risks analysed. In these analyses, we also included the ICD codes R96 (other sudden death, cause unknown), R98 (unattended death) and R99 (other ill-defined and unspecified causes of mortality). We

also analysed differences in the risk of a first myocardial infarction and fatal myocardial infarction as secondary outcomes.

Cofactors

Information was gathered at baseline on several potential covariates such as age and gender. All Cox proportional hazard models were adjusted for age and gender. To test whether the association between NOS1AP genotype and mortality or cardiovascular mortality was caused via an effect on the QTc interval, diabetes mellitus or hypertension, we also adjusted for these covariates. Diabetes mellitus was defined as any participant who had been diagnosed with diabetes mellitus at baseline. Diastolic and systolic blood pressure from the right upper arm were measured twice with a random-zero sphygmomanometer with the participant in sitting condition. The mean of the two readings was used to determine blood pressure levels. Hypertension was defined as use of antihypertensive drugs for the indication of high blood pressure, or a diastolic blood pressure of $\geq$90 mmHg, or a systolic blood pressure of $\geq$140 mmHg. The heart rate-corrected QT interval (QTc) was calculated from the ECG readings, using the Bazett's formula ($QTc = QT/\sqrt{RR}$). Since this CCB subcohort was nested in the Rotterdam Study, baseline characteristics were assessed before the time of the first prescription for a CCB. Because there was little reason to assume that this biased our results, these baseline characteristics are used in the analyses. In additional analyses we adjusted for the time-varying determinants heart failure, diabetes mellitus, sulphonylurea co-medication (glibenclamide, tolbutamide, gliclazide and glimepiride) and cardiovascular co-medication (loop diuretics, other diuretics, β-blockers and angiotensin converting enzyme-inhibitors/angiotensin II antagonists) at the time of event.

Genotyping

All participants were genotyped for the *NOS1AP* SNP rs10494366 T→G, which has previously been shown to be associated with a prolonged QTc interval [3]. This SNP was genotyped using Taqman assays C_1777074_10 (Applied Biosystems, Foster City, CA, USA) in 1 ng of genomic DNA extracted from leucocytes, as previously reported [16].

Statistical analysis

Deviation from Hardy–Weinberg equilibrium was tested using a χ^2 test. To test whether differences between genotypes were present at start of CCB therapy, we analysed differences in time from baseline to start of CCB therapy with Cox proportional hazard models and differences in starting dose with one-way ANOVA.

Multivariate Cox proportional hazard models were constructed for the outcomes occurring during follow-up. First, all-cause and cardiovascular mortality in the whole Rotterdam Study was analysed. Participants were followed from entrance in the Rotterdam Study, until death or end of the study period. Second, all-cause mortality, cardiovascular mortality, incident myocardial infarction and fatal myocardial infarction were analysed in participants who were prescribed CCBs. The date of the first prescription was regarded as start of follow-up. We analysed participants starting on dihydropyridine CCBs, nondihydropyridine CCBs, and the individual drugs amlodipine, nifedipine, verapamil and diltiazem separately.

Results

In the whole Rotterdam Study, 6571 blood samples from participants were available for analysis; 6292 people were successfully genotyped for the SNP rs10494366 and in 279 participants genotyping failed. The minor allele frequency was 0.36 (G allele). The genotype distribution of rs10494366 was in Hardy–Weinberg equilibrium in the Rotterdam Study ($\chi^2 = 1.04$, $P = 0.59$). No associations were found between *NOS1AP* genotype and all-cause mortality or cardiovascular mortality risks in the total group of participants, independent of whether they were prescribed CCBs or not. People with the TG or GG genotype had similar all-cause mortality risks [TG genotype hazard ratio (HR) 1.05, 95% confidence interval (CI) 0.96, 1.14, GG genotype HR 1.08, 95% CI 0.96, 1.22] and cardiovascular mortality risks (TG genotype HR 1.01, 95% CI 0.90, 1.14, GG genotype HR 1.04, 95% CI 0.88, 1.23) to those in the referent group with the TT genotype.

Of the 6292 genotyped people, 1113 (17.7%) were prescribed a first CCB during the study period and were included in the study cohort (Table 1). The genotype distribution of rs10494366 was in Hardy–Weinberg equilibrium in the study cohort ($\chi^2 = 0.45$, $P = 0.80$). No differences among genotypes were seen in time from enrolment in the Rotterdam Study to a first prescription for a CCB, or in prescribed daily dose of the first prescription for a CCB.

During follow-up, 79 of the 1113 participants (7.1%) who were prescribed CCBs for the first time during follow-up died while they were prescribed the CCB they started on. In participants with a first prescription for a dihydropyridine CCB, all-cause mortality risk was significantly higher in participants with the TG (HR 2.57, 95% CI 1.24, 5.34) or GG (HR 3.18, 95% CI 1.18, 8.58) genotype than in participants with the TT genotype (Table 2). No associations were found between *NOS1AP* and all-cause mortality for participants with a first prescription for the nondihydropyridine CCBs as a class or on verapamil or diltiazem individually.

In 54 of the 79 mortality cases, the cause of death was categorized as cardiovascular. In Table 3 the associations between *NOS1AP* genotypes and cardiovascular mortality are given. Here as well, cardiovascular mortality risk was significantly higher in participants with the TG (HR 3.51, 95% CI 1.41, 8.78) or GG (HR 6.00, 95% CI 1.80, 20.0) genotype with a first prescription for dihydropyridine CCB, than

Table 1

Characteristics of the study population of incident calcium channel blocker users ($n = 1113$)

Genotype	TT	TG	GG
Number	467	500	146
Gender, male (%)	44.8	40.2	43.8
Age, years (SD)	68.9 (7.9)	68.0 (7.3)	69.1 (7.5)
Follow-up time, years (SD)*	11.4 (2.9)	11.5 (2.9)	10.7 (3.8)
QTc, ms (SD)	430.2 (27.8)	431.3 (28.8)	434.9 (25.1)
Hypertension (%)	69.1	68.6	74.4
Diabetes mellitus (%)	7.6	6.9	5.6
Body mass index, kg m^{-2} (SD)	26.6 (3.6)	26.8 (3.5)	26.9 (4.1)
Serum creatinine, µmol l^{-1} (SD)	85.0 (25.1) ($n = 367$)	83.4 (16.5) ($n = 392$)	82.6 (15.5) ($n = 99$)
Start drug			
Dihydropyridine calcium channel blockers	283 (60.6%)	332 (66.4%)	86 (58.9%)
Nondihydropyridine calcium channel blockers	184 (39.4%)	168 (33.6%)	60 (41.1%)
Dihydropyridine calcium channel blockers			
Amlodipine	22.9%	28.2%	29.5%
Nifedipine	26.6%	27.4%	21.2%
Felodipine	1.3%	0.4%	1.4%
Isradipine	2.6%	5.0%	2.7%
Nicardipine	2.4%	3.0%	1.4%
Nisoldipine	0.9%	0.4%	0%
Nitrendipine	0.2%	0%	0%
Lacidipine	0.6%	0.2%	0.7%
Barnidipine	2.6%	1.4%	2.1%
Lercanidipine	0.4%	0.4%	0%
Mibefradil	0.2%	0%	0%
Nondihydropyridine calcium channel blockers			
Verapamil	11.6%	11.2%	13.0%
Diltiazem	27.8.%	22.4%	28.1%

*Follow-up time in the Rotterdam Study.

in participants with the TT genotype. No differences were found in participants starting on verapamil or diltiazem.

The HRs for both all-cause mortality and cardiovascular mortality after adjustment for QTc interval, hypertension or diabetes mellitus are given in Tables 2 and 3. Adjusting for these covariates or for heart failure, diabetes mellitus, sulphonylurea co-medication and cardiovascular co-medication at the time of the event (data not shown) did not change the results essentially.

We also analysed 34 cases of nonfatal and fatal myocardial infarction in the study population. Since numbers were too small to analyse the TG and GG genotype separately, these genotypes were grouped. In the participants who were prescribed dihydropyridine CCB the HR of any myocardial infarction ($n = 23$) for participants with the TG or GG genotype was 1.31 (95% CI 0.52, 3.31) compared with participants with the TT genotype. The risk of dying from a myocardial infarction ($n = 11$) was higher in patients with the TG or GG genotype (HR 6.69, 95% CI 0.83, 53.8), although not statistically significant.

Discussion

In our study of 1113 participants, we found a statistically significant three- to sixfold increased cardiovascular mortality risk for participants with a G allele at SNP rs10494366 while they were prescribed dihydropyridine CCBs. In the whole Rotterdam Study no differences were seen in cardiovascular mortality, indicating that the association between *NOS1AP* genetic variation and cardiovascular mortality is present only in participants who were prescribed dihydropyridine CCBs.

The precise mechanisms by which the common variation in the *NOS1AP* gene causes differences in mortality in participants who were prescribed dihydropyridine CCB is not known. Both nNOS, regulated by *NOS1AP*, and CCBs have an effect on intracellular calcium homeostasis. nNOS has negative feedback regulation of calcium release in the cytosol, because increases in calcium levels stimulate nNOS synthesis of NO, which in turn inhibits calcium release [7–11, 17, 18]. Although the effects of nNOS have been mostly assessed in the cardiomyocyte, calcium plays a vital role in many other cells.

Differences in all-cause and cardiovascular mortality were found only for the dihydropyridine CCBs and not for verapamil and diltiazem, although modest sample sizes preclude definitive conclusions. The clinical relevance of our findings could be high because 16.5% of our population used a dihydropyridine CCB at any time during follow-up. Dihydropyridine CCBs have a higher affinity for vascular calcium channels, whereas verapamil and diltiazem have a

Table 2

Association between *NOS1AP* genotype and all-cause mortality ($n = 79$) in 1113 incident calcium channel blocker users

	Cases	Model 1* HR	(95% CI)	Model 2† HR	(95% CI)	Model 3‡ HR	(95% CI)
Dihydropyridine calcium channel blockers ($n = 52$)							
TT	12	Ref.		Ref.		Ref.	
TG	30	**2.57**	**(1.24, 5.34)**	**2.50**	**(1.20, 5.19)**	**2.50**	**(1.19, 5.22)**
GG	10	**3.18**	**(1.18, 8.58)**	**3.18**	**(1.18, 8.61)**	**3.25**	**(1.19, 8.85)**
Amlodipine							
TT	4	Ref.		Ref.		Ref.	
TG	9	1.47	(0.45, 4.84)	1.48	(0.45, 4.86)	1.25	(0.38, 4.18)
GG	4	2.65	(0.63, 11.1)	2.39	(0.58, 9.89)	2.56	(0.61, 10.8)
Nifedipine							
TT	7	Ref.		Ref.		Ref.	
TG	16	**3.48**	**(1.19, 10.2)**	**3.68**	**(1.22, 11.1)**	**3.95**	**(1.28, 12.2)**
GG	5	2.65	(0.53, 13.3)	2.54	(0.50, 12.8)	2.27	(0.42, 12.1)
Nondihydropyridine calcium channel blockers ($n = 27$)							
TT	13	Ref.		Ref.		Ref.	
TG	9	0.83	(0.35, 2.01)	0.81	(0.34, 1.98)	0.80	(0.33, 1.96)
GG	5	0.94	(0.30, 2.97)	0.95	(0.30, 3.01)	1.02	(0.29, 3.54)
Verapamil							
TT	5	Ref.		Ref.		Ref.	
TG	4	0.65	(0.15, 2.86)	0.56	(0.12, 2.57)	0.46	(0.09, 2.35)
GG	3	0.33	(0.03, 3.15)	0.50	(0.04, 6.22)	0.38	(0.02, 5.67)
Diltiazem							
TT	8	Ref.		Ref.		Ref.	
TG	5	0.76	(0.24, 2.41)	0.76	(0.24, 2.41)	0.73	(0.22, 2.37)
GG	2	0.73	(0.15, 3.53)	0.75	(0.15, 3.63)	0.73	(0.12, 4.22)

*Model 1: adjusted for age and gender. †Model 2: adjusted for age, gender and QTc interval. ‡Model 3: adjusted for age, gender, QTc interval, hypertension and diabetes mellitus.

Table 3

Association between *NOS1AP* genotype and cardiovascular mortality ($n = 54$) in 1113 incident calcium channel blocker users

	Cases	Model 1* HR	(95% CI)	Model 2† HR	(95% CI)	Model 3‡ HR	(95% CI)
Dihydropyridine calcium channel blockers ($n = 38$)							
TT	7	Ref.		Ref.		Ref.	
TG	22	**3.51**	**(1.41, 8.78)**	**3.40**	**(1.36, 8.51)**	**3.33**	**(1.32, 8.39)**
GG	9	**6.00**	**(1.80, 20.0)**	**5.91**	**(1.77, 19.7)**	**6.38**	**(1.38, 22.2)**
Amlodipine							
TT	2	Ref.		Ref.		Ref.	
TG	6	2.39	(0.47, 12.1)	2.41	(0.47, 12.3)	2.23	(0.44, 11.3)
GG	3	4.49	(0.73, 27.8)	2.99	(0.47, 19.3)	3.23	(0.48, 21.7)
Nifedipine							
TT	4	Ref.		Ref.		Ref.	
TG	12	**4.98**	**(1.32, 18.9)**	**5.22**	**(1.34, 20.3)**	**5.90**	**(1.49, 23.4)**
GG	5	**11.0**	**(1.13, 107)**	8.80	(0.84, 92.0)	**14.7**	**(1.17, 184)**
Nondihydropyridine calcium channel blockers ($n = 16$)							
TT	8	Ref.		Ref.		Ref.	
TG	5	0.85	(0.26, 2.80)	0.83	(0.25, 2.75)	0.77	(0.23, 2.63)
GG	3	1.10	(0.21, 5.77)	1.10	(0.21, 5.83)	1.12	(0.18, 6.85)
Verapamil							
TT	3	Ref.		Ref.		Ref.	
TG	1	0.49	(0.04, 6.09)	0.43	(0.03, 5.29)	0.56	(0.03, 8.92)
GG	1	–§		–§		–§	
Diltiazem							
TT	5	Ref.		Ref.		Ref.	
TG	4	1.01	(0.25, 4.08)	1.02	(0.25, 4.10)	0.96	(0.21, 4.26)
GG	2	1.57	(0.27, 9.18)	1.48	(0.26, 8.45)	1.41	(0.19, 10.7)

*Model 1: adjusted for age and gender. †Model 2: adjusted for age, gender and QTc interval. ‡Model 3: adjusted for age, gender, QTc interval, hypertension and diabetes mellitus. §Numbers were too low to calculate hazard ratios.

higher affinity for the cardiac calcium channels. Verapamil and diltiazem are also used for the treatment of heart rhythm disturbances, such as atrial fibrillation, and angina pectoris, but adjusting for cardiovascular drugs co-prescribed with these indications did not change the results. It is suggested that dihydropyridine CCB relax coronary arteries by a NO-mediated mechanism [19, 20]. Although this has been attributed to the role of endothelial nitric oxide synthase, it is also possible that nNOS is involved. This may explain why differences were found for the dihydropyridine CCBs and not for verapamil and diltiazem.

Participants carrying a TG or GG genotype have a prolonged QTc interval, and therefore they might have an increased risk of arrhythmias and sudden cardiac death [21]. However, we do not think that this can explain our results. First, no associations between rs10494366 genotypes and all-cause mortality were seen in the whole Rotterdam Study. Second, adjusting for the QTc interval at baseline did not change the results materially. It is suggested that the CCBs isradipine, nicardipine, verapamil and diltiazem can cause QTc prolongation, although the evidence is weak [22, 23]. The number of participants in the study cohort starting on isradipine or nicardipine therapy was small, so any QTc-prolonging effect of these drugs could not have changed the results much. Recently, we identified an association between genetic variation in the *NOS1AP* gene and mortality in users of sulphonylurea [24]. Adjusting for diabetes mellitus, both at baseline and at the time of the event, and sulphonylurea use at the time of the event did not change the results either. Therefore, the effect of dihydropyridine CCBs on all-cause or cardiovascular mortality is not mediated by an effect on diabetes mellitus or prescribed sulphonylurea.

The risk of acute myocardial infarction was not increased, but the risk of dying from a myocardial infarction was increased, albeit nonsignificantly. Increased mortality in users of nifedipine with myocardial infarction has also been observed in two double-blind randomized clinical trials, but no genetic determinants were assessed [25, 26]. Although the number of cases was low and the results nonsignificant, this may be an interesting issue for further research.

In population-based studies, bias might affect the obtained results. We believe that bias in our study is minimal. Information in the Rotterdam Study is collected prospectively, without prior knowledge of the study hypothesis. Therefore information bias is unlikely. We identified all participants who started on CCB therapy during follow-up. Selection bias may have occurred if there were differences in severity of disease or in allocation to CCB therapy among genotypes at entry in the study cohort

caused by the *NOS1AP* polymorphism. However, the genotypes in this population were in Hardy–Weinberg equilibrium, and no differences were found in time to start of CCB therapy or starting dose. The absence of blood samples and difficulties with genotyping were probably independent of the genotype. It is also unlikely that confounding has influenced the results of our study, because all participants were incident users, and because physicians were unaware of the participant's genotype and could not base their drug choice on this information. In this study, drug use was calculated from filled prescriptions. In an earlier study published in this journal, we demonstrated that there was high agreement in the Rotterdam Study for filled cardiovascular chronic medication and actual drug use as stated by the patient during interview [27]. There is always the possibility that the results are a chance finding. However, we think that this is probably not the case in our study. First, these analyses are not part of a genome-wide association study, but we were testing an *a priori* hypothesis. Second, we found an association for dihydropyridine CCB users with both the TG genotype and the GG genotype, making a chance finding less likely. Given the small number of cases in our study, it is necessary that the results be replicated in further studies.

In the Caucasian population around 40% of the population has the TT genotype, whereas in Yoruba in Ibadan (Nigeria), Japanese in Tokyo and Han Chinese in Beijing only 10–15% of the population have the TT genotype [28]. As a consequence of this, the results of trials with dihydropyridine CCBs performed in a Caucasian population cannot be extrapolated unconditionally to other populations and vice versa. Regarding the polymorphism in the *NOS1AP* gene, it could be hypothesized that the risk of cardiovascular mortality in users of dihydropyridine CCB in Yoruba, Japanese and Chinese populations in general will be higher than in Caucasian populations.

To conclude, our results show that the genetic variation in the *NOS1AP* gene is associated with mortality risk in participants using dihydropyridine CCB. Participants with a TG or GG genotype at SNP rs10494366 have a higher all-cause and cardiovascular mortality risk than participants with the TT genotype. Because both the use of dihydropyridine CCBs and the allele frequencies of both alleles of the *NOS1AP* SNP rs10494366 are high, our results seem to be of substantial clinical importance, if replicated in further studies.

Competing interests

None to declare.

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