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ORIGINAL ARTICLE

Diuretic–gene interaction and the risk of myocardial infarction and stroke

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This study investigates whether the interaction between diuretics and alpha-adducin (ADD1) G460W or G-protein β3-subunit (GNB3) rs2301339 polymorphism modifies the risk of myocardial infarction (MI) or stroke. Data were used from the Rotterdam Study. The drug–gene interaction was determined with a Cox proportional hazard model with adjustment for each drug class as time-dependent covariates. The risk of MI in current users of low-ceiling diuretics with one or two copies of the ADD1 W-allele (hazard ration (HR) = 0.92) was similar compared to the expected joint effect of the W-allele and low-ceiling diuretics on a multiplicative scale ($1.04 \times 0.90 = 0.94$) (synergy index (SI):0.99; 95% confidence interval (CI): 0.43–2.27). No drug–gene interaction was found on the risk of stroke (SI:0.66; 95% CI:0.43–1.27). In addition, a trend towards an interaction was found between current use and the GNB3 rs230119 G/A polymorphism on the risk of MI (SI: 0.51; 95% CI: 0.23–1.15), whereas no interaction on the risk of stroke was found (SI: 0.84; 95% CI: 0.46–1.56).
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Introduction

Hypertension is a complex disease with a high prevalence and a risk factor for myocardial infarction (MI), stroke, congestive heart failure, end-stage renal disease, and peripheral vascular disease.[1–4] Antihypertensive therapy has been associated with a 35–40% reduction in stroke incidence and a 20–25% reduction in MI in clinical trials.[5] Even though there are effective antihypertensive drugs, it can be difficult to find the most appropriate pharmacological treatment for an individual patient.

Pharmacogenetics aims to understand how genetic variations contribute to the variation in response. Targeting treatment to the genetic components may enhance treatment efficacy and improve overall benefit, resulting in more effective blood pressure control and a lower incidence of hypertension-related morbidity.

Two genes that might influence the response to low-ceiling diuretics are the alpha-adducin (ADD1) and G protein β_3-subunit (GNB3) gene. The human ADD1 460W-allele can be considered as a candidate allele for hypertension, because it may affect blood pressure by increasing renal tubular reabsorption of sodium through the activation of Na^+,K^+-ATPase (adenosine triphosphatase). Compared with hypertensive patients who are homozygous for the 460G wild-type allele, hypertensive patients carrying at least one 460W-allele have a less steep pressure natriuresis slope. This means that they need a higher arterial pressure to

excrete the same amount of sodium after saline infusion.[6] Psaty *et al*.[7] reported that the W-allele was associated with a lower risk of MI or stroke in diuretic users compared with other antihypertensive drug users in an observational study. However, Davis *et al*.[8] found no significant drug–gene interaction on the risk of cardiovascular disease in a trial setting. In four nonrandomized trials, the interaction between the ADD1 G460W polymorphism and antihypertensive drugs on blood pressure response was evaluated.[9–12] Three studies, with partly the same study population, found a greater blood pressure reduction with the 460W-allele than with the 460G-allele,[9–11] whereas another study could not replicate this finding.[12] The other candidate gene, GNB3 mediates signal transduction across cell membranes.[13] In one trial, a positive association was found between the 825T-allele and the effect of hydrochlorothiazide on blood pressure. Mean declines in blood pressures was greater in TT than in CC homozygous patients, respectively.[14]

The objective of our study was to determine whether the risk of MI or stroke in hypertensive patients treated with low-ceiling diuretics is modified by the ADD1 G460W or GNB3 rs2301339 polymorphism.

Results

There were 4097 subjects with hypertension during follow-up. Of these 4097 persons, 707 persons were treated with low-ceiling diuretics at baseline and 1982 during follow-up. A subject may have contributed to one or more categories of antihypertensive drug classes during follow-up. In 99.1% ($n = 4062$) of the hypertensive individuals, genotypes were assessed for the ADD1 gene, and 97.8% ($n = 4008$) for the GNB3 gene. Tables 1 and 2 show the baseline characteristics stratified by ADD1 G460W and GNB3 rs2301339 G/A genotypes.

Myocardial infarction

In total, 196 subjects of the 4062 subjects, who were genotyped for the ADD1 G460W polymorphism, experienced an MI. Thirty-three subjects had an MI while they were treated with low-ceiling diuretics, of whom 22 had the GG genotype and 11 had the GW or WW genotype (see Table 3).

Subjects with the GG genotype who were currently treated with low-ceiling diuretics had similar risk of MI compared to subjects with the GG genotype who never used low-ceiling diuretics (hazard ratio (HR) = 1.04; 95% confidence interval (CI): 0.47–2.29) (see Table 3). Among subjects who never used low-ceiling diuretics, the GW or WW genotype was not associated with the risk of MI (HR = 0.90; 95% CI: 0.61–1.32). Subjects with the GW or WW genotype who were currently treated with low-ceiling diuretics had a similar risk of MI compared to subjects with the GG genotype who never used low-ceiling diuretics (HR = 0.92; 95% CI: 0.38–2.21). This HR was close to the expected value from the joint effect of the GW or WW genotype and low-ceiling diuretics on a multiplicative scale ($1.04 \times 0.90 = 0.94$). The synergy index (SI) was, therefore, close to 1 (SI = 0.99; 95% CI: 0.43–2.27) (see Table 4).

Of the 4008 subjects genotyped for the GNB3 rs2301339 G/A polymorphism, 192 subjects experienced an MI. Neither the genotype nor the use of a low-ceiling diuretic reduced significantly the risk of MI (see Table 3). The HR for the joint effect of the GA or AA genotype and low-ceiling diuretic on the multiplicative scale was lower than expected ($1.27 \times 1.10 = 1.40$). The SI was non-significantly reduced (SI = 0.51; 95% CI: 0.23–1.15) (see Table 4).

Additional analyses in which adjustments were made for systolic blood pressure level, diastolic blood pressure level, history of angina, use of acetylsalicylic acids, use of coumarins, use of nonsteroidal anti-inflammatory drugs (NSAIDs), use of anti-diabetic medication, history of stroke, and smoking yielded similar results and were therefore not shown. When the analysis was repeated with other antihypertensive drug classes (i.e. angiotensin converting enzyme (ACE) inhibitors, β-blockers, high-ceiling diuretics, or calcium antagonists), there was no significant drug–gene interaction with any of these antihypertensive drug classes with either of the polymorphisms on the risk of MI.

When we combined the risk alleles of the two polymorphisms, none of the associations with the risk of MI were significant (see Table 5). In addition, the SI was non-significant (SI = 0.78; 95% CI: 0.27–2.27).

Table 1 Baseline characteristics stratified by ADD1 G460W genotype

Characteristics	*GG (N = 2535)*	*W-allele (N = 1527)*
Gender (female)	1534 (60.5%)	925 (60.6%)
Age (years)	70.7 ± 9.1	70.4 ± 8.6
Stroke at baseline (yes)	85 (3.4%)	67 (4.4%)
MI at baseline (yes)	390 (15.4%)	240 (15.7%)
Diabetes (yes)	308 (13.0%)	173 (12.0%)
SBP (mmHg)	143.9 ± 22.3	142.9 ± 22.6
DBP (mmHg)	75.4 ± 11.9	74.6 ± 12.1[a]
BMI (kg/m^2)	26.9 ± 3.8	27.0 ± 3.7
Total cholesterol/high density cholesterol (mmol/l)	5.3 ± 1.7	5.3 ± 1.6
Smoking		
Current (yes)	461 (18.6%)	340 (22.9%)
Past (yes)	1067 (43.1%)	615 (41.4%)
Use of low-ceiling diuretic	453 (18.3%)	248 (16.2%)
Use of high-ceiling diuretic	194 (7.7%)	85 (5.6%)[a]
Use of β-blockers	559 (22.1%)	329 (21.5%)
Use of ACE inhibitors	222 (8.8%)	129 (8.4%)
Use of calcium channel blocker	247 (9.7%)	128 (8.4%)
Use of statins	64 (2.5%)	49 (3.2%)
Use of coumarins	120 (4.7%)	79 (5.2%)
Use of NSAID	236 (9.3%)	130 (8.5%)
Use of ASA/salicylate	365 (14.8%)	206 (13.5%)

Abbreviations: ACE, angiotensin converting enzyme; ADD, alpha-adducin; ASA, acetylsalicylic acids; BMI, body mass index; DBP, dibolic blood pressure; MI, myocardial infraction; NSAID, nonsteroidal anti-inflammatory drugs; SBP, systolic blood pressure.
[a]Significant difference between GG- and W-allele ($P < 0.05$).

Stroke

In total, 348 subjects who were genotyped for the ADD1 G460W polymorphism experienced a stroke during follow-up. Sixty-two subjects had a stroke when they were treated with a low-ceiling diuretic, of whom 44 had the GG genotype.

Table 2 Baseline characteristics stratified by GNB3 rs2301339

Characteristics	*GG (N = 1979)*	*A-allele (N = 2029)*
Gender (female)	1189 (60.1%)	1231 (60.7%)
Age (years)	70.6 ± 9.0	70.5 ± 8.8
Stroke at baseline (yes)	81 (4.1%)	70 (3.4%)
MI at baseline (yes)	306 (15.5%)	314 (15.4%)
Diabetes (yes)	231 (12.5%)	246 (12.9%)
SBP (mmHg)	143.1 ± 22.6	143.9 ± 22.3
DBP (mmHg)	74.9 ± 11.8	75.4 ± 12.2
BMI (m/kg^2)	27.0 ± 3.7	26.8 ± 3.8
Total cholesterol/high density cholesterol (mmol/l)	5.3 ± 1.6	5.4 ± 1.7
Smoking		
Current (yes)	388 (20.1%)	414 (20.9%)
Past (yes)	814 (42.2%)	844 (42.6%)
Use of low-ceiling diuretic	340 (17.2%)	354 (17.4%)
Use of high-ceiling diuretic	129 (6.5%)	147 (7.2%)
Use of β-blockers	406 (20.5%)	465 (22.9%)
Use of ACE inhibitors	166 (8.4%)	176 (8.7%)
Use of calcium channel blocker	188 (9.5%)	183 (9.0%)
Use of statins	52 (2.6%)	58 (2.9%)
Use of coumarins	96 (4.9%)	101 (5.0%)
Use of NSAID	182 (9.2%)	180 (8.9%)
Use of ASA/salicylate	283 (14.3%)	290 (14.3%)

Abbreviations: ACE, angiotensin converting enzyme; ADD, alpha-adducin; ASA, acetylsalicylic acids; BMI, body mass index; DBP, diabolic blood pressure; GNB3, G-protein β3-subunit; MI, myocardial infraction; NSAID, nonsteroidal anti-inflammatory drugs; SBP, systolic blood pressure.

To investigate the possible interaction between low-ceiling diuretic users and both polymorphism on the risk of stroke, participants were grouped by current use and genotype group (see Table 6). The drug–gene interaction between current use of low-ceiling diuretics and the G460W polymorphism on the risk of MI was non-significantly reduced (SI = 0.66; 95% CI: 0.43–1.27) (see Table 7). The interaction between current use of low-ceiling diuretics and the rs2301339 G/A polymorphism on the risk of stroke was also non-significant (SI = 0.84; 95%CI: 0.46–1.56) (see Table 7).

After inclusion of only subjects with ischemic strokes ($N = 188$), the SI remained non-significant (G460W; SI = 0.66; 95% CI: 0.33–1.33 and rs2301339 G/A; SI = 0.77; 95% CI: 0.31–1.89).

Additional analyses in which adjustments were made for systolic blood pressure level, diastolic blood pressure level, history of angina, use of acetylsalicylic acids, use of coumarins, use of NSAIDs, use of anti-diabetic medication, history of stroke, and smoking yielded similar results and

Table 4 Synergy indices for diuretic user with the ADD1 G460W polymorphism or GNB3 rs2301339 on the risk of MI

Polymorphism	*SI (95% CI)*[a]	*SI (95% CI)*[b]
G460W		
GW/WW versus GG	1.00 (0.44–2.27)	0.99 (0.43–2.27)
rs2301339 G/A		
GA/AA versus GG	0.49 (0.22–1.09)	0.51 (0.23–1.15)

Abbreviations: ADD, alpha-adducin; CI, confidence interval; GNB3, G-protein β3-subunit; MI, myocardial infraction; SI, synergy index.
[a]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and defined daily dose.
[b]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, defined daily dose, BMI, cholesterol level, statin use, and history of percutaneous transluminal coronary angioplasty, CABG, and MI.

Table 3 Association of low-ceiling diuretic use and ADD1 G460W polymorphism or GNB3 rs2301339 with myocardial infraction (MI) risk

Polymorphism	*Type of use*	*MI (N)*	*HR (95% CI)*[a]	*HR (95% CI)*[b]
G460W				
GG	No	103	1 (reference)	1 (reference)
GG	Current	22	1.00 (0.46–2.16)	1.04 (0.47–2.29)
GW/WW	No	60	0.87 (0.59–1.27)	0.90 (0.61–1.32)
GW/WW	Current	11	0.87 (0.37–2.06)	0.92 (0.38–2.21)
rs2301339 G/A				
GG	No	76	1 (reference)	1 (reference)
GG	Current	20	1.27 (0.59–2.74)	1.27 (0.57–2.86)
GA/AA	No	84	1.08 (0.76–1.55)	1.10 (0.76–1.59)
GA/AA	Current	12	0.69 (0.29–1.60)	0.74 (0.31–1.76)

Abbreviations: ADD, alpha-adducin; CI, confidence interval; GNB3, G-protein β3-subunit; HR, hazard ratio; MI, myocardial infraction.
[a]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and defined daily dose.
[b]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, defined daily dose, BMI, cholesterol level, statin use, and history of percutaneous transluminal coronary angioplasty, CABG, and MI.

Table 5 Drug–gene–gene interaction in diuretic users on the risk of MI

Polymorphism	*Type of use*	*(N)*	*HR (95% CI)*[a]	*HR (95% CI)*[b]
Rest	No	133	1 (reference)	1 (reference)
Rest	Current	28	1.01 (0.49–2.06)	1.02 (0.49–2.15)
GW/WW+GA/AA	No	30	1.02 (0.64–1.64)	1.06 (0.66–1.70)
GW/WW+GA/AA	Current	5	0.79 (0.27–2.28)	0.80 (0.27–1.37)

Abbreviations: CI, confidence interval; HR, hazard ratio; MI, myocardial infraction.
[a]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and defined daily dose.
[b]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, defined daily dose, systolic blood pressure level, diabetes mellitus, use of statins, use of acetylsalicylic acids, and history of coronary heart disease, stroke, and angina.

Table 6 Association of low-ceiling use and ADD1 G460W polymorphism or GNB3 rs2301339 with stroke risk

Polymorphism	*Type of use*	*Stroke (N)*	*HR (95% CI)*[a]	*HR (95% CI)*[b]
G460W				
GG	No	170	1 (reference)	1 (reference)
GG	Current	44	0.82 (0.46–1.46)	0.84 (0.46–1.54)
GW/WW	No	116	1.06 (0.78–1.43)	1.05 (0.77–1.44)
GW/WW	Current	18	0.65 (0.33–1.27)	0.59 (0.29–1.18)
rs2301339 G/A				
GG	No	127	1 (reference)	1 (reference)
GG	Current	28	0.78 (0.41–1.47)	0.81 (0.42–1.55)
GA/AA	No	150	1.14 (0.84–1.54)	1.16 (0.85–1.59)
GA/AA	Current	31	0.82 (0.44–1.53)	0.79 (0.42–1.50)

Abbreviations: ADD, alpha-adducin; CI, confidence interval; GNB3, G-protein β3-subunit; HR, hazard ratio.
[a]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and defined daily dose.
[b]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, defined daily dose, systolic blood pressure level, diabetes mellitus, use of statins, use of acetylsalicylic acids, and history of coronary heart disease, stroke, and angina.

Table 7 Synergy indices for diuretic user with the ADD1 G460W polymorphism or GNB3 rs2301339 on the risk of stroke

Polymorphism	*SI (95% CI)*[a]	*SI (95% CI)*[b]
G460W		
GW/WW versus GG	0.75 (0.40–1.40)	0.66 (0.43–1.27)
rs2301339 G/A		
GA/AA versus GG	0.93 (0.51–1.68)	0.84 (0.46–1.56)

Abbreviations: ADD, alpha-adducin; BMI, body mass index; CABG, coronary artery bypass grafting; CI, confidence interval; GNB3, G-protein β3-subunit; MI, myocardial infraction; SI, synergy index.
[a]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and defined daily dose.
[b]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, defined daily dose, BMI, cholesterol level, statin use, and history of percutaneous transluminal coronary angioplasty, CABG, and MI.

were therefore not shown. When the analysis was repeated with other antihypertensive drug classes (i.e. ACE inhibitors, β-blockers, high-ceiling diuretics, or calcium antagonists), there was no significant drug–gene interaction with any of these antihypertensive drug classes with either of these polymorphisms.

When we combined the risk alleles of the two polymorphisms, none of the associations with the risk of stroke were significant (see Table 8). In addition, the SI was non-significant (SI = 0.69; 95% CI: 0.32–1.49).

Discussion

In this study, current users of low-ceiling diuretics with at least one copy of the ADD1 460W-allele had no significantly reduced risk of MI or stroke compared to low-ceiling diuretic users with the GG genotype. With regard to the GNB3 rs23011339 G/A polymorphism, no interaction was found with the use of low-ceiling diuretics on the risk of stroke and a tendency towards an interaction on the risk of MI. There were more stroke cases during the use of a low-ceiling diuretic and, therefore, we had more power to detect a drug–gene interaction on the risk of stroke. Hence, it seems more likely that there is no drug–gene interaction with this polymorphism. The combination of both polymorphisms did not have a statistically significant influence on the risk

Table 8 Drug–gene–gene interaction in diuretic users on the risk of stroke

Polymorphism	*Type of use*	*(N)*	*HR (95% CI)*[a]	*HR (95% CI)*[b]
Rest	No	208	1 (reference)	1 (reference)
Rest	Current	69	0.80 (0.45–1.39)	0.81 (0.45–1.45)
GW/WW+GA/AA	No	49	1.37 (0.96–1.97)	1.35 (0.94–1.96)
GW/WW+GA/AA	Current	10	0.75 (0.34–1.64)	0.61 (0.27–1.39)

Abbreviations: CI, confidence interval; HR, hazard ratio.
[a]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, and defined daily dose.
[b]Adjusted for age, gender, other antihypertensive drugs, past exposure to antihypertensive drugs, defined daily dose, systolic blood pressure level, diabetes mellitus, use of statins, use of acetylsalicylic acids, and history of coronary heart disease, stroke, and angina.

of MI or stroke in low-ceiling diuretic users, which might be compatible with the absence of an interaction but also be explained by the fact that the number of MI and stroke cases with both risk alleles was small.

It is debatable whether the ADD1 G460W polymorphism influences the effect of low-ceiling diuretic therapy. In a large trial (GENHAT) in high-risk hypertensive persons, no significant interaction between the use of chlorthalidone compared to other antihypertensive drugs and the ADD1 G460W polymorphism was found.[8] Although, women carrying the W-allele may have an increased coronary heart disease risk if treated with chlorothalidone versus lisinopril or amlodipine. Psaty *et al*.[7] reported that users of low-ceiling diuretics carrying one or two copies of the W-allele might have a reduced risk of non-fatal MI or non-fatal stroke compared to users of other antihypertensive drugs in an observational study. These results were in contrast with the results obtained in our observational study. There were some differences between the two observational studies. For instance, we included fatal and non-fatal cases of MI and stroke and compared the use of low-ceiling diuretics versus non-users instead of users of other antihypertensive drugs.

Only one study investigated the association between the use of low-ceiling diuretics and a polymorphism in the GNB3 gene.[14] In this study, a greater blood pressure-lowering effect was seen in TT than in CC homozygotes when treated with diuretics. However, the contribution of these findings on blood pressure to cardiovascular risk remains uncertain. The distance between the 825C/T and rs2301339 G/A polymorphism is about 250 base pairs and therefore these polymorphisms are most likely in linkage disequilibrium with each other.

The main limitation of our study is the relatively small number of events. Therefore, the results should still be interpreted with caution and need to be replicated in other studies before definitive conclusions can be made. In addition, we compared the risk of MI or stroke in an observational setting instead of a clinical trial. An advantage of randomized controlled trials is that the random allocation to the intervention enhances the internal validity of a study by minimizing confounding. In our observation study, it was the choice of the physician whether a patient received antihypertensive drug treatment or not and the type of antihypertensive drug. Specific patients characteristics my have influenced this decision and could have biased our results. However, the interaction between low-ceiling diuretics and the ADD1 G460W or GNB3 rs2301339 G/A polymorphism is probably not influenced by this bias, as persons with hypertension are first started on thiazide diuretics in the Netherlands. This choice is made without knowledge of the genetic profile. Race could have been an additional confounder, however, given that less than 1% of the subjects had a different ethnic background, it is unlikely that this biased our results. As our populations consisted of $>99\%$ of Caucasians, our results can only be generalized to this group. Furthermore, only one SNP in per gene was investigated. An advantage of our study is that we investigated the interactions between polymorphisms in two genes, as it is most likely that more than one gene influences the protective effect of low-ceiling diuretics.

In conclusion, our results indicate that the ADD1 G460W polymorphism does not influence the effect of low-ceiling diuretics on the risk of MI or stroke. In addition, the results did not indicate a strong drug–gene interaction between the use of low-ceiling diuretics and the GNB3 rs230119 G/A polymorphism on the risk of stroke or MI. The results of the GNB3 rs230119 G/A polymorphism need to be replicated before definitive conclusions can be made.

Methods

Setting

The Rotterdam Study started in 1990 as a population-based prospective follow-up study. All 10 275 residents of the suburb Ommoord in Rotterdam, aged 55 years or older were invited to participate. In total, 7983 (78%) subjects gave written informed consent. The baseline measurements took place until 1993. The design of this population-based study has been described elsewhere.[15] Information was collected on age, gender, present health status, and medical history, including previous MI and stroke. All reported MIs or strokes at baseline were verified with medical records. During a physical examination, blood pressure, weight, and height were measured and blood was drawn for DNA extraction. Since the start of the Rotterdam Study, follow-up examinations have been carried out every 2–3 years. Blood pressure data from all three examination rounds were used.

Cohort and outcome definition

Only subjects with hypertension were included in this study. Therefore, follow-up started on the day that an elevated blood pressure was measured and/or the day that a first antihypertensive drug was prescribed, whichever came first. Elevated blood pressure was defined as systolic blood pressure $\geqslant$160 mmHg, and/or diastolic blood pressure $\geqslant$95 mmHg. MI and stroke cases between January 1, 1991 and July 1, 1991 were not included, as pharmacy records were not available before January 1, 1991, and this resulted in a drug history which is shorter than 6 months. The end of the study was set at January 1 2002. Follow-up ended on the date of the first MI (or first stroke for the analysis with stroke as primary outcome), or a censoring event (end of study period, moving out of the area, or death), whichever was earlier. All collected coronary events were verified by review of hospital discharge reports and letters from medical specialists, and classified as definitive and possible MI. Two research physicians independently coded events according to the International Classification of Diseases, 10th Revision (ICD-10).[16] MI was defined as ICD codes: I21. A medical expert in cardiovascular disease also reviewed all coded events for final classification.

Stroke was defined as ICD codes: K90. Stroke research physicians reviewed information on all possible strokes and transient ischemic attacks; an experienced stroke neurologist verified all diagnosis. Subarachnoid hemorrhages and retinal strokes were excluded. Ischemic strokes were diagnosed when a patient had typical symptoms and a computed tomography (CT) and magnetic resonance imaging (MRI) that was made within 4 weeks ruled out other diagnoses or when indirect evidence (deficit limited to one limb or completely resolved within 72 h, atrial fibrillation in the absence of anticoagulants) pointed at an ischemic nature of the stroke. Hemorrhagic stroke was diagnosed when a relevant hemorrhage was shown on CT or MRI scan, or when the subject permanently lost consciousness or died within hours after the onset of focal signs. If a stroke did not match these criteria, it was classified as unspecified.[17]

Exposure definition

Pharmacy records were available for approximately 99% of the cohort as of January 1, 1991. These records include the name of the drug, the day of dispensing, the dosage form, the number of units dispensed, the prescribed daily dose, and the Anatomical Therapeutic Chemical code of the drug.[18] The exposure of interest was low-ceiling diuretics (i.e. thiazides and combination of thiazides and potassium sparing agents).

When an MI or stroke occurred, the date was defined as the event date and the cumulative duration of use for current and past exposure of all antihypertensive drug classes on that date was calculated for all participants. Hereto, we first calculated each prescription length by dividing the number of dispensed tablets or capsules by the prescribed daily number. Each refill at the pharmacy which occurred within 7 days after last intake from the previous prescription was considered as a continuous drug episode. Current, past, and never used were defined as mutually exclusive categories. When the event fell within a usage period, the patient was considered as currently exposed, and the cumulative number of days of current use was calculated. Similarly for those who were not current users, but had used a representative of the drug group in the past, the number of days since last intake was calculated. Those who had not used a low-ceiling diuretic during the follow-up period were considered as non-users. For dose–effect associations, we used the defined daily dosages (DDD) which consist of the recommended daily dose for the indication of hypertension in an adult.

Genotyping

Genomic DNA was extracted from whole-blood samples using standard methods, described previously.[19] Samples were genotyped with TaqMan allelic discrimination Assays-By-Design (Applied Biosystems, Foster City, CA, USA). Forward and reverse primer sequences were 5′ GAG AAG ACA AGA TGG CTG AAC TCT 3′ and 5′ GTC TTC GAC TTG GGA CTG CTT 3′, and the minor groove binding probes were 5′ ATT CTG CCA TTC CTC 3′ (VIC) and 5′ ATT CTG CCA TTC CTC 3′ (FAM) for the ADD1 gene. Forward and reverse primer (antisense strand) sequences were 5′ GGC AGG GCT GCT TCT CA3′ and 5′ GCA AGC CGC TGC TCT CA 3′, and the minor groove binding probes were 5′ AAA CCA AGG AAG GGA CA 3′ (VIC) and 5′ ACC AAG GGA GGG ACA 3′ (FAM) for the GNB3 gene. The assays utilized 5 ng of genomic DNA and 2 μl reaction volumes. The amplification and extension protocol was as follows: an initial activation step of 10 min at 95°C preceded by 40 cycles of denaturation at 95°C for 15 s and annealing and extension at 50°C for 60 s. Allele-specific fluorescence was then analyzed on an ABI Prism 7900HT Sequence Detection System with SDS v 2.1 (Applied Biosystems, Foster City, CA, USA).

Potential confounders

For the analysis with MI as an outcome, we considered age, gender, systolic/diastolic blood pressure, body mass index (BMI), current and past smoking, and cholesterol level (total cholesterol/high density cholesterol) at baseline as potential confounders. Adjustments for history of stroke, history of MI, history of percutaneous transluminal coronary angioplasty, history of coronary artery bypass grafting, statin use, coumarin use, ASA use, NSAID use, nitrate use, use of antidiabetic medication, history of angina, past and current use of other antihypertensive drugs, and the defined daily dose were made as time-dependent covariates. History of angina was defined as the use of two or more prescriptions of nitrate. In addition, we adjusted for the combined use of other antihypertensive drug classes by adding each antihypertensive drug class separately in the model for past and current users.

For the analysis with stroke as an end point, we considered the same potential confounders, but combined history of MI, history of percutaneous transluminal coronary angioplasty,

and history of coronary artery bypass grafting in one variable (coronary heart disease).

Statistical analyses

The outcomes MI and stroke were analysed separately because of their different aetiology. Both events were evaluated using a Cox proportional hazard model with time-varying exposure for each antihypertensive drug class separately. We created non-cumulative time-dependent categorical variables (yes/no) for current and past use of antihypertensive drugs and follow-up time was the time-axis of the model. Non-use of low-ceiling diuretics served as a reference. The associations were expressed as hazard ratios (HR) with 95% CIs.

We calculated the SI, which is the ratio of the HR in susceptibles (e.g. with the W-allele) to the HR in non-susceptibles (with the GG genotype). To investigate the SI between the G460W polymorphism and low-ceiling diuretics, one dummy variable was added to the model: W-allele (0/1) $\times$ low-ceiling diuretics (0/1). An SI of one means that the HR in the two subgroups is the same and that there is no interaction on a multiplicative scale. An SI grater than one means that the joint effect of gene and drug is larger than expected from the product of their individual effect.[20]

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Duality of interest

None declared.

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