Diuretic therapy, the alpha-adducin variant, and the risk of myocardial infarction or stroke in persons with treated hypertension

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Diuretic Therapy, the α-Adducin Gene Variant, and the Risk of Myocardial Infarction or Stroke in Persons With Treated Hypertension

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Context A genetic variant in α-adducin has been associated with renal sodium reabsorption and salt-sensitive hypertension. Whether this genetic variant modifies the effect of diuretic therapy on the incidence of myocardial infarction (MI) and stroke is unknown.

Objectives To estimate the interaction between α-adducin and diuretic therapy on the risk of MI or stroke. Specifically, we hypothesized that in participants with treated hypertension, the risk of MI or stroke associated with diuretic use would be lower in carriers of the adducin variant than in carriers of the adducin wild-type genotype.

Design, Setting, and Participants Population-based case-control study of patients enrolled in a health maintenance organization, treated pharmacologically for hypertension, and genotyped as homozygous carriers of the adducin wild-type genotype or carriers of 1 or 2 copies of the *Trp460* variant allele. Cases had a first nonfatal MI (n=206) or stroke (n=117) between January 1995 and December 1998. Controls (n=715) were a stratified random sample of pharmacologically treated hypertensive patients who were matched to MI cases by age, sex, and calendar year.

Main Outcome Measure Risk of the combined outcome of first nonfatal MI or stroke.

Results The adducin variant was present in more than one third of the participants. Among the 653 carriers of the adducin wild-type genotype, diuretic therapy was not associated with the risk of MI or stroke (odds ratio [OR], 1.09; 95% confidence interval [CI], 0.78-1.52). Among the 385 carriers of the adducin variant allele, diuretic therapy was associated with a lower risk of the combined outcome of MI and stroke than other antihypertensive therapies (OR, 0.49; 95% CI, 0.32-0.77). The OR in carriers of the adducin variant was less than half of the OR in carriers of the wild-type genotype ($P=.005$). The case-control synergy index (SI) was 0.45 (95% CI, 0.26-0.79) for the combined outcome of MI and stroke. The point estimates of the diuretic-adducin interaction were similar in separate analyses of MI (SI, 0.41; 95% CI, 0.21-0.80) and stroke (SI, 0.53; 95% CI, 0.24-1.19). The diuretic-adducin interaction was not confounded by traditional cardiovascular risk factors, was specific to diuretic therapy but not present for other major antihypertensive drug classes, and did not differ substantially between subgroups defined by age, sex, race, diabetes, and history of cardiovascular disease.

Conclusions In carriers of the adducin variant, diuretic therapy was associated with a lower risk of combined MI or stroke than other antihypertensive therapies. If these findings are confirmed in other studies, this large subgroup of the hypertensive population may be especially likely to benefit from low-dose diuretic therapy.

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OVER CENTURIES AND ACROSS populations, a large number of polymorphisms have appeared in genes that are now said to code for drug receptors,[1] drug-metabolizing enzymes,[2] and drug-effector pathways.[3] Under a variety of historical selection pressures, some of their variant alleles became common long before the appearance of modern pharmacotherapies. By the early 1990s, about 25 million persons in the United States were taking antihypertensive medications.[4] This massive population exposure to prescription drugs provides the opportunity for common or powerful drug-gene interactions to occur.[5]

One candidate is the potential interaction between diuretics and the α-adducin gene, whose Gly460Trp variant has been associated with renal sodium retention and a salt-sensitive form of hypertension in some populations. A polymorphism in the rat α-adducin gene

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was first described as a cause of hypertension in a series of elegant experiments on the Milan hypertensive strain [6-8] A cytoskeletal protein, adducin is a heterodimer or heterotetramer of α and β subunits that is critical for the assembly of the actin-spectrin network and has been implicated in cell-signal transduction [9,10] In humans, a Gly460Trp polymorphism of the α-adducin gene is associated with blood pressure levels or the prevalence of hypertension in some but not all populations [11-20] Carriers of 1 or 2 copies of the variant *Trp460* allele display high rates of renal tubular sodium reabsorption [21] The blood pressure responses, both to diuretics and to infused saline, are more pronounced in participants with the variant adducin allele than in those homozygous for the wild type [11,22] Moreover, the phenotype of salt sensitivity, independent of blood pressure, has been associated with an increased risk of cardiovascular events [23]

On the basis of this evidence, we initiated a population-based case-control study in participants with pharmacologically treated hypertension to assess the interaction between diuretic therapy and the adducin variant on the incidence of myocardial infarction (MI) and stroke The a priori hypothesis was that among pharmacologically treated hypertensive patients, the risk of MI or stroke associated with diuretic use would be lower in carriers of the adducin variant than in carriers of the wild-type genotype

METHODS

Setting

The study setting was the Group Health Cooperative (GHC, Seattle, Wash), a health maintenance organization with an enrollment of more than 400000 persons The methods have been described previously [24,25] The study was reviewed and approved by human subjects committees at both GHC and the University of Washington

Identification of Cases and Controls

Cases were GHC enrollees who had pharmacologically treated hypertension and survived an incident MI or stroke between January 1995 and December 1998 Potential cases were identified from the computerized discharge abstracts for the 2 Group Health hospitals and the GHC claims databases, which include bills for all services provided by non-GHC physicians and health care facilities Events criteria were adapted from the Cardiovascular Health Study for both MI and stroke [26,27] All strokes, both ischemic and hemorrhagic, were included Controls were a stratified random sample of GHC enrollees with pharmacologically treated hypertension, and they were sampled from the GHC computerized enrollment files on the basis of person time, which ensures that the odds ratio (OR) approximates the relative risk [28] Controls were frequency matched to the MI cases by age (within decade), sex, and calendar year of the index date (defined below) at a ratio of at least 2 to 1 for men and at least 3 to 1 for women The MI cases were used to set the matching targets because there were more MI cases than stroke cases within each age-sex-calendar-year stratum Controls met the same eligibility criteria as the cases, but they had not had an MI or stroke before their index dates All participants provided written informed consent

Index Dates and Eligibility

All participants had an index date For the cases, the index date was the date of admission for the first acute MI or stroke, and for the controls, the index date was a computer-generated random date within the same calendar year for which they had been chosen as controls For all participants, we collected information about risk factor data available only before the index date This approach ensured comparability between cases and controls in the assessment of risk factors and eligibility criteria All participants were GHC enrollees aged 30 to 79 years at their index dates, they were members of the GHC for 1 year or had made at least 4 visits with a GHC clinician during the year prior to the index date, and based on the ambulatory medical record, they had a physician diagnosis of pharmacologically treated hypertension Cases whose index event was a complication of a procedure were not eligible for the study Additionally, we excluded patients (1) who were not currently taking at least 1 antihypertensive medication at their index date (for instance, noncompliant hypertensive patients), (2) whose blood specimens did not yield an adducin genotype, (3) who had a history of congestive heart failure, and (4) who had had a previous MI or stroke

Data Collection

Data collection included a review of the GHC outpatient medical record, a telephone interview, and a venous blood sample from consenting participants Based on the medical record, research assistants determined eligibility and collected information about the following risk factors for coronary heart disease blood pressure and pulse, height and weight, cholesterol level, smoking status, family history, marital status, and use of health services, medical conditions such as angina, hypertension, diabetes, congestive heart failure, stroke, and peripheral vascular disease Cardiovascular disease was defined as a history of angina, claudication, or vascular procedures, including coronary artery bypass graft, angioplasty, carotid endarterectomy, and peripheral vascular bypass Research assistants were not blinded to case-control status, but they were not aware of the research hypothesis

Methods of Assessing Antihypertensive Medication Use

The GHC computerized pharmacy database served as the primary source of information about antihypertensive drug therapies Since 1976, the GHC pharmacy database has included a record for all prescriptions dispensed to GHC enrollees Each pharmacy record contains a patient identifier, the drug type and dose, the date, the quantity dispensed, and dosing instructions For determining the current use

Table 1. Eligible Myocardial Infarction and Stroke Cases and Controls

	No. (%) of Cases		
	Myocardial Infarction	Stroke	No. (%) of Controls
Included in primary analysis	206 (71)	117 (67)	715 (75)
Exclusion criteria			
Heart failure	13 (4)	10 (6)	37 (4)
Previous myocardial infarction	0 (0)	13 (7)	64 (7)
Previous stroke	28 (10)	0 (0)	33 (3)
No current antihypertensive medicine use	43 (15)	33 (19)	104 (11)
No adducin genotype available	0 (0)	1 (1)	5 (1)
Total	**290 (100)**	**174 (100)**	**958 (100)**

of each medication at the index date, we searched the pharmacy data for a prescription immediately preceding the reference date For example, when a participant (who was at least 80% compliant) received enough pills to last until the index date, he/she was counted as a potential current user of the drug, the participant also had to be classified as a user for at least 30 days prior to the index date, otherwise, the participant was counted as a nonuser of the drug This definition of current use, which specified a minimum duration of 30 days of use, thus excluded current users who had just started the medication For 80% compliance, a participant who received 100 pills with instructions to take 1 pill per day was classified as a current user for 125 days (from 100/0 8) after the prescription dispensing date In preplanned sensitivity analyses, we reanalyzed data that defined current use assuming 100% rather than 80% compliance

Individual drugs were grouped into major common classes diuretics, β-blockers, angiotensin-converting enzyme (ACE) inhibitors, calcium-channel blockers, and other vasodilators Diuretics included both loop and thiazide diuretics During the study period, only 2 controls received angiotensin-receptor blockers, which were grouped with ACE inhibitors

Blood Collection and Laboratory Assays

A blood specimen was drawn from the antecubital vein into tubes containing EDTA and processed White blood cells were shipped on dry ice to the laboratory in Leiden, the Netherlands DNA was extracted using standard salting-out procedures [29] The status of the adducin variant was assayed using standard polymerase chain reaction–restriction-fragment-length polymorphism genotyping methods The forward mutagenic primer was 5′GGGGCGACGAAGCTcCaGAGGAA3′ Nucleotides in lower case differ from the sequence of the gene, and they create a *Bst*XI recognition site in the presence of the variant *Trp460* allele The reverse primer was 5′GGCTGGATTCCCAAAGCCTCC3′ The presence of the *Trp460* allele was assessed by the occurrence of this additional *Bst*XI restriction site in the polymerase chain reaction fragment Laboratory personnel were blinded both to case-control status and to antihypertensive drug-therapy status

Exposure Definitions and Statistical Analysis

Based on the computerized pharmacy data, participants' diuretic use was classified as current or not current at their index dates All patients who did not meet criteria for current use of diuretics were currently taking 1 or more other antihypertensive drug therapies at their index dates All participants were also classified either as homozygous carriers of the adducin wild-type (normal) genotype or as carriers of 1 or 2 copies of the α-adducin variant *Trp460* allele

In comparing case and control characteristics, we used the *t* test or analysis of variance for continuous variables and the χ^2 test or Fisher exact text for categorical variables The ORs were estimated from the cross product of the 2 × 2 table for case-control status by exposure status, and their 95% confidence intervals (CIs) were estimated in the standard way [30,31] Logistic regression was used for multivariable analysis The ORs were also calculated separately in the 2 strata defined by adducin genotype Formal tests for interaction were performed with both case-control and case-only methods [32,33] When assumptions are met,[33] case-only studies are more efficient and powerful than case-control methods [34] Both methods estimate the synergy index (SI), which is the ratio of the OR in those with the variant to the OR in those without the variant The case-only SI is calculated from the cross product of the exposure and genotype among the cases [33] An SI equal to 1 means that the ORs in the 2 subgroups are the same and that there is no interaction on the multiplicative scale An SI of less than 1 represents an interaction—for instance, the risk of MI or stroke associated with diuretic use is smaller in carriers of the adducin variant than in carriers of the wild type All statistical tests were 2-tailed Sensitivity analyses included not only several definitions of diuretic use, dose, type, and duration of use, but also subgroup analyses defined by age, sex, race, diabetes, and history of cardiovascular disease Analysis was performed using SPSS statistical software (Version 10, SPSS Inc, Chicago, Ill)

RESULTS

The primary analysis included 206 MI cases, 117 stroke cases, and 715 controls (TABLE 1) Among the controls, the adducin variant was in Hardy-Weinberg equilibrium [35] TABLE 2 summarizes the patient characteristics of the 3 groups Cases and controls differed in expected ways For example, diabetes, previous angina, family history of MI, systolic blood pressure, current smoking, total cholesterol, high-density lipoprotein cholesterol, and glucose level were risk factors for MI Since controls were frequency matched to the

MI cases, the mean age of stroke cases was higher than that of the controls The prevalence of the adducin variant was high (≥35%) in all groups

When cases and controls were analyzed separately (TABLE 3), the adducin genotype was not associated with most other risk factors For instance, most recent and pretreatment systolic and diastolic blood pressures did not differ significantly between carriers of the variant allele and carriers of the wild type This was true for both the cases and the controls While the mean numbers of diuretic prescriptions were similar in adducin-variant and wild-type carriers, the prevalence of current diuretic use differed significantly by ad-

Table 2. Characteristics of Myocardial Infarction (MI) and Stroke Cases and Controls[a]

Characteristic	MI Cases (n = 206)	Stroke Cases (n = 117)	Controls (n = 715)	*P* Value: MI Cases vs Controls	*P* Value: Stroke Cases vs Controls
Age y	63 5 (10 6)	69 8 (7 8)	64 5 (10 1)	23	< 001
Women %	37 9	59 8	48 1	01	02
Black %	3 4	1 7	2 9	74	43
Current smoking %	15 5	13 7	9 9	03	24
Nonsedentary %	69 9	59 8	74 3	22	002
Married %	78 2	68 4	73 4	17	26
Diabetes %	24 8	22 2	13 1	< 001	01
Prior disease/procedure %					
Angina	26 2	14 5	12 4	< 001	54
Percutaneous coronary intervention or coronary artery bypass graft	7 3	7 7	4 3	10	14
Cardiovascular disease	30 6	17 9	14 8	< 001	39
Family history of MI	41 7	20 5	26 7	< 001	15
No of antihypertensive medicines taken %					
1	63 1	53 3	62 0	76	17
2	30 1	35 9	32 3		
3	6 3	7 7	4 9		
4	0 5	2 6	0 8		
Body mass index kg/m^2	31 2 (5 8)	29 6 (6 3)	30 3 (6 3)	09	24
Years in Group Health Cooperative	17 8 (12 3)	21 3 (13 3)	21 2 (10 8)	< 001	96
No of visits in the prior year	6 5 (6 0)	7 0 (5 6)	5 6 (4 9)	04	005
Most recent blood pressure mm Hg					
Systolic	144 0 (19 6)	150 7 (21 2)	141 0 (18 3)	04	< 001
Diastolic	82 4 (10 3)	83 3 (10 8)	82 6 (10 5)	83	47
Pretreatment blood pressure mm Hg					
Systolic	165 0 (21 4)	169 4 (24 1)	160 6 (19 7)	04	003
Diastolic	99 4 (11 2)	101 1 (13 1)	98 7 (10 0)	58	12
Duration of treated hypertension y	9 8 (6 6)	14 0 (9 3)	10 9 (7 7)	06	001
Pulse beats/min	75 2 (11 2)	75 5 (12 4)	74 2 (11 5)	29	25
Cholesterol level					
mg/dL	240 5 (44 1)	243 5 (49 6)	226 6 (44 0)	< 001	< 001
mmol/L	6 2 (1 1)	6 3 (1 2)	5 8 (1 1)		
High density lipoprotein cholesterol level					
mg/dL	42 3 (12 1)	47 3 (15 6)	48 0 (15 1)	< 001	64
mmol/L	1 1 (0 3)	1 2 (0 4)	1 2 (0 4)		
Glucose level					
mg/dL	129 8 (56 9)	130 6 (58 3)	114 5 (43 5)	< 001	005
mmol/L	7 2 (3 2)	7 2 (3 2)	6 4 (2 4)		
Potassium mEq/L	4 26 (0 4)	4 18 (0 5)	4 17 (0 4)	01	75
Creatinine level					
mg/dL	1 10 (0 3)	1 20 (0 9)	1 08 (0 4)	41	16
μmol/L	97 1 (24 9)	106 2 (83 1)	95 2 (32 6)		
α Adducin genotype %					
Homozygous wild type (*GG*)	62 1	59 0	63 8	21	02
Heterozygous (*GT*)	35 4	40 2	31 5		
Homozygous variant (*TT*)	2 4	0 9	4 8		

[a]Values are expressed as mean (SD) unless otherwise indicated Control to case matching ratios were higher for women than men by design

ducin genotype among the cases but not the controls.

During the study period, the number of pills dispensed for a typical diuretic prescription lasted about 100 days (at 100% compliance). Among the controls, participants classified as current diuretic users had received an average of 38.8 diuretic prescriptions, while participants classified as not current users of diuretics had received an average of 13.7 diuretic prescriptions ($P<.001$). In other words, the average duration of past diuretic use at 100% compliance was about 10.6 years for current use vs 3.7 years for noncurrent use. Among the cases, the mean numbers of diuretic prescriptions were 34.0 for current users vs 13.9 for noncurrent users ($P<.001$). Among current diuretic users, the case-control difference of 4.8 diuretic prescriptions was not significant ($P=.28$).

Considered individually, neither diuretic use nor the adducin variant was associated with case-control status (TABLE 4). The primary analysis to assess the drug-gene interaction appears in TABLE 5. Among wild-type carriers, the use of diuretics was not associated

Table 3. Association of the α-Adducin Variant With Characteristics of Participants*

Characteristic	Controls			Myocardial Infarction and Stroke Cases		
	Wild Type (n = 456)†	Variant (n = 259)‡	*P* Value	Wild Type (n = 197)†	Variant (n = 126)‡	*P* Value
Women, %	50.7	54 1	38	42 1	51.6	.10
Black, %	3.3	2.3	.46	3.0	2.4	.72
Diabetes, %	14 7	10.4	.11	22.3	26.2	.43
History of angina, %	12.9	11.6	.60	21.8	22 2	.93
Any cardiovascular disease, %	15.1	14.3	.76	24.9	27.8	.56
Current smoking, %	11.4	7.3	.08	15.2	14.3	.82
Antihypertensive medication, %						
Diuretics	45.6	49 4	.33	47.7	32.5	.007
β-Blockers	32 9	32.8	.98	34.0	44.4	06
Calcium-channel blockers	25.4	25 9	.90	30.5	24 6	.25
Angiotensin-converting enzyme inhibitors	33.1	34.0	81	34.0	33.3	90
Other vasodilators	6.1	5.0	.54	9.6	4.8	.11
No. of antihypertensive medications taken, %						
1	62.7	60.6	.84	55 3	66.7	.21
2	32.0	32 8		35 0	27.8	
3	4.6	5 4		8.1	4.8	
4	0.7	1.2		1.5	0.8	
No. of prescriptions for diuretics	25.4 (27.1)	25.7 (26.6)	.88	23.6 (27.2)	20.3 (26.8)	.30
Most recent blood pressure, mm Hg						
Systolic	141.1 (18.8)	140.9 (17.5)	.87	146.8 (19.7)	146.0 (21.5)	.76
Diastolic	82.5 (10.4)	82.7 (10.6)	.77	83.5 (10.1)	81.6 (11.1)	.13
Pretreatment blood pressure, mm Hg						
Systolic	160.5 (19.5)	160.7 (20 0)	.95	165.2 (21.9)	167.8 (23.3)	37
Diastolic	98 7 (10.1)	98.8 (9 9)	.95	100.5 (11.3)	99 1 (12.8)	.38
Years receiving hypertension treatment	10.7 (7.7)	11.2 (7.9)	.36	11.5 (8.1)	11.1 (7.6)	65
Pulse, beats/min	74.9 (11.3)	73.1 (11.8)	05	76.8 (12.0)	73 0 (10.7)	.004
Creatine level						
mg/dL	1.07 (0.4)	1.08 (0.3)	.77	1.15 (0.7)	1.11 (0.3)	.55
μmol/L	94 9 (35.1)	95.6 (27.6)		101.8 (65.1)	98.1 (28 7)	
Glucose level						
mg/dL	115.0 (43.8)	113.6 (43.1)	.68	129.5 (58.1)	131.0 (56.4)	.82
mmol/L	6.4 (2.4)	6.3 (2.4)		7.2 (3.2)	7.3 (3.1)	
Cholesterol level						
mg/dL	227.9 (46.1)	224.5 (39.9)	.32	243.4 (44.6)	238.7 (48.4)	.38
mmol/L	5.9 (1.2)	5.8 (1.0)		6.3 (1.2)	6 2 (1.3)	
High-density lipoprotein cholesterol level						
mg/dL	47 5 (15 1)	48.8 (15.2)	.25	43.7 (13.5)	44.8 (13.8)	.49
mmol/L	1.3 (0.4)	1.3 (0.4)		1.1 (0.4)	1.2 (0.4)	
No. of visits in prior year	5.4 (4.9)	5.8 (4.9)	.26	6.4 (5.8)	7.1 (6.0)	.31
Years in Group Health Cooperative	21 2 (10 5)	21.4 (11.3)	.84	18.9 (12.8)	19.3 (12.6)	.79

*Values are expressed as mean (SD) unless otherwise indicated.
†Homozygous *GG*
‡Either or both *GT* or homozygous *TT*

with the risk of MI or stroke (OR, 1.09; 95% CI, 0.78-1.52). Among those with noncurrent use of diuretics, the adducin variant was associated with a modest increase in risk (OR, 1.56; 95% CI, 1.09-2.23). In the absence of an interaction (on a multiplicative scale), the expected joint effects of the adducin variant and current diuretic use would have been 1.70 (the product of the individual ORs, 1.09×1.56). But the point estimate for the adducin-variant carriers who were taking diuretics was lower than expected for the combined outcome of MI and stroke (OR, 0.77; 95% CI, 0.51-1.17).

The analyses stratified on the adducin genotype appear on the right side of Table 5. Among the 653 carriers of the adducin wild type, diuretic therapy was not associated with the risk of MI or stroke (OR, 1.09; 95% CI, 0.78-1.52). But among the 385 carriers of the adducin variant, diuretic therapy was associated with a lower risk of MI or stroke than other antihypertensive therapies (OR, 0.49; 95% CI, 0.32-0.77). The case-control estimate of the SI was 0.45 (95% CI, 0.26-0.79). The case-only estimate of the SI was similar at 0.53 (95% CI, 0.33-0.84). Both the case-control and case-only SIs indicated a significant interaction between diuretic therapy and the adducin variant on the risk of MI or stroke ($P = .005$ and $P = .007$, respectively).

When MI and stroke were considered separately, the SI point estimates were similar. The case-control SI for MI alone was 0.41 (95% CI, 0.21-0.80) and for stroke was 0.53 (95% CI, 0.24-1.19); their 95% CIs were widely overlapping. Adjustment for age, sex, race, smoking, and diabetes had little effect on the case-control SIs for risk of MI or stroke, or for the combined outcome of MI and stroke (TABLE 6). Additional adjustment for cholesterol level and systolic blood pressure had trivial effects on the SIs (Table 6).

The adjusted case-control SIs for the analysis assuming 80% compliance differed little from those assuming 100% compliance (TABLE 7). For diuretic dose, type, and duration of use, the differences between the SIs were within the play of chance. The case-control SIs did not differ between those who were taking only 1 medication and those who were taking 2 or more medications. The interaction between diuretic use and the adducin variant was specific to diuretics. There was no significant interaction between the adducin variant and any of the other major classes of antihypertensive medications (Table 7).

Table 4. Association of Diuretic Use and α-Adducin Variant Individually With Case-Control Status*

	MI and Stroke			MI Only			Stroke Only		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
Current diuretic use									
No	188	379	1.0	123	379	1.0	65	379	1.0
Yes	135	336	0.81 (0.62-1.06)	83	336	0.76 (0.56-1.04)	52	336	0.90 (0.61-1.34)
Adducin variant									
Wild type	197	456	1.0	128	456	1.0	69	456	1.0
Variant	126	259	1.13 (0.86-1.48)	78	259	1.07 (0.78-1.48)	48	259	1.23 (0.82-1.83)

*MI indicates myocardial infarction; OR, odds ratio; and CI, confidence interval.

Table 5. Interactions Between Diuretic Use and α-Adducin Variant on Risk of First Nonfatal Myocardial Infarction (MI), Stroke, or Both*

Diuretic Use	Adducin Variant†	No. of Cases	No. of Controls	OR (95% CI)	P Value	Study Population	No. of Subjects	Measure	Point Estimate (95% CI)	P Value
MI and Stroke										
Not current	Wild type	103	248	1.0		Adducin variant carrier	385	Case-control OR	0.49 (0.32-0.77)	.002
Current	Wild type	94	208	1.09 (0.78-1.52)	.62	Adducin wild-type carrier	653	Case-control OR	1.09 (0.78-1.52)	.62
Not current	Variant	85	131	1.56 (1.09-2.23)	.01	All cases and controls	1038	Case-control SI	0.45 (0.26-0.79)	.005
Current	Variant	41	128	0.77 (0.51-1.17)	.23	Cases only	323	Case-only SI	0.53 (0.33-0.84)	.007
MI Only										
Not current	Wild type	68	248	1.0		Adducin variant carrier	337	Case-control OR	0.43 (0.25-0.74)	.002
Current	Wild type	60	208	1.05 (0.71-1.56)	.80	Adducin wild-type carrier	584	Case-control OR	1.05 (0.71-1.56)	.80
Not current	Variant	55	131	1.53 (1.01-2.32)	.04	All cases and controls	921	Case-control SI	0.41 (0.21-0.80)	.009
Current	Variant	23	128	0.66 (0.39-1.10)	.11	Cases only	206	Case-only SI	0.47 (0.26-0.86)	.01
Stroke Only										
Not current	Wild type	35	248	1.0		Adducin variant carrier	307	Case-control OR	0.61 (0.33-1.16)	.13
Current	Wild type	34	208	1.16 (0.70-1.92)	.57	Adducin wild-type carrier	525	Case-control OR	1.16 (0.70-1.92)	.57
Not current	Variant	30	131	1.62 (0.95-2.76)	.07	All cases and controls	832	Case-control SI	0.53 (0.24-1.19)	.13
Current	Variant	18	128	1.00 (0.54-1.83)	.99	Cases only	117	Case-only SI	0.62 (0.29-1.31)	.21

*OR indicates odds ratio; CI, confidence interval; and SI, synergy index.
†Wild type is homozygous for wild-type allele; variant is heterozygous or homozygous for *Trp460* allele.

In additional sensitivity analyses (TABLE 8), the case-control SIs did not differ significantly between subgroups defined by age, sex, and presence of cardiovascular disease or diabetes. Although the number of blacks was small, the SIs for both events combined were similar (0.43 for nonblacks and 0.40 for blacks). Despite multiple testing, there was no statistical evidence of a second-order interaction. The most extreme difference, SIs of 0.19 in men and 0.61 in women for the combined outcome of MI and stroke, was within the play of chance ($P = .07$).

Table 6. Effect of Serial Adjustments on Case-Control Synergy Indices for the Interaction Between α Adducin Variant and Diuretic Use on the Risks of First Nonfatal Myocardial Infarction (MI), Stroke, or Both*

	MI and Stroke		MI Only		Stroke Only	
Covariate†	SI (95% CI)	*P* Value	SI (95% CI)	*P* Value	SI (95% CI)	*P* Value
None	0.45 (0.26-0.79)	.005	0.41 (0.21-0.80)	.009	0.53 (0.24-1.19)	.13
Age	0.45 (0.26-0.78)	.005	0.41 (0.21-0.80)	.009	0.51 (0.22-1.16)	.11
Sex	0.45 (0.26-0.79)	.005	0.42 (0.22-0.83)	.01	0.50 (0.22-1.15)	.10
Race	0.45 (0.26-0.79)	.005	0.42 (0.21-0.82)	.01	0.50 (0.22-1.15)	.10
Smoking	0.45 (0.26-0.80)	.006	0.42 (0.22-0.83)	.01	0.51 (0.22-1.16)	.11
Diabetes	0.46 (0.26-0.82)	.008	0.44 (0.22-0.87)	.02	0.49 (0.21-1.14)	.10
Any cardiovascular disease	0.45 (0.25-0.79)	.006	0.41 (0.21-0.83)	.01	0.49 (0.21-1.14)	.10
Total cholesterol	0.45 (0.25-0.80)	.007	0.42 (0.21-0.83)	.01	0.49 (0.21-1.15)	.10
Systolic blood pressure‡	0.43 (0.24-0.77)	.004	0.40 (0.20-0.81)	.01	0.46 (0.20-1.09)	.08

SI indicates case control synergy index; CI, confidence interval.
†Each covariate was added to a model that contained the covariate(s) listed above it.
‡Most recent measurement taken.

The interaction was more pronounced among homozygotes for the *Trp460* allele. Among participants taking diuretics, none of the MI or stroke cases and 16 controls were homozygous for the *Trp460* allele. Among the homozygotes, the OR for both events combined was 0 ($P = .02$, Fisher exact test). The point estimate of the SI was lower for nonhemorrhagic stroke (SI, 0.45, 95% CI, 0.18-1.09) than for hemorrhagic stroke (SI, 0.90, 95% CI, 0.10-7.74), although neither of the 2 SIs individually, nor the difference between them, was significant.

In additional analyses, diuretic use and the adducin variant were not associated with most recent or pretreatment systolic or diastolic blood pressure, and 2-way analysis of variance provided no evidence of an interaction between diuretic use and the adducin variant on blood pressure ($P = .37$). In separate MI analyses, the inclusion of cases (n=28, Table 1) and controls (n=33) who had had a prior stroke had trivial effects on the SI estimates. Similarly in stroke analyses, the inclusion

Table 7. Interaction Between the Adducin Variant and Aspects of Diuretic Use or Use of Other Antihypertensive Agents on the Risks of First Nonfatal Myocardial Infarction (MI), Stroke, or Both*

	MI and Stroke		MI Only		Stroke Only	
Variable	No. of Subjects	SI (95% CI)	No. of Subjects	SI (95% CI)	No. of Subjects	SI (95% CI)
Compliance with diuretics						
80%	1038	0.45 (0.25-0.79)	921	0.41 (0.21-0.83)	832	0.49 (0.21-1.14)
100%	1038	0.52 (0.30-0.89)	921	0.46 (0.24-0.89)	832	0.61 (0.28-1.35)
Diuretic dose†						
≤Modal	915	0.43 (0.23-0.81)	814	0.46 (0.22-0.96)	727	0.39 (0.15-1.00)
>Modal	681	0.55 (0.22-1.41)	610	0.33 (0.09-1.22)	549	0.92 (0.26-3.26)
Type of diuretic†						
Thiazide	974	0.51 (0.28-0.93)	868	0.52 (0.25-1.06)	782	0.51 (0.21-1.26)
Loop	631	0.20 (0.06-0.70)	555	0.10 (0.02-0.56)	494	0.38 (0.08-1.90)
Duration of diuretic use†‡						
<37 prescriptions	788	0.43 (0.19-0.95)	735	0.41 (0.15-1.16)	701	0.43 (0.15-1.23)
≥37 prescriptions	817	0.45 (0.23-0.88)	770	0.40 (0.18-0.86)	688	0.77 (0.29-2.01)
No. of antihypertensive medicines						
1	636	0.50 (0.22-1.14)	543	0.46 (0.17-1.22)	506	0.52 (0.15-1.84)
≥2	402	0.42 (0.16-1.11)	348	0.33 (0.10-1.21)	326	0.60 (0.16-2.69)
Adducin variant interaction with						
β Blockers	1038	1.41 (0.79-2.51)	921	1.17 (0.69-2.35)	832	2.00 (0.86-4.68)
Angiotensin converting enzyme inhibitors	1038	1.00 (0.56-1.80)	921	1.21 (0.60-2.44)	832	0.66 (0.27-1.60)
Calcium channel blockers	1038	0.79 (0.42-1.48)	921	1.06 (0.51-2.23)	832	0.48 (0.18-1.27)

*Models were adjusted for age, sex, race, diabetes, cardiovascular disease, and current smoking. SI indicates case control synergy index; CI, confidence interval.
†Subjects who were not exposed to the category of interest were excluded from the analysis of those who were exposed (ie, subjects who took thiazides were excluded from the analysis of loop diuretics).
‡The number of 37 prescriptions is equivalent to a duration of diuretic use of approximately 10 years.

of cases (n=13) and controls (n=64) who had had a prior MI also had trivial effects on the SI estimates.

COMMENT

In this population-based case-control study, the adducin variant was present in about one third of hypertensive participants. There was a significant interaction between the presence of the adducin *Trp460* variant and the use of diuretics on the risk of the combined outcome of first nonfatal MI or stroke. Among the 653 carriers of the adducin wild-type genotype, diuretic therapy was not associated with risk of MI or stroke (OR, 1.09; 95% CI, 0.78-1.52). Among the 385 carriers of the adducin variant allele, diuretic use was associated with a lower risk of the combined outcome of MI or stroke (OR, 0.49; 95% CI, 0.32-0.77) than use of other antihypertensive medications. The OR in carriers of the adducin variant was less than half of the OR in carriers of wild-type genotype ($P=.005$). The case-control SI was 0.45 (95% CI, 0.26-0.79). The point estimates of this drug-gene interaction were similar in separate analyses of MI (SI, 0.41; 95% CI, 0.21-0.80) and stroke (SI, 0.53; 95% CI, 0.24-1.19). The diuretic-adducin interaction was not confounded by traditional cardiovascular risk factors, was specific to diuretic therapy but not present for other major antihypertensive drug classes, and did not differ between subgroups defined by age, sex, race, diabetes, and cardiovascular disease.

This study had a number of limitations. Potential alternative explanations for the findings of genetic association studies include uncontrolled confounding from traditional risk factors or ethnic/racial differences between cases and controls and linkage disequilibrium.[35] While we performed genotyping for a well-studied adducin polymorphism, it is possible that the actual causative locus may be represented by another adducin nucleotide variant or variants that are in linkage disequilibrium with the *Trp460* allele. This study focused on only 1 single nucleotide polymorphism. However, single nucleotide polymorphisms, which are common in genes related to hypertension,[36] may fail to capture biological effects of haplotypes, which represent all the polymorphisms present on a single maternal or paternal chromosomal segment.[37] While the adjusted case-control SIs were similar for MI and stroke (0.41 and 0.49, respectively, Table 6), the findings for stroke alone did not reach conventional levels of statistical significance ($P=.10$), and the study lacked power to evaluate differences between nonhemorrhagic and hemorrhagic stroke (SIs of 0.45 and 0.90, respectively). Moreover, the case participants in this study represented survivors of an MI or stroke, and it is possible that the genotype or a gene-environment interaction may affect survival rather than disease incidence. If, for instance, the joint effects of diuretic therapy and the adducin variant were associated with a high case-fatality rate, a case-control study of nonfatal events might provide a biased estimate of the interaction.

Although the computerized GHC pharmacy data measured prescriptions filled rather than drugs taken, these prospectively collected pharmacy data provided a powerful resource for estimating antihypertensive drug use in an unbiased fashion for all cases and controls. In this study, drug use was defined a priori as current use at the index date. Importantly, current diuretic use was associated with an average duration of use of about 10 years, which was almost 3

Table 8. Sensitivity Analyses of the Adjusted Case-Control Synergy Indices for the Interaction Between the α-Adducin Variant and Diuretic Use on the Risks of First Nonfatal Myocardial Infarction (MI), Stroke, or Both[a]

	MI and Stroke		MI Only		Stroke Only	
Population	No. of Subjects	SI (95% CI)	No. of Subjects	SI (95% CI)	No. of Subjects	SI (95% CI)
Adjusted model	1038	0.45 (0.25-0.79)	921	0.41 (0.21-0.83)	832	0 49 (0.21-1.14)
Race						
Not black	1008	0.43 (0.24-0.78)	893	0.40 (0.20-0.80)	809	0.50 (0.21-1.17)
Black†	30	0.40 (0.01-17.8)	28	0.60 (0.07-37.7)	23	NA
History of cardiovascular disease						
No	848	0.49 (0.26-0 94)	752	0.46 (0 20-1.02)	705	0.58 (0.23-1 47)
Yes	190	0.33 (0 10-1.16)	169	0.33 (0.08-1.33)	127	0.19 (0 02-1.53)
Diabetes						
No	867	0.49 (0.26-0.91)	776	0.51 (0.24-1.11)	712	0.42 (0.17-1.07)
Yes	171	0.31 (0.07-1.31)	145	0.20 (0 04-1 04)	120	0.63 (0.07-5.93)
Sex						
Men	546	0.19 (0.08-0.49)	499	0.22 (0.08-0.60)	418	0.16 (0.03-0.89)
Women	492	0.61 (0.26-1.39)	422	0.58 (0.20-1 71)	414	0.58 (0.20-1.70)
Age, y						
<65	468	0.29 (0.11-0.75)	448	0 28 (0.10-0.78)	359	0.30 (0.04-2.19)
≥65	570	0.57 (0.27-1.19)	473	0.56 (0.21-1.46)	473	0.57 (0.22-1.48)

[a]Models were adjusted for age, sex, race, diabetes, cardiovascular disease, and current smoking. Models stratified on a factor (such as diabetes) were not adjusted for that factor. All strata are mutually exclusive, and none of the synergy indices (SIs) differs between strata. Even for the most extreme difference, both events in men vs women, $P = .07$ for SIs of 0 19 and 0.61. CI indicates confidence interval, NA, not estimatable.
†Synergy indices are unadjusted.

times longer than participants who were not taking diuretics at the index date.

In some but not all clinical studies, the adducin variant has been associated with prevalent hypertension or mean levels of blood pressure.[11-20] In this study of participants with pharmacologically treated hypertension, genotype was not associated with mean levels of blood pressure in either the cases or the controls (Table 3). Moreover, blood pressure level did not appear to be a mechanism of the adducin-diuretic interaction (Table 6). We do not know by what mechanism diuretic use may preferentially reduce the risk of MI or stroke in hypertensive patients with the adducin variant. Nonetheless, the effect of diuretics, which promote renal sodium excretion, is the opposite of the physiological effect of the adducin variant, which promotes renal sodium reabsorption.[10,21]

The phenotype of salt sensitivity, independent of blood pressure, has been associated with an increased risk of cardiovascular events.[23] Diuretic therapy in people with salt sensitivity and hypertension may decrease the incidence of cardiovascular events through mechanisms other than the direct lowering of blood pressure. In the Antihypertensive and Lipid-Lowering to prevent Heart Attack Trial,[38] systolic blood pressure was 2 to 3 mm Hg higher in those randomized to doxazosin than in those randomized to low-dose diuretics; yet the risk of heart failure was twice as high in the doxazosin arm as in the diuretic arm. Although the α-adducin variant appeared to be a good candidate gene (in part on the basis of blood pressure effects), blood pressure differences may not be a good surrogate for the effects of drugs on cardiovascular end points.[39]

Several monogenic forms of high blood pressure, such as glucocorticoid-remediable aldosteronism and Liddle syndrome,[6,40] are so rare that they do not contribute measurably to the burden of hypertensive disease in humans. Essential hypertension, generally mild-to-moderate elevations of blood pressure in the population, has been associated with several genetic polymorphisms.[6,7,41-43] This research has advanced our understanding of the biological and molecular etiologies of high blood pressure. Identifying the genes responsible for variation in or regulation of blood pressure may even provide new opportunities for the design of novel drugs.[44] Nonetheless, thousands of prescription medications are already on the market. In 1994, 2.2 million hospitalized persons experienced serious adverse drug reactions in the United States, and 106 000 had fatal adverse drug reactions.[45] Work in pharmacogenetics can perhaps also improve the safety and efficacy profile of commonly used medications. Drug-gene interactions for ACE inhibitors and the *ACE* gene have been reported, for instance, for renal outcomes in nondiabetic patients with nephropathy.[46]

The long-term goal of research in the area of pharmacogenetics is to help clinicians individualize treatment for their patients and select drug therapies that maximize either effectiveness, or safety, or both. If the adducin variant identifies a subset of hypertensive patients who are particularly likely to benefit from diuretic therapy, it is reasonable to evaluate whether screening hypertensive patients for selected genetic polymorphisms may be indicated when selecting antihypertensive therapies and perhaps even to inquire in future clinical trials whether diuretic therapy may reduce the risk of cardiovascular events in nonhypertensive carriers of the adducin variant. The findings of this study need to be confirmed in other settings, and randomized clinical trials of drug therapy for hypertension would be an ideal setting for case-only studies because drug use and genotype are, by design, independent.[32,33] If the adducin findings are confirmed, or if other drug-gene interactions are identified, clinicians may eventually screen hypertensive patients for selected genetic variants that help characterize an individual's expected risk or benefit from specific antihypertensive therapies for outcomes such as myocardial infarction, stroke, and heart failure.

Currently, low-dose diuretics together with β-blockers are recommended as the first-line pharmacological therapy for hypertension by the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.[47] The data in this study suggest that among carriers of the adducin wild-type genotype, diuretics are comparable with other antihypertensive medications; but among carriers of the adducin variant, diuretics are associated with a lower risk of MI and stroke than other antihypertensive agents. Regardless of genotype, in other words, diuretics are safe and effective in preventing devastating complications such as MI, stroke, and heart failure.[38,48] They remain the preferred first-line medication for the pharmacological treatment of high blood pressure.

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