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Original Contribution

Renin-Angiotensin System Haplotypes and the Risk of Myocardial Infarction and Stroke in Pharmacologically Treated Hypertensive Patients

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The products of the renin-angiotensin system (RAS) play an important role in the pathogenesis of cardiovascular disease. Studies examining RAS gene variants and cardiovascular disease have focused on single-nucleotide polymorphisms (SNPs) rather than haplotypes, which better characterize the patterns of genetic variation. The authors conducted a population-based, case-control study at Group Health (Seattle, Washington) between 1995 and 1999 to determine whether common haplotypes in the angiotensinogen gene (*AGT*), the renin gene, the angiotensin-converting enzyme gene, and the angiotensin II receptor type 1 and receptor type 2 genes were associated with the risk of myocardial infarction and stroke among pharmacologically treated hypertensive patients. SNP discovery was done using 23 European-origin samples. Thirty tagSNPs (the minimum sets of SNPs that capture most of the haplotype diversity within a block) were genotyped in cases and controls. Haplotypes were inferred using the program PHASE (http://www.stat.washington.edu/stephens/software.html). The authors used weighted logistic regression to estimate associations and conducted a permutation test to estimate the probability of a chance finding. *AGT* haplotype B was associated with the risk of myocardial infarction (odds ratio = 1.58, 95% confidence interval: 1.06, 2.35); however, results were not statistically significant given the number of tests performed (permutation $p = 0.17$). In this case-control study, RAS gene haplotypes were not significantly associated with increased risks of myocardial infarction or stroke.

cerebrovascular accident; genetics; hypertension; myocardial infarction

Abbreviations: CI, confidence interval; OR, odds ratio; RAS, renin-angiotensin system; SNP, single-nucleotide polymorphism.

The ''common disease/common variant'' hypothesis (1), which predicts that common, modest risk alleles will be responsible for common disease, makes the discovery and study of common variants in candidate genes of public health interest (2). One such set of candidate genes is that of the renin-angiotensin system (RAS), whose proteins, enzymes, and receptors play an important role in the pathogenesis of cardiovascular disease (3, 4). Inhibition of the

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RAS has antiatherogenic properties that have been demonstrated in animal models (3), and clinical trials show that the pharmacologic inhibition of the RAS with angiotensin-converting enzyme inhibitors and angiotensin II receptor type 1 antagonists decreases cardiovascular events (5–7). Some studies suggest that variants such as the Met235Thr single-nucleotide polymorphism (SNP) and the G-6A promoter SNP in the angiotensinogen gene (*AGT*) and the insertion-deletion variant in the angiotensin-converting enzyme gene (*ACE*) are associated with cardiovascular risk, but null findings have also been reported (8–14). Much of the research has focused on SNPs rather than haplotypes, which better characterize the common patterns of variation in a population (15).

We conducted a case-control study using highly informative SNPs to infer haplotypes to examine the association of common patterns of genetic variation in five RAS genes with the risk of cardiovascular disease. Specifically, we hypothesized that common haplotypes in the *AGT*, renin gene (*REN*), *ACE,* and angiotensin II receptor type 1 gene (*AGTR1*) and type 2 gene (*AGTR2*) would be associated with the risk of myocardial infarction or stroke in subjects with pharmacologically treated hypertension.

MATERIALS AND METHODS

Setting

The setting was Group Health, a health maintenance organization located in Seattle, Washington, with over 400,000 enrollees. The methods have been described previously (16–18). The study was approved by the human subjects committee at Group Health. Study subjects gave signed informed consent.

Identification of cases and controls

Cases were Group Health enrollees who had pharmacologically treated hypertension and survived an incident myocardial infarction or stroke from January 1995 through December 1999. Potential cases were identified from computerized discharge abstracts for the two Group Health hospitals and claims databases for non-Group Health facilities. Events criteria were adapted from the Cardiovascular Health Study for both myocardial infarction and stroke (19, 20). All strokes, both ischemic and hemorrhagic, were included. Controls were population based; that is, control subjects were selected from the same population from which the cases arose. Controls were a stratified random sample of Group Health enrollees with pharmacologically treated hypertension sampled from the Group Health computerized enrollment files on the basis of person-time, a procedure that ensures that the odds ratio approximates the relative risk (21). Controls were frequency matched to the myocardial infarction cases by decade of age, sex, and calendar year of identification at a ratio of at least 2:1 for men and at least 3:1 for women. The myocardial infarction cases were used to set matching targets because there were more myocardial infarction cases than stroke cases in each age/sex/index year stratum; thus, the selected controls were less well matched to the stroke cases. Controls met the same eligibility criteria as the cases, and they did not have either a previous myocardial infarction or a previous stroke. 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 myocardial infarction 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. All participants were 30–79 years of age at the index date, had at least four visits before the index date, and had pharmacologically treated hypertension on the index date. To be eligible, subjects had to be alive at the time of the study. Of the 2,008 eligible cases and controls who were asked to give a blood sample, 1,819 (91 percent) participated. The participation rates were 92 percent among controls, 88 percent for myocardial infarction cases, and 87 percent for stroke cases. An additional 205 (11 percent of the 1,819) subjects were excluded because of a prior myocardial infarction or stroke (12 percent controls, 8 percent myocardial infarctions, and 11 percent strokes), leaving 1,614 subjects to be included in the analysis.

Data collection

Data collection included a review of the Group Health outpatient medical record, a telephone interview, and a venous blood sample. The blood samples were obtained specifically for research. Study phlebotomists traveled to consenting participants' homes or other locations selected by the participant to draw their blood. Research assistants determined eligibility and collected information about risk factors for coronary heart disease from subjects' medical records for the period before the index date. The data collected included blood pressure and pulse; height and weight; cholesterol level, smoking status, family history, marital status, and use of health services; and medical conditions. Cardiovascular disease was defined as a history of angina, claudication, or vascular procedures.

Blood collection and laboratory assays

A blood specimen was drawn from the antecubital vein. Samples were drawn into tubes containing ethylendiaminetetraacetic acid, processed, and extracted using standard salting-out procedures (22). Investigators at the University of Washington Department of Genome Sciences used TaqMan (Roche Molecular Systems, Inc., Pleasanton, California) to assay the minimum sets of SNPs that capture most of the haplotype diversity within a block (termed ''tagSNPs'') in cases and controls. Researchers performing the genotyping were blinded to case-control status.

SNP discovery and tagSNP selection

SNP discovery was performed by the SeattleSNPs Program for Genomic Applications at the University of Washington (http://pga.mbt.washington.edu/). Each of the five RAS genes was sequenced in a panel of 46 European-American

TABLE 1. Single nucleotide polymorphism discovery and tagSNP* selection

Genes†	Region sequenced		Total SNPs discovered (no.)	Common SNPs (minor allele frequency ≥10%) (no.)	tagSNPs (no.)	Common SNPs marked‡ (no.)	Coverage (%)
	Upstream (base pairs)	Downstream (base pairs)					
AGT	2,790	830	84	57	8	57	100
REN	1,503	1,122	46	21	5	19	90
ACE	2,355	700	43	27	2	27	100
AGTR1	2,674	1,191	129	76	12	74	97
AGTR2	1,852	1,085	10	8	3	8	100

* tagSNP, minimum set of single-nucleotide polymorphisms (SNPs) that captures most of the haplotype diversity within a block.

† *AGT*, angiotensinogen; *REN*, renin; *ACE*, angiotensin-converting enzyme; *AGTR1*, angiotensin II receptor type 1; *AGTR2*, angiotensin II receptor type 2.

‡ The number of SNPs with a minor allele frequency of 10% or greater that were highly correlated ($r^2 \geq 0.64$) with or "marked" by the selected tagSNP.

chromosomes purchased from the Coriell Cell Repository (Camden, New Jersey). Sequencing included the whole gene (introns and exons) and approximately 2 kilobases upstream and 1 kilobase downstream (table 1). Only SNPs with a minor allele frequency greater than or equal to 10 percent were considered in the analysis. The LDselect program (23) was used to select a set of tagSNPs that were genotyped in the entire sample.

Statistical analysis

Haplotypes were inferred from the tagSNPs by use of the program PHASE 2.0 (24). Because of the uncertainty inherent in the estimation of haplotypes from unphased genotype data in unrelated individuals, cases and controls could have more than one possible haplotype pair, and the probability associated with each haplotype pair was used as a weight in the analysis. Logistic regression models, adjusted for the matching variables, were thus clustered on the case-control identification number to account for correlation within cases and controls who had multiple possible haplotype pairs (25). We analyzed the X-linked *AGTR2* separately for males and females. We assumed an additive model on the log scale for haplotype analyses and coded subjects as having zero, one, or two copies of each haplotype. Haplotypes observed at a frequency of less than 2.5 percent were grouped into a single category (26). The most common haplotype was used as the reference group, and the score test was used for global tests of association (26). The power to detect an odds ratio of 1.50 (our definition of ''modest'' risk) for a haplotype with a frequency of 20 percent was 82 percent with 349 myocardial infarction cases and 1,063 controls and 64 percent with 202 stroke cases and 1,063 controls. All statistical tests were two tailed. We made no statistical correction for testing hypotheses for five genes and two phenotypes. However, we did perform a permutation analysis, randomly permuting case-control status 5,000 times, to further evaluate our findings. In secondary analyses, we examined the association between the events and the following: individual SNPs; haplotypes adjusted for history of cardiovascular disease, diabetes, race, systolic blood pressure, antihypertensive therapy, total cholesterol, smoking status, and the matching variables; haplotypes in subgroups defined by race (Caucasians only vs. non-Caucasians), age, sex, diabetes, and history of cardiovascular disease; and haplotypes modeled dominantly or recessively. We also examined the association of haplotypes and stroke within each stroke subtype. We report any findings that differed from the primary analysis. Statistical analyses were performed using STATA, version 8.2, software (StataCorp LP, College Station, Texas).

RESULTS

Table 2 summarizes the characteristics of the 349 myocardial infarction cases, 202 stroke cases, and 1,063 controls. Cases and controls differed in a predictable manner. For instance, diabetes, high systolic blood pressure, and high total cholesterol were risk factors for myocardial infarction.

The *AGT* gene

Table 3 displays the results for the *AGT* gene. *AGT* haplotype B, which included the minor allele of the tagSNP rs5051 (rs5051 is the G-6A polymorphism; it is in complete linkage disequilibrium with the Met235Thr polymorphism), was associated with an increased risk of myocardial infarction (odds ratio (OR) = 1.58, 95 percent confidence interval (CI): 1.06, 2.35), and the global test of haplotype association was significant ($p = 0.018$). Haplotypes A, C, D, and E, which also included the minor allele of rs5051, however, were not associated with myocardial infarction risk (haplotype D also included the minor allele of the tagSNP marking the Thr174Met polymorphism). In subgroup analyses, females with an extra copy of haplotype E had a higher risk of myocardial infarction compared with females with an extra copy of haplotype G (OR = 1.73, 95 percent CI: 1.20, 2.49) (global test $p = 0.007$). Two *AGT* SNPs,

TABLE 2. Characteristics of Group Health-treated hypertensive myocardial infarction cases, stroke cases, and controls, Seattle, Washington, 1995 and 1999

Characteristic*	Controls ($n = 1,063$)	Cases	
		Myocardial infarction ($n = 349$)	Stroke ($n = 202$)
Age (years)	64.1 (10.3)	64.5 (10.5)	68.0 (8.9)
Sex (% male)	53.5	60.2	42.1
Race (% Caucasian)	91.1	89.7	91.6
Current smoking (%)	11.1	16.3	15.3
Years in Group Health Cooperative	21.6 (10.6)	18.4 (12.2)	20.6 (13.0)
No. of visits in year prior to index	5.8 (5.0)	6.9 (6.2)	7.1 (5.9)
Diabetes (%)	14.1	24.4	23.3
Body mass index (kg/m^2)	30.3 (6.3)	30.4 (6.0)	30.0 (6.5)
History of congestive heart failure (%)	3.0	6.3	5.9
History of cardiovascular disease (%)†	12.6	26.1	13.9
Duration of treated hypertension (years)	10.1 (7.7)	8.9 (7.3)	10.6 (8.4)
No. of antihypertensive medications	1.3 (0.8)	1.3 (0.8)	1.3 (0.9)
Current diuretic user (%)	42.6	36.1	39.6
Current beta blocker user (%)	29.8	28.9	32.2
Current angiotensin-converting enzyme-inhibitor user (%)	29.9	28.4	29.7
Current calcium channel blocker user (%)	21.4	25.5	21.3
Current vasodilator user (%)	5.6	5.4	7.9
Most recent systolic blood pressure (mmHg)	141.5 (18.5)	144.0 (19.9)	150.1 (22.5)
Most recent diastolic blood pressure (mmHg)	82.5 (10.7)	82.5 (11.16)	83.9 (12.2)
Untreated systolic blood pressure (mmHg)	161.0 (19.4)	165.7 (20.8)	167.9 (22.1)
Untreated diastolic blood pressure (mmHg)	98.9 (10.1)	99.3 (11.9)	100.0 (11.8)
Total cholesterol (mmol/liter)	5.7 (1.1)	6.0 (1.2)	6.0 (1.2)
High density lipoprotein cholesterol (mmol/liter)	1.3 (0.4)	1.1 (0.3)	1.3 (0.4)

* Values are expressed as means (standard deviation) unless otherwise indicated.

† History of angina, claudication, or vascular procedures, including coronary bypass, angioplasty, carotid endarterectomy, or peripheral vascular bypass.

rs4762 and rs2493132, violated the Hardy-Weinberg equilibrium assumption among controls. No violations were noted when these analyses were limited to Caucasian control patients. Individually, no *AGT* tagSNP was associated with the risk of myocardial infarction or stroke (Web table 1). (This information is described in the first of 10 supplementary tables; each is referred to as ''Web table'' in the text and is posted on the *Journal*'s website (http://aje.oxfordjournals.org/).). Results of the other exploratory analyses, including the analysis limited to Caucasian patients, the analysis excluding patients with a history of cardiovascular disease, and the fully adjusted analysis (Web table 2), were similar to those of the main analysis.

The *REN* gene

Table 4 displays the results for the *REN* gene. None of the six common *REN* haplotypes was associated with the risk of myocardial infarction. An extra copy of *REN* haplotype A increased the risk of stroke (OR = 1.78, 95 percent CI: 1.01, 3.13) relative to an additional copy of *REN* haplotype D, although globally, there was not a haplotype association ($p = 0.058$). In subgroup analyses, an extra copy of *REN* haplotype E more than doubled the risk of stroke relative to haplotype D in subjects younger than 65 years (OR = 2.40, 95 percent CI: 1.34, 4.28) (global test $p = 0.007$). In other exploratory analyses, including the analysis limited to Caucasian patients, the analysis excluding patients with a history of cardiovascular disease, the SNP analysis (Web table 3), and the fully adjusted haplotype analysis (Web table 4), results were similar to those of the main analysis. Two *REN* SNPS, rs11571078 and rs6676670, violated the Hardy-Weinberg equilibrium assumption among controls. No violations were noted when these analyses were limited to Caucasian control patients.

The *ACE* gene

Table 5 displays the results for the *ACE* gene. None of the three common *ACE* haplotypes was associated with the risk of myocardial infarction. An extra copy of haplotype A, which included the *ACE* deletion variant, decreased the risk

TABLE 3. Angiotensinogen gene haplotypes and risk of myocardial infarction and stroke among Group Health-treated hypertensive patients, adjusted for age, sex, and index year, Seattle, Washington, 1995–1999

	Haplotypes*								Haplotype frequency (%)			Myocardial infarction		Stroke	
	rs5051†	rs3789679	rs2004776	rs4762‡	rs3789670	rs2493132	rs2478523	rs7079	Controls ($2n = 2,126$)	Myocardial infarction cases ($2n = 698$)	Stroke cases ($2n = 404$)	Odds ratio§	95% confidence interval	Odds ratio§	95% confidence interval
A	A	C	A	C	A	A	C	C	14	12	14	0.90	0.67, 1.22	1.01	0.72, 1.43
B	A	C	A	C	G	G	C	C	4	6	5	1.58	1.06, 2.35	1.24	0.75, 2.05
C	A	C	G	C	G	G	C	C	6	4	6	0.74	0.49, 1.12	0.97	0.62, 1.50
D	A	C	G	T	G	G	C	C	11	10	10	0.87	0.64, 1.18	0.82	0.55, 1.22
E	A	T	A	C	G	G	T	C	8	9	9	1.01	0.72, 1.41	1.00	0.67, 1.48
F	G	C	G	C	G	A	T	C	22	27	18	1.23	0.97, 1.56	0.77	0.56, 1.05
G	G	C	G	C	G	A	T	A	29	28	31	Referent		Referent	
Rare									5	4	6	0.81	0.53, 1.24	1.12	0.70, 1.81
Global test¶												$p = 0.018$		$p = 0.557$	

* The underscored letters indicate the minor alleles for each single-nucleotide polymorphism.
† rs5051 is the G-6A polymorphism. It is in complete linkage disequilibrium with the Met235Thr polymorphism.
‡ rs4762 is the Thr174Met polymorphism.
§ Multiple-haplotype model. Odds ratios for an additional copy of specified haplotype versus an additional copy of haplotype G adjusted for the other haplotypes.
¶ Score test for multiple haplotypes.

TABLE 4. Renin gene haplotypes and risk of myocardial infarction and stroke among Group Health-treated hypertensive patients, adjusted for age, sex, and index year, Seattle, Washington, 1995–1999

	Haplotypes*						Haplotype frequency (%)			Myocardial infarction		Stroke	
	rs11571078	rs6676670	rs10900555	rs5705	rs2272237	rs2368564	Controls ($2n = 2,126$)	Myocardial infarction cases ($2n = 698$)	Stroke cases ($2n = 404$)	Odds ratio†	95% confidence interval	Odds ratio†	95% confidence interval
A	C	C	C	A	G	G	3	3	4	1.14	0.69, 1.90	1.78	1.01, 3.13
B	C	C	C	C	C	A	12	12	11	0.97	0.73, 1.30	1.01	0.69, 1.47
C	C	C	T	A	C	G	18	18	18	0.95	0.75, 1.21	1.02	0.76, 1.38
D	C	C	T	A	G	G	32	33	31	Referent		Referent	
E	C	A	C	A	C	G	18	18	22	1.01	0.79, 1.30	1.30	0.97, 1.75
F	T	C	T	A	C	A	13	13	8	0.98	0.74, 1.30	0.68	0.44, 1.05
Rare							4	3	5	0.81	0.52, 1.27	1.22	0.73, 2.03
Global test‡										$p = 0.969$		$p = 0.058$	

* The underscored letters indicate the minor alleles for each single-nucleotide polymorphism.
† Multiple-haplotype model. Odds ratios for an additional copy of specified haplotype versus an additional copy of haplotype D adjusted for the other haplotypes.
‡ Score test for multiple haplotypes.

TABLE 5. Angiotensin-converting enzyme gene haplotypes and risk of myocardial infarction and stroke among Group Health-treated hypertensive patients, adjusted for age, sex, and index year, Seattle, Washington, 1995–1999

Haplotypes*				Haplotype frequency (%)			Myocardial infarction		Stroke	
	rs4295	rs4330	rs4362†	Controls ($2n = 2{,}126$)	Myocardial infarction cases ($2n = 698$)	Stroke cases ($2n = 404$)	Odds ratio‡	95% confidence interval	Odds ratio‡	95% confidence interval
A	G	A	T	16	16	12	0.99	0.76, 1.28	0.64	0.45, 0.90
B	G	C	C	42	44	47	Referent		Referent	
C	C	A	T	38	36	37	0.93	0.77, 1.13	0.88	0.70, 1.11
Rare				4	4	4	1.05	0.68, 1.62	1.09	0.63, 1.89
Global test§							$p = 0.886$		$p = 0.069$	

* The underscored letters indicate the minor alleles for each single-nucleotide polymorphism.
† rs4362 marks the angiotensin-converting enzyme gene (*ACE*) insertion-deletion variant.
‡ Multiple-haplotype model. Odds ratios for an additional copy of specified haplotype versus an additional copy of haplotype B adjusted for the other haplotypes.
§ Score test for multiple haplotypes.

of stroke by 30 percent relative to an extra copy of haplotype B, which included the insertion (OR = 0.64, 95 percent CI: 0.45, 0.90), but globally there was not a haplotype association ($p = 0.069$). In exploratory analyses, including the analysis limited to Caucasian patients, the analysis excluding patients with a history of cardiovascular disease, the SNP analysis (Web table 5), and the fully adjusted haplotype analysis (Web table 6), results were similar to those of the main analysis.

The *AGTR1* gene

Table 6 displays the results for the *AGTR1* gene. An extra copy of haplotype G increased the risk of stroke relative to an extra copy of haplotype C (OR = 1.62, 95 percent CI: 1.10, 2.37), but globally there was not a haplotype association ($p = 0.059$). We observed no associations between *AGTR1* haplotypes and myocardial infarction risk. In subgroup analyses, patients with a history of cardiovascular disease and an extra copy of haplotype E had a risk of myocardial infarction more than three times higher than patients with an extra copy of haplotype C (OR = 3.65, 95 percent CI: 1.23, 10.79) (global test $p = 0.023$). In the SNP analysis (Web table 7), the fully adjusted haplotype analysis (Web table 8), the analysis limited to Caucasian patients, and the analysis excluding patients with a history of cardiovascular disease, results were similar to those of the main analysis.

The *AGTR2* gene

Table 7 displays the results for the *AGTR2* gene. None of the *AGTR2* haplotypes was associated with the risk of myocardial infarction or stroke in men or women. In the subgroup analyses, the risk of stroke was lower for women with cardiovascular disease and an extra copy of haplotype A or C relative to those with an extra copy of haplotype D (OR = 0.01, 95 percent CI: 0.00, 0.29, and OR = 0.03, 95 percent CI: 0.00, 0.44, respectively) (global test $p = 0.023$). Non-White men with an extra copy of haplotype C had a higher risk of myocardial infarction than did non-White men with an extra copy of haplotype D (OR = 6.88, 95 percent CI: 1.48, 31.88) (global test $p = 0.028$). In the SNP analysis (Web table 9), the fully adjusted haplotype analysis (Web table 10), the analysis limited to Caucasian patients, and the analysis excluding patients with a history of cardiovascular disease, results were similar to those of the main analysis.

Permutation analysis

We performed a permutation analysis to determine the probability of observing a p value of 0.018 or less if there were no genetic effects. Randomly permuting case-control status, we obtained a permutation corrected p value of 0.165, suggesting that the findings should not be considered significant (at the 0.05 threshold) given the number of associations tested.

DISCUSSION

In this case-control study of pharmacologically treated hypertensive patients, we observed an uncorrected global association between the *AGT* haplotypes and myocardial infarction risk ($p = 0.018$). In particular, *AGT* haplotype B, which included the minor allele of the tagSNP marking the Met235Thr and G-6A polymorphisms, was associated with an increased risk of myocardial infarction (OR = 1.58, 95 percent CI: 1.06, 2.35). We also observed global trends between stroke and haplotypes in the *REN* ($p = 0.058$), *ACE* ($p = 0.069$), and *AGTR1* ($p = 0.059$) genes. The direction of the point estimates was generally similar for both myocardial infarction and stroke, and confidence intervals surrounding the haplotype-myocardial infarction and haplotype-stroke point estimates overlapped. Results of the permutation test, which estimated the probability of a finding due to chance alone, suggest that none of these results should be considered statistically significant.

TABLE 6. Angiotensin II receptor type 1 gene haplotypes and risk of myocardial infarction and stroke among Group Health-treated hypertensive patients, adjusted for age, sex, and index year, Seattle, Washington, 1995–1999

	Haplotypes*												Haplotype frequency (%)			Myocardial infarction		Stroke	
	rs275651	rs1492078	rs3772633	rs12721279	rs3772630	rs4681442	rs1492099	rs2675511	rs389566	rs2638355	rs1800766	rs5182	Controls ($2n = 2,126$)	Myocardial infarction cases ($2n = 698$)	Stroke cases ($2n = 404$)	Odds ratio†	95% confidence interval	Odds ratio†	95% confidence interval
A	A	T	T	A	T	G	C	A	T	C	T	C	3	2	4	0.68	0.40, 1.15	1.41	0.80, 2.51
B	A	T	T	A	T	G	T	G	A	T	C	C	5	5	6	0.88	0.59, 1.31	1.29	0.80, 2.08
C	T	C	T	G	T	G	C	A	T	C	T	C	22	25	19	Referent		Referent	
D	T	C	T	G	T	G	C	A	T	C	T	T	19	20	21	0.97	0.77, 1.22	1.36	0.99, 1.87
E	T	C	T	G	T	G	C	A	A	C	T	T	3	2	1	0.70	0.41, 1.17	0.60	0.28, 1.28
F	T	C	C	A	C	G	C	A	T	C	T	C	3	2	2	0.85	0.54, 1.34	0.83	0.43, 1.60
G	T	C	C	A	C	G	C	A	T	C	T	T	9	10	13	1.02	0.75, 1.40	1.62	1.10, 2.37
H	T	T	T	A	C	A	C	A	T	C	T	C	4	3	3	0.69	0.46, 1.06	0.86	0.50, 1.48
I	T	T	T	A	C	A	C	A	T	C	T	T	3	3	4	0.84	0.54, 1.31	1.60	0.92, 2.78
Rare													29	27	29	0.86	0.69, 1.08	1.20	0.90, 1.60
Global test‡																$p = 0.560$		$p = 0.059$	

* The underscored letters indicate the minor alleles for each single-nucleotide polymorphism.
† Multiple-haplotype model. Odds ratios for an additional copy of specified haplotype versus an additional copy of haplotype C adjusted for the other haplotypes.
‡ Score test for multiple haplotypes.

The study had a number of strengths. Discovery was based on sequencing the whole gene including the regions approximately 2 kilobases upstream and 1 kilobase downstream of transcription, and the proportion of the common variants that were captured by the tagSNP approach was high (90–100 percent). We studied common RAS haplotypes rather than focusing on individual SNPs and, thus, were able to characterize the association of events with multiple variants across a gene acting in combination (27). The study design was population based, and data collection was thorough.

Our results are compatible with those of other investigators in that they provide little support for the common variant/common disease hypothesis with respect to RAS polymorphisms and cardiovascular disease. Some studies have reported associations between common RAS polymorphisms and cardiovascular disease, but null findings are also prevalent. The deletion variant of the *ACE* insertion-deletion variant, which is the most widely studied polymorphism of all the RAS variants, has been associated with an increased risk of myocardial infarction in some, but not all, studies. A meta-analysis of *ACE* insertion-deletion association studies, which found an association of the DD genotype with myocardial infarction risk (OR = 1.21, 95 percent CI: 1.11, 1.32) relative to the II or ID genotype, also showed that the association was nullified when small studies were excluded (OR = 0.99, 95 percent CI: 0.88, 1.12) (8, 28), and another recent study showed that the *ACE* insertion-deletion polymorphism did not modify antihypertensive response (29). A meta-analysis of studies examining the association between the *ACE* insertion-deletion polymorphism and ischemic cerebrovascular disease showed that the DD allele was associated with an increased stroke risk (OR = 1.18, 95 percent CI: 1.01, 1.37), but again, most of the positive results were observed in small studies (8). Divergent results have also been reported for polymorphisms in the other RAS genes. Both positive and null results have been reported for the association between the *AGTR1* A1166C variant and cardiovascular disease (11, 12, 30–38) and the association between the *AGT* Thr174Met variant and cardiovascular disease (9, 37, 39, 40). Variants in the *AGTR2* and *REN* genes have been less well studied with respect to cardiovascular risk (41, 42).

Several studies have suggested an association between the *AGT* Met235Thr variant, which is in complete linkage disequilibrium with the G-6A promoter variant, and cardiovascular disease (23–28), but a recent meta-analysis of 63 studies of Met235Thr showed that the polymorphism was associated with circulating *AGT* levels and hypertension risk but not with ischemic heart disease or myocardial infarction risk (43). In our study, these SNPs were not associated with myocardial infarction or stroke; their effects in the haplotype analysis varied. The minor allele of rs5051 (the G-6A promoter polymorphism which marked the Met235Thr polymorphism) was present in haplotype B, which had an odds ratio of 1.58 for myocardial infarction, but it was also present in haplotypes with odds ratios close to unity. This suggests that, if there really is an association between the *AGT* gene and myocardial infarction risk, haplotypes, as opposed to SNPs, may be important.

TABLE 7. Angiotensin II receptor type 2 gene haplotypes and risk of myocardial infarction and stroke among Group Health-treated hypertensive patients, adjusted for age, sex, and index year, Seattle, Washington, 1995–1999

Haplotypes*				Haplotype frequency (%)†			Myocardial infarction		Stroke	
	rs5193	rs11091046	rs12845035	Controls	Myocardial infarction cases	Stroke cases	Odds ratio‡	95% confidence interval	Odds ratio‡	95% confidence interval
Females										
A	T	C	G	24	28	23	0.98	0.50, 1.94	0.91	0.42, 1.93
B	G	A	G	23	20	26	0.56	0.25, 1.24	1.22	0.54, 2.72
C	G	A	C	25	23	23	0.63	0.31, 1.31	0.77	0.35, 1.68
D	G	C	G	27	30	27	Referent		Referent	
Global test§							$p = 0.339$		$p = 0.697$	
Males										
A	T	C	G	24	24	17	1.18	0.75, 1.85	0.73	0.36, 1.46
B	G	A	G	23	23	26	1.18	0.75, 1.87	1.29	0.68, 2.46
C	G	A	C	26	30	30	1.31	0.84, 2.03	1.15	0.63, 2.10
D	G	C	G	27	23	27	Referent		Referent	
Global test§							$p = 0.691$		$p = 0.430$	

* The underscored letters indicate the minor alleles for each single-nucleotide polymorphism.

† For females, $2n = 986$, 278, and 234 for controls, myocardial infarction cases, and stroke cases, respectively; for males, $n = 568$, 210, and 85 for controls, myocardial infarction cases, and stroke cases, respectively.

‡ Multiple-haplotype model. Odds ratios for an additional copy of specified haplotype versus an additional copy of haplotype D adjusted for the other haplotypes.

§ Score test for multiple haplotypes.

This study had a number of limitations. Potential alternative explanations for the findings of genetic association studies include uncontrolled confounding from unmeasured ethnic-racial differences between cases and controls and false positives. We focused on common haplotypes and could have missed a genetic mechanism that was due to a number of rare mutations in a specific location. That the observed associations were between the more rare haplotypes and events diminishes the importance of our findings. Our study population was limited to treated hypertensive patients with insurance who had visited their providers at least four times. Results may generalize poorly to other populations.

In a population of pharmacologically treated, hypertensive patients, we observed no statistically significant associations between RAS gene haplotypes and myocardial infarction or stroke risk. We observed a few associations in subgroup analyses. However, because of the small sample size of the subgroups, the multiple tests conducted, and the lack of a priori hypotheses, subgroup findings should be considered hypothesis generating. This study provides little support for the common variant/common disease hypothesis with respect to RAS polymorphisms and cardiovascular disease.

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