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Genetic Variation in ACE-related pathways associated with Sudden Cardiac Arrest Risk

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Abstract

Background—Angiotensin converting enzyme (ACE)-related pathways influence arrhythmias and sudden cardiac arrest (SCA) risk.

Objective—We investigated whether genetic variation in ACE-related pathways are associated with SCA risk. Because these pathways are sex-dependent and influenced by estrogen, we examined these genotype-SCA associations in the full study population, and tested for interaction with gender.

Methods—In a population-based case-control study set in King County WA, we genotyped 211 SCA cases (mean age 59, 80% male) and 730 age- and gender-matched controls of European descent for 47 single nucleotide polymorphisms (SNPs) in eight genes (ACE, AGT, REN, AGTR1, AGTR2, ACE2, KNG1, BDKRB2). We examined association of SNPs and haplotypes with SCA risk using logistic regression.

Results—AGTR1 SNP rs1492099 (allele frequency=15%) was associated with decreased SCA risk (OR=0.62, 95%CI=0.4–0.9). Haplotype variation in AGTR2 was associated with SCA risk (global haplotype test p=0.001), with haplotype 2 (allele frequency=27%) associated with increased risk (OR=1.26, 95%CI=1.1–1.5). There was interaction with gender on SCA risk for variation in KNG1 (interaction p-value range=0.0004–0.017 for 6/8 SNPs). KNG1 SNP rs710448 (allele frequency=42%) was associated with decreased risk (OR=0.44, 95%CI=0.3–0.8) among women but not men. Other SNPs and haplotypes in the eight genes examined were not associated with SCA risk after multiple testing correction.

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Conclusions—Variation in AGTR1 and AGTR2 are associated with SCA risk in a population-based case-control study. There was evidence of interaction with gender on SCA risk for variation in KNG1. Our findings, if replicated, suggest that variation in genes in ACE-related pathways influence SCA risk.

Keywords

sudden death; cardiac arrest; epidemiology; genetics; polymorphism; renin-angiotensin system

Introduction

Sudden cardiac arrest (SCA) accounts for over 300,000 deaths in the United States each year.[1] Although the majority of SCA events in the general population are due to underlying coronary artery disease, SCA frequently presents as the first and only manifestation of previously unrecognized ischemic heart disease. A family history of SCA is associated with a doubled SCA risk, suggesting a genetic susceptibility.2

The classic renin-angiotensin system (RAS), as well as two other intimately related systems where the angiotensin converting enzyme (ACE) plays a central role – kallikrein-kininogen and angiotensin converting enzyme 2 (ACE2) pathways – influence arrhythmias and sudden cardiac arrest risk. In animal models, the end products of all three pathways influence ischemia-reperfusion injury arrhythmias. [3–6] In population studies, higher renin levels are associated with increased risk of cardiovascular disease mortality.7 Furthermore, in randomized clinical trials, blocking these pathways with ACE inhibitors, angiotensin receptor blockers, or aldosterone inhibitors decreases risk of SCA.[8–10] Although, basic, clinical, and epidemiologic studies suggest that ACE-related pathways influence SCA risk, whether genetic variation in genes in these pathways is associated with SCA risk in humans has not previously been explored.

We examined variation in eight genes in these three ACE-related pathways for association with SCA risk in a population-based case-control study of SCA. Two of these genes are on the X-chromosome, and components of these pathways are sex-dependent and influenced by estrogen.[11] We, therefore, examined these associations in the full study population, and then tested for an interaction with gender.

Methods

Study Subjects

The Cardiac Arrest Blood Study (CABS) is a population-based case-control study of the determinants of cardiac arrest in the community. Cases were out-of-hospital SCA patients attended by paramedics in Seattle and suburban King County, Washington, between October 1988 and September 2005. We defined SCA as a sudden pulseless condition in an otherwise stable individual presumed due to a malignant cardiac arrhythmia in the absence of evidence of a non-cardiac cause of cardiac arrest. Cases, aged 25–74, were identified prospectively by emergency medical service (EMS) personnel. In addition to EMS incident reports, we reviewed information provided by spousal interview, death certificates, medical examiner reports, and autopsy reports when available, to exclude patients with evidence of a non-cardiac condition as cause of cardiac arrest. Sixty-six percent of the SCA events were witnessed. Because a primary aim of the initial CABS study focused on dietary fatty acids, cases were restricted to those without clinically-recognized heart disease to minimize the possibility of bias from changing diet due to the knowledge of presence of heart disease. We excluded nursing home residents to avoid misclassification as to etiology of death. Furthermore, cases were restricted to married individuals, in order to obtain spousal

information on the cases' risk factors and comorbidities. The spouses of approximately 85 percent of eligible case patients agreed to participate. Twenty-one percent of cases survived to hospital discharge.

Control subjects, matched to cases on age and sex with the same eligibility criteria, were randomly selected from the community by random-digit dialing. The spouses of approximately 60% of controls subjects agreed to participate. Spouses completed a questionnaire on comorbidities and cardiac risk factors for case and control subjects. Details of the CABS recruitment experience are described elsewhere.[12]

The current study of genetic variants is limited to those of European descent, as there were too few participants of other ancestry to perform meaningful analyses. In total, 218 case and 740 control European-American subjects were available for this study, of whom 211 cases and 730 controls were successfully genotyped (see below).

The University of Washington Human Subject Review Committee approved the study protocol and all study subjects or their proxy signed an informed consent.

Gene Selection

Figure 1 describes the ACE-related pathways. In the classic RAS system, angiotensinogen (AGT) is cleaved to angiotensin I (AngI) by renin (REN). AngI is then cleaved by ACE to angiotensin II (AngII), which exerts its effect through binding angiotensin receptor types I (AGTR1) and II (AGTR2).13 In the ACE2 pathway, AngI is converted to angiotensin I-9 (AngI-9) by ACE2. AngI-9 is then converted to angiotensin I-7 (AngI-7) by ACE.14 Similarly, AngII is hydrolyzed to AngI-7 by ACE2. In a parallel system, kallikrein converts kininogen (KNG1) to bradykinin (Figure 1). Bradykinin acts on the bradykinin receptors including bradykinin receptor type 2 (BDRK2) to exert its effects.[15] Bradykinins are inactivated by ACE. We selected eight genes (AGT, REN, ACE, AGTR1, AGTR2, ACE2, KNG1, and BDRK2) in these three ACE-related pathways to examine for association with SCA risk.

SNP Selection

Common variants of the RAS genes were identified based on resequencing data from SeattleSNPs (http://pga.gs.washington.edu). DNA from 46 European-American chromosomes was analyzed. The LDSelect algorithm (University of Washington, http://pga.gs.washington.edu) and visual haplotype methods were used to identify tag single nucleotide polymorphisms (SNPs) to describe genetic variation with an $r^2>0.80$ with MAF>10%.[16] TagSNPs are a set of maximally informative SNPs identified by assessing the correlation between SNPs, grouping them into bins according to the specified correlation cutoff, and selecting one tagSNP from each group for genotyping. This method allows for complete coverage of a gene while reducing the number of SNPs that must be assayed. SNPs of particular interest based on literature reviews were also included. Details of the SNPs selected and genotyped, including their genomic context, MAF, etc, can be found in Supplemental Table.

Blood collection and genotyping

Paramedics obtained blood specimens from the cases in the field after essential emergency medical care had been provided and either the patient was clinically stable, or resuscitation had proven ineffective. Blood specimens from controls were obtained at the time of the interview. Blood was collected in tubes containing EDTA, and DNA was extracted from white blood cells using standard phenol extraction procedures. Genotyping was performed by SeattleSNPs through a Genotyping for Young Investigators award. Genotyping was

performed using BeadArray technology with a GoldenGate custom panel (Illumina, San Diego, CA). 55 SNPs were genotyped among 218 cases and 740 controls. Seven case subjects and 10 control subjects failed genotyping. Hence, 211 cases and 730 controls were included in the analyses. Six of the 55 SNPs failed genotyping. The remaining 49 SNPs had 99.9% of nucleotide pairs successfully called. Two pairs of SNPs were in strong linkage disequilibrium ($r^2>0.85$) and therefore one SNP from each pair was dropped from the analyses. All staff and laboratory personnel were blinded to case–control status.

Statistical methods

Statistical analyses were carried out using STATA 8.2. We compared the distribution of risk factors among cases and controls using t-test for continuous variables and χ^2 test for categorical variables.

Hardy-Weinberg equilibrium was assessed among all control participants (autosomal genes) and among female control participants (X-linked genes) by chi-squared test. Hardy-Weinberg exact p-value was deemed significant if $p<0.01$. We examined the degree of linkage disequilibrium (LD) between the variants using $r^2=D^2/p_1p_2q_1q_2$.

Genotype-SCA association was assessed using logistic regression to obtain odds ratios adjusted for the sampling variables of age, sex, and event year. Statistical significance was assessed using the likelihood ratio test. An additive (codominant) genetic model was used for all autosomal analyses. For X-linked genes, similarly an additive model was used. Male subjects have a single copy of X-linked genes. Males were assumed to be the equivalent of homozygous females, and were coded 0 (wild-type) or 2 (variant). Female subjects have two copies of X-linked genes but undergo X-inactivation. Therefore, they similarly manifest only one copy of the gene. For heterozygous female participants, X-inactivation was assumed to be random. Females were therefore coded 0 (homozygous wild-type), 1 (heterozygotes), or 2 (homozygous variant).

All analyses were first performed in the full study sample and then we looked for interaction between gender and genetic variation on the outcome of SCA. Using a likelihood ratio test, we formally tested for an interaction using nested models with and without the interaction term. Where an interaction was found, we present results stratified by gender.

Each SNP in each gene was analyzed separately. For genes where multiple SNPs were associated with SCA risk, analyses were performed including two SNPs in the model (the SNP being examined and the SNP with the most significant p-value) in order to assess whether the SNP being examined contributes to risk beyond the association of the variant with the most extreme p-value.

Permutation tests were used to adjust for multiple tests of SNPs within a gene. We randomly permuted case-control status 10,000 times to determine the likelihood that our findings were due to multiple comparisons.

Haplotypes and their frequencies were estimated using the program PHASE (version 2.0; UW, Seattle; http://stat.washington.edu/stephens/software.html). Haplotypes observed at a frequency of less than 2 percent were grouped into a single category. For the analyses among women where there were fewer cases, haplotypes with 5 or fewer observed cases were grouped into a single category. A global Wald test of all haplotype terms assessed the null hypothesis of no association between a gene's haplotypes and the outcome. The most common haplotype was arbitrarily selected as the reference group for the global Wald test. The Huber/White/sandwich estimator of variance was used in place of the traditional calculation in order to specify that the observations are independent across individuals but

not necessarily within individuals. The number of copies of a given haplotype (0, 1 or 2) was entered as a novel risk factor into the weighted logistic regression model, where individual's SCA outcome was modeled as a function of inferred haplotype pairs, weighted by their estimated probabilities to allow for haplotype uncertainty. For the haplotype analysis, to obtain an estimate of relative risk, each haplotype was modeled separately.

Statement of Responsibility

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

The study included 211 cases of SCA without previously diagnosed heart disease and 730 age- and gender-matched controls. The average age of the cases was 59 years with 80% being male (Table 1). Given the sampling, mean age and sex distribution were similar in cases and controls. All subjects were European American. Traditional cardiovascular risk factors including current smoking, diabetes, hypertension, and family history of myocardial infarction or SCA, were more prevalent among cases than controls (Table 1). All variants included in the analyses were in Hardy-Weinberg equilibrium (Supplementary Table).

We assessed for association with SCA using both SNP and haplotype analyses. Table 2 lists (1) genes examined, (2) the number of SNPs examined per gene, (3) the number of SNPs identified with raw p-values less than 0.05, (4) the likelihood that a SNP with the lowest p-value would be identified by chance using permutation, (5) a global p-value for haplotype analyses, and (6) the likelihood that a genotype-gender interaction identified was due to chance using permutation. Of the eight genes examined, using either SNP or haplotype analyses, variation in two (AGTR1 and AGTR2) showed an association with SCA risk among both men and women, and variation in KNG1 was associated with SCA risk among women (Table 2).

AGTR1

Three of the eleven SNPs examined in AGTR1 were associated with SCA risk (Table 3). SNP rs1492099 (minor allele frequency (MAF)=15%) was associated with 38% decreased risk of SCA per copy of minor allele (odds ratio (OR)=0.62, 95% confidence-interval (CI)=0.4–0.9). The other two SNPs (rs2933249 and rs1800766) were in moderate LD with this variant (r^2=0.26–0.65). Including rs1492099 in the model with the other SNPs individually attenuated their association with SCA risk (Table 3) while the association with rs1492099 was minimally altered. After correction for multiple tests using a permutation test, rs1492099 remained significantly associated with SCA risk (p=0.044).

Given the large number of variants in AGTR1, 87 haplotypes were identified. Haplotype variation in this gene was not associated with SCA risk.

AGTR2

None of the three SNPs examined in AGTR2, located on the X chromosome, were associated with SCA risk (Table 4). Four haplotypes defined the common variation in AGTR2. Haplotype variation in AGTR2 was associated with SCA risk (global Wald test p=0.001). In particular, haplotype 2 (allele frequency=27%) was associated with a 26% increased risk of SCA for each copy present (OR=1.26, 95%CI=1.1–1.5). In sensitivity analyses, haplotype variation remained associated with SCA risk after dropping the rare haplotype group.

KNG1

Of the eight SNPs examined in KNG1, six showed evidence of interaction with gender for the outcome of SCA risk, interaction p-values ranging from 0.016–0.00037 (Table 5). Correcting for multiple comparisons using a permutation test, it was unlikely that this gender-genotype interaction was due to chance (p=0.003). Among women, five of eight SNPs were associated with lower risk of SCA (Table 6). SNP rs710448 (MAF=42%) was associated with 56% decreased risk of SCA (OR=0.44, 95%CI=0.3–0.8, p=0.003). The other four SNPs were in mild to moderate LD with this variant (r^2=0.20–0.57). Including rs710448 in the model with the other SNPs individually attenuated their association with SCA risk (Table 6), while the association with rs710448 was minimally altered. After correction for multiple tests using a permutation test, rs710448 remained significantly associated with SCA risk (p=0.02).

Fifty haplotypes were constructed for KNG1. KNG1 haplotype variation was associated with SCA risk among women (Table 6), primarily driven by the findings among less common haplotypes. After combining haplotypes with 5 or fewer cases into one group, haplotype variation remained associated with SCA risk (global Wald test p=0.003).

Among men, two SNPs (rs1621816 and rs5030093) were associated with increased SCA risk (Table 5), but neither association remained significant after multiple testing correction.

The above associations were minimally altered by adjustment for history of diabetes or hypertension: AGTR1 SNP rs1492099 (adjusted OR 0.62, 95%CI 0.43–0.88); AGTR2 Hap2 (adjusted OR 1.27, 95%CI 1.07–1.52); and KNG1 SNP rs710448 (adjusted OR 0.44, 95%CI 0.25–0.79, women only analysis).

Other Genes

We did not find an association with variation in AGT, ACE, ACE2, and BDRK2. One variant in the REN gene was associated with SCA risk (rs6676670), but did not remain significant after multiple testing correction (Supplemental Table).

Discussion

The results of this study show that common variation in three genes in ACE-related pathways are associated with SCA risk. Variants in both angiotensinogen receptors, types 1 and 2, were associated with SCA risk. Interestingly, there was strong evidence of interaction by gender for KNG1 on SCA risk. Variation in KNG1 was associated with lower SCA risk among women, but not among men.

Our findings of an association of SCA risk with genetic variation in ACE-related pathways are consistent with prior studies suggesting that these pathways play an important role in ventricular arrhythmias and SCA. In mice, cardiac-restricted ACE overexpression results in conduction defects, connexin dysregulation, and susceptibility to induced ventricular arrhythmias.[17, 18] Similarly, transgenic mice with increased cardiac ACE2 expression have connexin downregulation and a high incidence of sudden death due to ventricular fibrillation.[19] Conversely, adenovirus-mediated kallikrein gene delivery in a rat ischemia/reperfusion model decreased the incidence of ventricular fibrillation.[20] The end products of all three pathways influence ischemia-reperfusion injury arrhythmias in animal models. AngII promotes arrhythmias via the AGTR1, while both proarrhythmic and protective effects have been ascribed to AngI-7 and bradykinin.[3–6] Furthermore, AngII acts on a number of ion-handling proteins, which may account for the increased risk of arrhythmia during RAS activation. For example, AngII inhibits delayed rectifier potassium currents in cardiac muscle, and decreases cardiac sodium channel SCN5A transcription and current.[21,

[22] In humans, higher renin levels are associated with increased risk of cardiovascular disease mortality in population studies.[7] Importantly, blocking the RAS system with ACE inhibitors, angiotensin receptor blockers, or aldosterone inhibitors decreases risk of SCA in randomized clinical trials.[8–10]

Variation in KNG1, part of the kallikrein-kininogen pathway, showed a notable interaction with gender on its association with SCA risk. Variation in this gene was associated with lower risk of SCA in women, but not in men. Interestingly, kininogen levels differ between the genders and are responsive to estrogen.[23] In mice, estrogen increases kininogen levels in tissue and serum, whereas testosterone does not.[23, 24] Furthermore, the region near the transcription start site of KNG1 contains a consensus for the core sequence of the estrogen response element.[25] Our finding of a sex interaction with KNG1 deserves further investigation.

The two SNPs in KNG1 and AGTR1 found to be associated with SCA risk are common. KNG1 SNP rs710448 had a MAF=42% among our controls, which is similar to that found in HapMap (dbSNP http://www.ncbi.nlm.nih.gov/SNP/) among those of European descent (MAF=41%). AGTR1 rs1492099 had a MAF=15% among controls in our population (European-descent HapMap population MAF=16%). A review of the literature and of bioinformatics databases using the curated data aggregated by the SNPLogic website (http://snplogic.org/index.php) indicates that little is known about these SNPs, or the SNPs they tag. Both these SNPs are intronic and may lie in regions involved in transcriptional regulation.[26]

There are limited data on functional effects of variants in AGTR1, AGTR2, or KNG1, or on potential associations between these genetic variants and risk of SCA. One SNP in AGTR1, rs5186 (1166A>C), was found to be associated with prolongation of the corrected QT interval in a population of adults with end-stage renal disease.[27] This SNP is not in LD with our top SNP, rs1492099 (r^2=0.0006 in our population). The rs5186 SNP was found more commonly among survivors of malignant ventricular arrhythmias than among controls when in combination with the ACE insertion/deletion polymorphism, but was not independently associated with ventricular arrhythmias.[28] This variant was not independently associated with SCA in our study.

It is unclear whether the association of common ACE-related pathway genetic variants with risk of SCA is related to intermediate phenotypes (e.g. myocardial infarction, hypertension, diabetes) or represents novel associations that point toward other pathways that may influence the risk of SCA. It is notable that adjustment for hypertension and diabetes did not alter these results. Furthermore, the association of variation in these genes with other SCA risk factors, such as diabetes, hypertension, myocardial infarction, and heart failure, has been examined in prior studies and consistent associations have not been found.[29–33] Interestingly, several studies suggest that variation in ACE-related genes is associated with risk of atrial fibrillation, further supporting a role of variation in genes in these pathways in arrhythmias.[34, 35]

Several limitations to the interpretation of our findings deserve consideration. First, although population-based, CABS is a case-control study of married adults, aged 24–75, without clinically recognized heart disease, who were attended by the emergency medical system personnel. Because of these eligibility restrictions, a proportion of SCA events in Seattle and King County were not included. Second, despite rigorous prospective case ascertainment methods, there is likely heterogeneity of the SCA phenotype. Heterogeneity of outcome may lead to an underestimate of the true association. Furthermore, these studies were performed in adults of European descent. Whether these findings generalize to all SCA cases needs to

be examined. Additionally, population admixture is a potential concern with genetic association studies. Fine population stratification that may result in a spurious association cannot be excluded.

We examined 8 genes in these pathways. A number of other genes in these complex pathways were not examined, and need to be explored. Furthermore, common SNPs and haplotypes were examined. Our study design will not assess risk associated with rarer variants or with other types of genetic variation, such as insertion/deletions. Additionally, larger studies are needed to more fully assess potential interactions with modulators of the ACE-related pathways such as treatment with ACE inhibitor medications, as well as to explore allele-allele interactions. Furthermore, although biologic plausibility exists for the role of ACE-related genes in SCA, it is possible that the associations observed reflect other susceptibility loci in LD with these variants. Finally, it is possible that the associations of ACE-related genetic variants and SCA risk identified in this study are due to chance. It is important that these findings be replicated in other populations.

Conclusion

In summary, this study shows an association of common genetic variation in ACE-related pathways with SCA risk. Identifying those at increased risk of SCA and developing new therapies is a clinical and public health challenge. The findings of this investigation, if replicated in other populations, support the need for further investigation of ACE-related pathways in arrhythmogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of abbreviations

SCA	sudden cardiac arrest
RAS	renin-angiotensin system
SNP	single nucleotide polymorphism
MAF	minor allele frequency
LD	linkage disequilibrium

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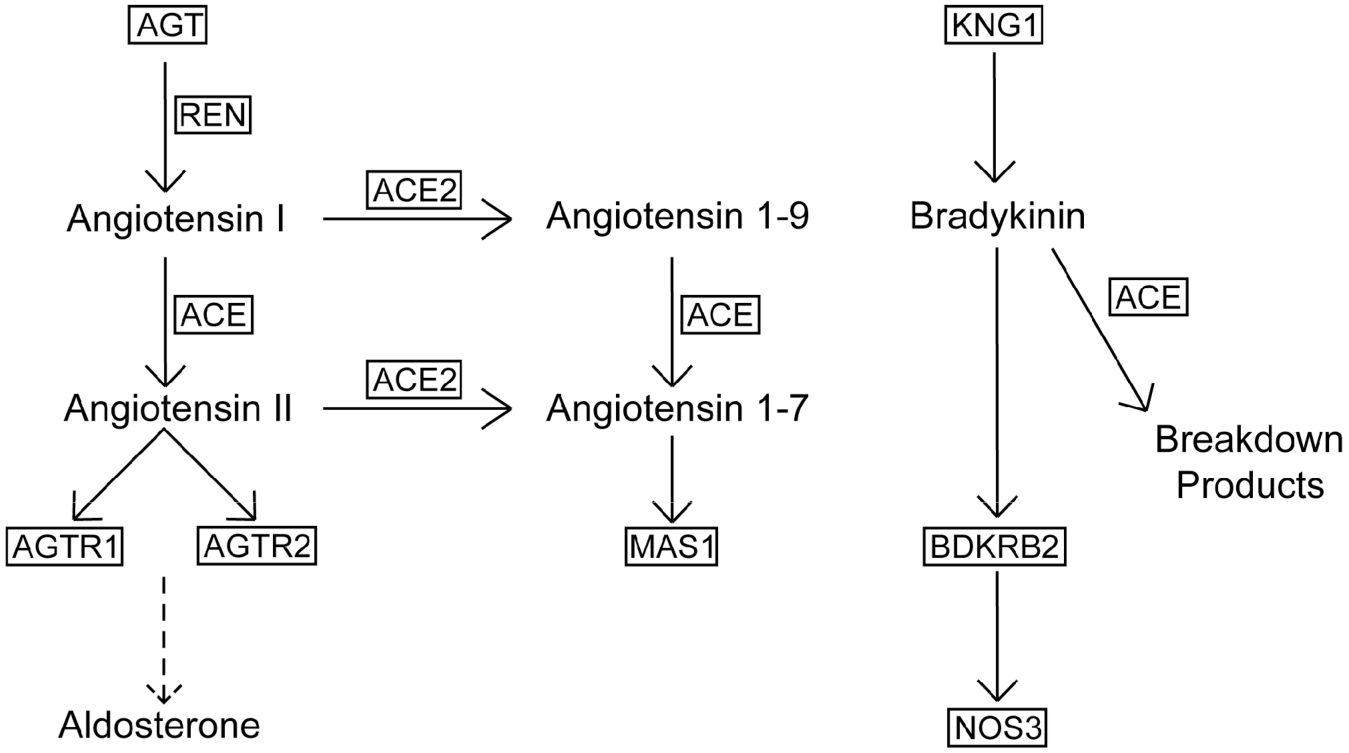

Figure 1. ACE-Related Pathways

Adapted from WikiPathways and KEGG Pathways

In the classic RAS system, angiotensinogen (AGT) is cleaved to angiotensin I (AngI) by renin (REN). AngI is then cleaved by ACE to angiotensin II (AngII), which exerts its effect through binding angiotensin receptor types I (AGTR1) and II (AGTR2).13 In the ACE2 pathway, AngI is converted to angiotensin I-9 (AngI-9) by ACE2. AngI-9 is then converted to angiotensin I-7 (AngI-7) by ACE.14 Similarly, AngII is hydrolyzed to AngI-7 by ACE2. In a parallel system, kallikrein converts kininogen (KNG1) to bradykinin. Bradykinin acts on the bradykinin receptors including bradykinin receptor type 2 (BDRK2) to exert its effects.15 Bradykinins are inactivated by ACE. We selected eight genes (AGT, REN, ACE, AGTR1, AGTR2, ACE2, KNG1, and BDRK2) in these three ACE-related pathways to examine for association with SCA risk.

Table 1

Characteristics of Case and Control Subjects

	Cases [n=211]	Controls [n=730]	P-value
Age (years)	59	58	-
Male (%)	80%	78%	-
BMI (kg/m^2)	25.8	25.6	0.09
Diabetes (%)	9%	4%	0.004
Hypertension (%)	25%	15%	<0.001
Current Smoker (%)	26%	10%	<0.001
Family History (%)	54%	41%	0.001

Table 2

Summary Results of Single SNP and Haplotype Analysis

Gene	Number of SNPs Examined	Number of Significant SNPs	SNP Permutation P-value	Haplotype Global Test P-value	Gender-genotype Interaction Permutation P-value
REN	5	1	0.13	0.092	0.57
AGTR1	11	3	**0.043**	0.19	0.27
AGTR2	3	0	0.11	**0.001**	0.18
ACE2	4	0	0.99	0.34	0.45
BDRK2	5	0	0.78	0.82	0.09
AGT	7	0	0.57	0.52	0.26
ACE	4	0	0.45	0.19	0.85
KNG1	8	0	0.86	0.53	**0.003**
KNG1 (M)	8	2	0.15	0.18	--
KNG1 (F)	8	5	**0.021**	**0.003**	--

M = male subjects only, F = female subjects only

Table 3

Association of AGTR1 with SCA

SNP / Haplotype	rs2933249	rs3772633	rs2276736	rs2640543	rs718858	rs1492099	rs389566	rs2638355	rs18007766	rs5182	rs5186	Haplotype Frequency in Controls (%)	Single Haplotype OR (95% CI)
Hap1	G	A	A	G	G	G	A	G	A	**A**	A	21%	1.01 (0.79–1.30)
Hap2	G	A	A	G	G	G	A	G	A	G	A	8%	1.14 (0.80–1.63)
Hap3	G	A	A	G	G	G	A	G	A	G	**C**	15%	1.22 (0.93–1.60)
Hap8	G	A	A	G	G	G	**T**	G	A	**A**	A	2%	1.10 (0.55–2.22)
Hap39	G	A	**G**	**A**	**A**	G	A	G	A	**A**	A	5%	1.52 (0.98–2.35)
Hap54	G	**G**	**G**	G	G	G	A	G	A	**A**	A	11%	0.84 (0.58–1.20)
Hap72	**A**	A	A	**A**	G	G	A	G	A	G	**C**	4%	1.11 (0.64–1.90)
Hap77	**A**	A	A	**A**	G	**A**	A	G	A	**A**	A	3%	0.72 (0.37–1.42)
Hap85	**A**	A	A	**A**	G	**A**	**T**	**A**	**G**	**A**	A	3%	0.39 (0.16–0.93)
Hap86	**A**	A	A	**A**	G	**A**	**T**	**A**	**G**	G	A	4%	0.56 (0.29–1.07)
Rare												25%	0.96 (0.75–1.22)
MAF %	19%	18%	32%	33%	14%	15%	25%	17%	16%	48%	30%		

Haplotype	SNP	rs2933249	rs3772633	rs2276736	rs2640543	rs718858	rs1492099	rs3895666	rs2638355	rs18007666	rs5182	rs5186	Haplotype Frequency in Controls (%)	Single Haplotype OR (95% CI)
Single SNP OR (95%CI)		0.71 (0.53-0.96)	0.91 (0.67-1.22)	1.12 (0.88-1.42)	0.90 (0.72-1.14)	1.27 (0.93-1.71)	0.62 (0.43-0.88)	0.80 (0.61-1.04)	0.74 (0.54-1.01)	0.70 (0.51-0.97)	0.96 (0.77-1.19)	1.14 (0.90-1.45)		
Single SNP P-value		**0.023**	0.52	0.36	0.38	0.13	**0.005**	0.09	**0.055**	**0.028**	0.70	0.28		
Adjusted P-value[1]		0.98							0.81	0.43				
Permutation Test							**0.044**							
Haplotype Global Wald Test P-value														0.19

[1] Adjusted for rs1492099. All models adjusted for age, sex, and index year. Minor alleles are shown in bold.

Table 4

Association of AGTR2 GENE with SCA

Haplotype	SNP rs3736556	rs5193	rs6608590	Haplotype Frequency in Controls (%)	Single Haplotype OR (95% CI)
Hap2	T	C	G	27%	**1.26 (1.06–1.50)**
Hap1	T	C	**A**	19%	0.92 (0.74–1.14)
Hap3	T	**A**	G	24%	0.90 (0.73–1.10)
Hap4	**A**	C	**A**	29%	0.87 (0.72–1.05)
Rare				0.07%	5.69 (2.07–15.64)
MAF (%)	29%	24%	49%		
Single SNP OR (95%CI)	0.91 (0.75-1.10)	0.93 (0.76-1.13)	0.85 (0.72-1.01)		
Single SNP p-value	0.32	0.44	0.06		
Haplotype Global Wald Test P-value:					**0.0011**

All models adjusted for age, sex, and index year. Minor alleles are shown in bold.

Table 5

Variant – Gender Interaction in KNG1 on the Outcome of SCA

SNP	OR (95% CI) among men	OR (95% CI) among women	Interaction P–Value
rs1621816	**1.35 (1.04–1.75)**	**0.50 (0.27–0.91)**	**0.0031**
rs1656922	1.00 (0.78–1.28)	0.85 (0.54–1.36)	0.59
rs5030039	1.22 (0.92–1.61)	**0.53 (0.28–0.99)**	**0.017**
rs4686799	0.86 (0.64–1.16)	1.43 (0.83–2.46)	0.14
rs710448	1.27 (0.98–1.64)	**0.44 (0.25–0.78)**	**0.00037**
rs5030091	1.26 (0.97–1.62)	**0.52 (0.31–0.89)**	**0.0053**
rs5030093	**1.35 (1.01–1.80)**	0.60 (0.33–1.09)	**0.011**
rs266760	1.20 (0.90–1.60)	0.55 (0.30–1.01)	**0.016**

Table 6

Association of KNG1 with SCA Among Women

SNP Haplotype	rs1621816	rs16656922	rs5030039	rs4686799	rs710448	rs5030091	rs5030093	rs266760	Haplotype Frequency, Controls (%)	Single Haplotype OR (95% CI) Model 1	Single Haplotype OR (95% CI) Model 2
Hap21	A	G	A	G	A	A	G	G	25%	**1.85 (1.08–3.16)**	**1.85 (1.08–3.16)**
Hap1	A	**A**	A	**A**	A	A	G	G	19%	1.71 (0.96–3.03)	1.71 (0.96–3.03)
Hap17	A	**A**	**G**	G	**G**	**G**	G	G	3%	0.75 (0.14–4.17)	
Hap27	A	G	A	G	**G**	**G**	G	G	4%	0.67 (0.19–2.36)	
Hap29	A	G	A	G	**G**	**G**	**A**	**A**	8%	0.65 (0.24–1.74)	
Hap30	A	G	**G**	G	**G**	A	G	G	2%	0.62 (0.10–3.74)	0.39 (<0.001)[1]
Hap37	**G**	**A**	**G**	G	**G**	A	G	G	4%	0.01 (0.00–0.11)	
Hap39	**G**	**A**	**G**	G	**G**	**G**	G	G	5%	0.71 (0.19–2.58)	
Hap42	**G**	**A**	**G**	G	**G**	**G**	**A**	**A**	12%	0.78 (0.36–1.70)	
Hap43	**G**	G	A	G	A	A	G	G	3%	0.01 (0.00–0.04)	
Hap46	**G**	G	A	G	A	**G**	**A**	**A**	5%	0.75 (0.25–2.25)	
Rare									4%	0.69 (0.20–2.41)	
MAF (%)	32%	50%	28%	23%	42%	47%	29%	30%			

SNP	rs1621816	rs16956922	rs5030039	rs4686799	rs710448	rs5030091	rs5030093	rs266760			
Haplotype									**Haplotype Frequency, Controls (%)**	**Single Haplotype OR (95% CI) Model 1**	**Single Haplotype OR (95% CI) Model 2**
Single SNP OR (95%CI)	0.50 (0.27-0.91)	0.85 (0.54-1.36)	0.53 (0.28-0.99)	1.43 (0.83-2.46)	0.44 (0.25-0.78)	0.52 (0.31-0.89)	0.60 (0.33-1.09)	0.55 (0.30-1.01)			
Single SNP P-value	**0.018**	0.51	**0.035**	0.21	**0.0028**	**0.014**	0.08	**0.047**			
Adjusted p-value[2]	0.35		0.90			0.68		0.95			
Permutation Test					**0.02**						
Haplotype Global Wald Test P-value:										<0.001	**0.0034**

All models adjusted for age, sex, and index year. Minor alleles are shown in bold. Model 1 collapses haplotypes with allele frequency less than 2% into "rare" haplotype group. Model 2 collapses haplotypes with 5 or fewer cases into "rare" haplotype group.

[1] Odds ratio for "rare" haplotype group which includes all haplotypes except for haplotypes 1 and 21.

[2] Adjusted for rs710448.