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Association between polymorphisms in the β2-adrenergic receptor gene with myocardial infarction and ischemic stroke in women

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Summary

Results from studies investigating the association between polymorphisms in the β2-adrenergic receptor gene (*ADRB2*) and cardiovascular disease (CVD) are controversial. Using haplotype-based analysis, we have previously shown a protective effect of the Gly16-Gln27-Ile164 haplotype on myocardial infarction in men. We sought to replicate these findings in women and further investigated, whether the gene variants exert differential effects on myocardial infarction and ischemic stroke. We performed a prospective study among 25,224 women, participating in the Women's Health Study and free of CVD at study entry. We had information on polymorphisms Gly16Arg, Gln27Glu, and Thr164Ile in the *ADRB2*. Incident CVD was self-reported and confirmed after medical record review. We used proportional hazards models to investigate the association between genotypes and haplotypes with any myocardial infarction, any ischemic stroke, and CVD death. During a mean of 11.8 years of follow-up, 274 myocardial infarctions, 299 ischemic strokes, and 159 CVD deaths occurred. Among the whole cohort genotype- and haplotype-based analyses did not show an association for any of the gene variants with any of the CVD outcomes. When we focused on Caucasian women, the haplotype-based analysis, however, suggested an inverse association of the haplotype Gly16-Gln27-Thr164 with incident myocardial infarction (multivariable-adjusted hazard ratio 0.75; 95%CI 0.58−0.97; p=0.03). We did not find associations in the haplotype-based analyses with incident ischemic stroke or CVD death. Our results suggest that the haplotype Gly16-Gln27-Thr164 is associated with reduced risk of incident myocardial infarction but not ischemic stroke in Caucasian women and suggests differential pathophysiologies for myocardial infarction and stroke.

Keywords

β2-adrenergic receptor gene; ischemic stroke; myocardial infarction; polymorphism

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Introduction

Cardiovascular disease (CVD) accounts for almost 30% of deaths worldwide. It is the leading cause of morbidity and mortality in the western world and represents a heavy social and economic burden. While hypertension and environmental factors like nutrition are well known risk factors for CVD, the genetic basis contributing to CVD remains largely obscure. However, understanding the genetics of CVD is of importance for primary and secondary prevention and might enable individualized treatment strategies for optimal drug response.

The β2-adrenergic receptor (ADRB2) belongs to the superfamily of membrane bound G-protein-coupled receptors (1) and the signalling cascade plays an important role in both cardiovascular and metabolic diseases (2). β2-adrenergic receptors are highly polymorphic (3). Three exonic polymorphisms in the β2-adrenocptor gene (*ADRB2*) are functionally relevant (4); Arg16Gly and Gln27Glu have been shown to affect receptor down-regulation, while Thr164Ile affects G-protein coupling (5-7).

Recent investigations have addressed the role of these *ADRB2* polymorphisms in cardiovascular and metabolic disease. However, studies examining an association with hypertension (8-13), ischemic stroke (14,15), myocardial infarction (14,16-18), and weight gain (10,11) are controversial. The discrepancies between these studies may in part be due to study design, gender composition and ethnicity of the study populations. In addition, genetic heterogeneity of the various ischemic vascular events may hamper the delineation of the contribution of single genetic variants.

Using a haplotype-based analysis, we have previously found a protective association between the Gly16-Gln27-Ile164 haplotype and myocardial infarction among men (18). We thus sought to replicate our previous findings in women and to explore whether the association differs by type of ischemic vascular event. Specifically, we investigated the primary hypothesis that the exonic *ADRB2* gene variants Gly16Arg (rs1042713), Gln27Glu (rs1042714) and Thr164Ile (rs1800888) are associated with incident CVD events including myocardial infarction, ischemic stroke, and death due to CVD in a large prospective cohort of over 25,000 apparently healthy women from the Women's Health Study (WHS).

Subjects and Methods

Study Population

The WHS was a randomized, placebo-controlled trial designed to test the benefits and risks of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer among apparently healthy women. The design, methods, and results have been described in detail previously (19). Briefly, a total of 39,876 U.S. female health professionals aged ≥45 years at baseline in 1993 without a history of CVD, cancer, or other major illnesses were randomly assigned to active aspirin (100 mg on alternate days), active vitamin E (600 IU on alternate days), both active agents, or both placebos. All participants provided written informed consent and the Institutional Review Board of Brigham and Women's Hospital approved the WHS. Baseline information was self-reported and collected by a mailed questionnaire that asked about many cardiovascular risk factors and lifestyle variables. Twice in the first year and yearly thereafter, participants were sent follow-up questionnaires asking about study outcomes and other information during the study period. For this analysis, we included follow-up information from the time of randomization through March 31st, 2007. As of this date, morbidity and mortality follow-up was >97 % complete.

Blood samples were collected in tubes containing EDTA from 28,345 participating women prior to randomization. Of those, 28,023 were genotyped for *ADRB2* polymorphisms. After excluding 2,799 participants with missing genotype information on the *ADRB2* at baseline, we were left with information on 25,224 women for this analysis.

DNA genotyping of the ADRB2 polymorphisms (rs1042713-Gly16Arg, rs1042714-Gln27Glu, rs1800888-Thr164Ile)

Genotype determination for the *ADRB2* polymorphisms (Gly16Arg, Gln27Glu, Thr164Ile) was accomplished using multiplex PCR and immobilized probe-based assays developed for multilocus variant detection (Roche Molecular Systems, Alameda, CA) essentially as described elsewhere (20). Genotype assignment was performed using the proprietary Roche Molecular Systems StripScan image processing software. To confirm genotype assignment, scoring was carried out by two independent observers. Discordant rates (<2%) were resolved by a further joint reading and, where necessary, by a repeat genotyping. All results were scored blinded to cardiovascular outcome status.

Ascertainment of cardiovascular disease

During follow-up, participants self-reported cardiovascular events. Medical records were obtained for all cardiovascular events and reviewed by an Endpoints Committee of physicians. Nonfatal stroke was confirmed if the participant had a new focal-neurologic deficit of sudden onset that persisted for >24 hours, and then classified into major subtypes (ischemic, hemorrhagic, or unknown) based on available clinical and diagnostic information with excellent interrater agreement (21). Participants with non-ischemic stroke events were censored at the time of event. The occurrence of myocardial infarction was confirmed if symptoms met World Health Organization criteria and if the event was associated with abnormal levels of cardiac enzymes or abnormal electrocardiograms ([1] in absence of left bundle branch block, left ventricular hypertrophy and Wolff-Parkinson-White Syndrome any of the following: (i) evolution of Q-waves in 2 or more contiguous leads, (ii) new ST elevation in 2 or more contiguous leads, (iii) new ST depression in 2 or more contiguous leads, (iv) new symmetric T-wave inversion or ”pseudonormalization” in 2 or more contiguous leads; or [2] new left bundle branch block). Cardiovascular deaths were confirmed by review of autopsy reports, death certificates, medical records, or information obtained from next of kin or family members.

Our two primary outcomes were nonfatal ischemic stroke and nonfatal myocardial infarction. We also evaluated death due to ischemic CVD.

Statistics

We compared baseline characteristics of participants with respect to their genotype status of Gly16Arg, Gln27Glu, and Thr164Ile using chi-square test for categorical variables and Wilcoxon rank sum for continuous variables.

Genotype and allele frequencies were determined for the whole cohort. We then calculated the Hardy-Weinberg-Equilibrium for each of the polymorphisms. Linkage disequilibrium (LD) was examined as described by Devlin and Risch, using Lewontin's *D'* as the LD measure (22).

Our primary hypothesis was that the three exonic *ADRB2* gene variants (Gly16Arg, Gln27Glu, Thr164Ile) are associated with incident CVD events including myocardial infarction, ischemic stroke, and death due to CVD. We used Cox proportional hazards models to evaluate the association between each of the polymorphisms and the outcomes. We built additive models to investigate the association of the gene variants with incident CVD events. This model

assumes that the risk for carriers of the heterozygous genotype for developing the outcome is half way between carriers of the homozygous genotypes. The advantage of this model is that the strength of genotype-phenotype association is expressed in a single parameter (beta estimate) and statistical tests for detecting a relationship have only one degree of freedom (23). We calculated hazard ratios (HRs) and 95% confidence intervals (CIs). In multivariable analyses, we adjusted for the following covariates: age (continuous), body mass index (continuous), history of hypertension (yes, no), diabetes (yes, no), physical activity/exercise (rarely/never, less than once/week, 1−3 times/week, 4 or more times/week), postmenopausal hormone use (never, past, current), history of oral contraceptive use (yes, no), smoking (never, past, current <15 cigarettes/day, current ≥15 cigarettes/day), alcohol consumption (rarely/never, 1−3 drinks/month, 1−6 drinks/week, >1 drinks/day), family history of myocardial infarction before age 60 (yes, no) and randomized aspirin assignment (yes, no).

We incorporated a missing value indicator if the number of women with missing information on covariates was ≥100 or imputed a value otherwise.

We tested the proportionality assumption of the Cox proportional hazards model by including an interaction term for each of the polymorphisms with time and found no statistically significant violation.

Haplotype inference and estimation from genotype data were determined using PHASE v2.1.1 (24). In addition, the possible associations between haplotypes and the pre-defined clinical outcomes were examined using a haplotype-based Cox proportional hazards model (25).

We further performed analyses stratified by race to account for potential race-specific genetic effects. We conducted single marker and haplotype analyses among Caucasian women. However, the number of non-Caucasian women was too small to perform meaningful analyses. In further exploratory analyses, we investigated gene-environment interactions by successively adding an interaction term of haplotypes with each covariate to the multivariable models. For the purpose of building the interaction term we categorized covariates as indicated above and body mass index as <25, 25−<30, 30−<35, and ≥35 kg/m^2. All analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC). All p-values were two-tailed and we considered a $p<0.05$ as statistically significant. Since we sought to replicate previous findings and evaluated biologically plausible associations between variants in only one gene and CVD, we did not further adjust p-values.

Results

The baseline characteristics of women according to *ADRB2* genotype status are presented in Table 1. Compared to the wild-type allele, women carrying the variant allele of the Gln27Glu less frequently had a history of hypertension, tended to consume alcohol more frequently, and were more frequently past smokers. All the other baseline characteristics of the study participants were similar across the *ADRB2* genotypes.

The observed genotype distribution for marker Gln27Glu was in Hardy-Weinberg-Equilibrium (chi-square with 1 degree of freedom: p-value: 0.17). However, the genotype distributions for markers Gly16Arg and Thr164Ile (chi-square with 1 degree of freedom: p-value: 0.003 and 0.048) were in Hardy-Weinberg Disequilibrium, which is most likely due to the large sample size, rendering the test statistic susceptible to minor allele frequency variations. The allele frequencies for the 3 polymorphisms were as follows: Gly16Arg – 62.7% (Gly16), 37.3% (Arg16); Gln27Glu – 57.6% (Gln27), 42.4% (Glu27); Thr164Ile – 98.7% (Thr164), 1.3% (Ile164). The polymorphisms tested were in strong linkage disequilibrium among each other (Gly16Arg-Gln27Glu: D'=0.99; Gly16Arg-Thr164Ile: D'=1.0; Gln27Glu-Thr164Ile: D'=1.0), comparable to other large studies.

During a mean of 11.8 years of follow-up (298,904 person-years), we confirmed 274 myocardial infarctions, 299 ischemic strokes, and 159 deaths due to CVD.

Since the results from the age-adjusted and from the multivariable-adjusted models were very similar for the single marker analyses and for the haplotype analyses, we only report the results from the multivariable-adjusted models.

Table 2 summarizes the results from the single-marker analyses among the whole cohort assuming an additive mode. We did not find an association between any of the three polymorphisms and any of the ischemic vascular outcome categories. The results did not change when we considered a dominant or recessive mode.

The inferred haplotype frequencies >1% for the whole cohort were as follows: 19.2% (Gly16-Gln27-Thr164), 1.3% (Gly16-Gln27-Ile164), 42.3% (Gly16-Glu27-Thr164); 37.2% (Arg16-Gln27-Thr164).

Table 3 summarizes the haplotype-based analysis for the whole cohort, considering all three polymorphisms, with Gly16-Glu27-Thr164 as the reference. The effect estimates suggest that carriers of the Gly16-Gln27-Thr164 haplotype may be at decreased risk of myocardial infarction (HR=0.80; 95%CI 0.63−1.02; p=0.07) and at increased risk for ischemic stroke (HR=1.24; 95%CI 1.00−1.54; p=0.05). However, the results were not statistically significant. We did not find evidence for an association between the haplotypes tested and death due to CVD.

To address the issue of potential race specific genetic effects of the *ADRB2*-CVD association, we only focused on Caucasian women for further analyses (n=23,814). The number of non-Caucasian women and the CVD outcomes among this group was too small to perform meaningful analyses (n=1,410).

Among Caucasian women we also did not find an association between any of the three polymorphisms and any of the ischemic vascular outcome categories in the single marker analysis assuming an additive mode (Table 4). The results did not change when we considered a dominant or recessive mode.

Table 5a summarizes the haplotype-based analysis among Caucasian women, considering all three polymorphisms, with Gly16-Glu27-Thr164 as the reference. We found that carriers of the Gly16-Gln27-Thr164 haplotype were at a significantly decreased risk of myocardial infarction. The multivariable-adjusted HR was 0.75 (95% CI 0.58−0.97; p=0.03). However, did not find evidence for an association between the haplotypes tested and ischemic stroke, and death due to CVD.

In Table 5b we present the haplotype-based analysis, based on only the more common polymorphisms Gly16Arg and Gln27Glu, with Gly16-Glu27 as the reference. The results were similar to those obtained from the 3-SNP-analysis. We found that carriers of the Gly16-Gln27 haplotype were at a significantly decreased risk of myocardial infarction. The multivariable-adjusted HR was 0.77 (95% CI 0.60−0.98; p=0.04). Again, we did not find evidence for an association between the haplotypes tested and ischemic stroke, and death due to CVD.

Further exploratory analysis did not reveal gene-environment interactions of *ADRB2* haplotypes (Gly16-Gln27-Thr164 haplotype vs. all other haplotypes) with any of the covariates on myocardial infarction (all p for interaction >0.05).

Discussion

In this large study of over 25,000 women, we did not find a single-marker or haplotype association between three functionally relevant polymorphisms in the *ADRB2* gene and incident myocardial infarction, ischemic stroke, or death due to cardiovascular disease. When we focused on Caucasian women only, single marker analyses also did not indicate an association. However, further analysis using haplotype information indicated a 25% reduced risk of myocardial infarction among carriers of the Gly16-Gln27-Thr164 haplotype, a pattern not observed for the outcomes ischemic stroke and CVD death.

Using a nested case-control study design, we have previously shown a protective association of the Gly16-Gln27-Ile164 haplotype with myocardial infarction among US men (odds ratio 0.18; p=0.017) (18). Our results suggest that genetic variants in the *ADRB2* gene are also important among Caucasian women and in addition demonstrate the association of the Gly16-Gln27-Thr164 haplotype with incident myocardial infarction. Thus, the results from the present and from the prior study, suggest that the first two alleles (Gly16-Gln27) are important in conveying a protective association with myocardial infarction. This is supported by findings from our analyses with just the Gly16Arg and Gln27Glu polymorphisms. This 2-SNP-analysis indicated that the Gly16-Gln27 haplotype mediates the reduced risk of myocardial infarction. In contrast, none of the genotypes and haplotypes appeared to alter the risk for ischemic stroke. Further exploratory analysis did not reveal significant gene-environment interactions between *ADRB2* haplotypes and any of the covariates with regard to myocardial infarction.

Only one prior prospective study investigated the association of the Arg16Gly and Gln27Glu polymorphisms with incident overall CVD, ischemic stroke and myocardial infarction (14). The authors found that the Glu27 allele was associated with a lower risk of myocardial infarction (HR 0.82, 95% CI 0.70−0.95), but not ischemic stroke or overall CVD. A study among Italian men (26) supports these results (14) and reports a protective association of the Glu27 allele with myocardial infarction among dyslipidemic young patients. Other studies did not find an association of any of these *ADRB2* genotypes with myocardial infarction in whites (16,17) and Japanese (27).

Studies investigating the role of *ADRB2* gene variants in ischemic stroke are scarce. In a case-control study of the three polymorphisms the Glu27 allelic variant was associated with an increased risk for ischemic stroke (15). A previous study (14) using prospective data and our results do not support these findings. Other studies investigating hypertension (8-13) and weight gain (10,11) as intermediate phenotypes for CVD are equally controversial.

There is also no clear association between *ADRB2* variants and venous thromboembolism. While the Gln27Glu polymorphism was reported to be associated with an increased risk in men (28), other studies did not support this (29,30), and one study did not find an association between the Gly16Arg and Gln27Glu polymorphisms with venous thromboembolism (31).

Several reasons may account for the discrepancies among existing studies examining the association between *ADRB2* variants and CVD. First, only one previous study used follow-up data allowing conclusions about incident CVD (14). Second, the other studies have a case-control design and choosing controls may be prone to selection bias leading to spurious associations or obscuring true associations. Third, analyses of single polymorphisms may yield different results than haplotype analyses (14,15,17,18,27,32). The impact, a particular genotype of a single polymorphism in a highly polymorphic gene like the *ADRB2* has on a certain phenotype like myocardial infarction is also determined by other polymorphic loci within that gene. Therefore, whether an association between a single genotype and a phenotype is found also depends on the other genotypes. However, analysis of certain allelic patterns, i.e. haplotypes, draws a more comprehensive picture of a gene-phenotype association. Fourth,

cardiovascular disorders, including myocardial infarction and ischemic stroke, are pathophysiologically complex and genetically heterogeneous, involving aspects of altered coagulation, increased blood lipid levels, oxidative stress, endothelial dysfunction etc. Thus, variants in a single gene only have a small to moderate effect on CVD (33) and differential processes may account for ischemic stroke and myocardial infarction. This may be further supported by findings that biomarkers like c-reactive protein are more closely related with ischemic stroke than with coronary heart disease (34). Furthermore, we have recently shown, that the association of migraine with ischemic stroke and myocardial infarction is differentially modified according the 677C>T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene (35). In addition, potential associations of gene variants, single polymorphisms or haplotypes, with certain outcomes are also determined by variants in other genes. Finally, race-specific genetic effects may add to this complexity, as shown by our findings. Taken together, unveiling the complex *ADRB2*-CVD association necessitates a study design using haplotypes and follow-up data on specific outcomes in a homogenous cohort, consisting of a well-defined ethnicity.

Our study has several strengths, including a large number of participants and outcome events, long follow-up, prospective design, and use of standardized questionnaires, which may reduce confounding. Furthermore, an Endpoints Committee of physicians confirmed all outcome events after medical record review.

Several limitations of our study should be considered when interpreting our results. First, the possibility of false positive results in association studies needs to be considered. We only found one protective association in the multivariable haplotype analysis with a p-value of 0.03. However, we sought to replicate previous findings and evaluated biologically plausible associations between variants in only one gene and CVD. Specifically, we have previously found a similar pattern of association between the Gly16-Gln27-Ile164 haplotype and myocardial infarction in a comparable study in men (18). Thus, we did not further adjust p-values for the present study. In addition, epidemiological evidence supports our findings. This includes that the effect estimates are clearly pointing towards a protective association and larger numbers of outcomes would likely have yielded smaller p-values. Second, the genotype distribution of the Gly16Arg and the Thr164Ile polymorphisms deviated from Hardy-Weinberg-Equilibrium. Sampling bias and genotyping errors are the most frequent reasons, however, we consider these scenarios unlikely due to the prospective character of our cohort and the stringent genotyping protocol. In case of the Thr164Ile polymorphism, the most likely reason is the rarity of the minor allele and the large sample size. Deviation of the Gly16Arg genotype distribution may be caused by the fact that the WHS only includes women older than 45 years, who were otherwise healthy, thus not representing the whole female population. Third, we only found an *ADRB2*-myocardial infarction association among Caucasian women age ≥45. Hence, generalizability to other populations may be limited. While the number of non-Caucasian women in our cohort was too small to perform meaningful analyses, our findings argue in favor of race-specific genetic effects of *ADRB2* variants. Further, given the controversy among previous studies a more homogeneous cohort may help to discern a genotype/haplotype association. Finally, experimental studies have provided evidence for the functional relevance of the three non-synonymous polymorphisms in *ADRB2* investigated. While Arg16Gly and Gln27Glu aggravate β2-adrenergic receptor downregulation, Thr164Ile impairs G-protein coupling (5-7). In addition, β2 adrenergic receptors (ADRB2) play an important role in the pathogenesis of cardiovascular disorders (2). However, association studies like the present one only examine the possible association between a phenotype and the actually tested polymorphisms and haplotypes. Thus, we cannot exclude the possibility that examination of a different polymorphism(s) –not in linkage disequilibrium with the variants tested– might lead to different results.

Our data further support the potential involvement of the *ADRB2* gene variation in the pathophysiology of incident myocardial infarction, but not ischemic stroke, and death due to CVD. Our exploratory analyses do not indicate significant *ADRB2* haplotype-environment interactions with regard to myocardial infarction. These results need to be confirmed in other prospective studies with detailed information on incident cardiovascular events. Further research should focus on potential gene-gene interactions of the *ADRB2*-CVD association and the interrelationships between *ADRB2*, thrombosis and CVD.

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Table 1

Baseline characteristics according to genotype status of the Gly16Arg, Gln27Glu, and Thr164Ile polymorphisms in the *ADRB2* gene among women in the Women's Health Study (n=25,224) *

Characteristics	Gly16Arg genotype			Gln27Glu genotype			Thr164Ile genotype		
	Gly16Gly16 (n=9,815)	Arg16Gly16 (n=12,013)	Arg16Arg16 (n=3,396)	Gln27Gln27 (n=8,418)	Gln27Glu27 (n=12,215)	Glu27Glu27 (n=4,591)	Thr164Thr164 (n=24,592)	Thr164Ile164 (n=624)	Ile164Ile164 (n=8)
Age, mean (SD), y	54.6 (7.0)	54.8 (7.1)	54.7 (7.2)	54.7 (7.1)	54.8 (7.1)	54.6 (7.0)	54.7 (7.1)	55.1 (7.5)	56.9 (11.9)
Body mass index, mean (SD), kg/m^2	25.9 (5.0)	25.9 (5.0)	25.9 (4.9)	26.0 (5.0)	25.9 (5.0)	25.9 (4.9)	25.9 (5.0)	26.0 (5.0)	26.6 (4.1)
History of hypertension	24.5	25.2	25.8	25.8†	25.0†	23.6†	25.0	25.5	25.0
Diabetes	2.5	2.4	2.4	2.7	2.4	2.3	2.4	2.6	0.0
Family history of myocardial infarction before age 60	11.5	11.6	11.0	11.5	11.5	11.2	11.4	12.3	12.5
Physical activity									
Never	36.6	38.3	36.4	36.9	38.1	36.4	37.4	36.9	37.5
<1/week	19.3	19.6	20.1	19.9	19.6	18.9	19.6	20.5	0.0
1−3/week	32.3	31.0	31.5	31.7	31.1	32.4	31.5	31.7	50.0
≥4/wk	11.8	11.1	12.0	11.5	11.2	12.3	11.5	10.9	12.5
Postmenopausal hormone therapy									
Never	48.2	48.1	49.6	48.5	48.2	48.2	48.4	47.0	62.5
Past	9.0	9.43	8.9	9.4	9.1	8.9	9.1	10.6	12.5
Current	42.9	42.5	41.5	42.1	42.7	42.9	42.5	42.5	25.0
Oral contraceptive use									
No	30.1	30.5	30.7	30.4	30.8	29.2	30.3	33.7	37.5
Yes	69.5	69.1	68.9	69.1	68.8	70.4	69.3	66.0	62.5
Not sure	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	0.0
Alcohol consumption									
Never	43.8	44.1	45.5	45.3†	43.9†	43.0†	44.2	42.5	62.5
1−3 drinks/month	13.1	13.3	13.5	13.6†	13.0†	13.3†	13.3	11.4	0.0
1−6 drinks/week	33.1	32.0	30.1	31.1†	32.5†	33.4†	32.1	36.9	25.0
≥1 drink/day	10.0	10.6	10.9	10.0†	10.7†	10.3†	10.4	9.3	12.5
Smoking status									

Characteristics	Gly16Arg genotype			Gln27Glu genotype			Thr164Ile genotype		
	Gly16Gly16 (n=9,815)	Arg16Gly16 (n=12,013)	Arg16Arg16 (n=3,396)	Gln27Gln27 (n=8,418)	Gln27Glu27 (n=12,215)	Glu27Glu27 (n=4,591)	Thr164Thr164 (n=24,592)	Thr164Ile164 (n=624)	Ile164Ile164 (n=8)
Never	51.6	51.8	52.1	52.1†	51.6†	51.7†	51.8	49.7	62.5
Past	37.2	36.7	35.8	36.2†	37.1†	37.0†	36.7	39.7	25.0
Current <15 cigs/day	3.8	4.4	5.1	4.9†	4.0†	3.9†	4.3	4.2	0.0
Current ≥15 cig/day	7.4	7.1	7.0	6.8†	7.3†	7.4†	7.2	6.4	12.5

Numbers may not add up to 100 due to missing values and rounding.

The distribution of all other characteristics according to genotype of Gly16Arg, Gln27Glu, and Thr164Ile did not differ significantly (*p*-values from chisquare test categorical variables and Wilcoxon rank sum test for continuous variables were all >0.05)

*data are expressed as percentages unless otherwise stated.

†chisquare (2df) $p<0.05$

Table 2

Multivariable-adjusted* hazard ratios (HRs) and 95% confidence intervals (CIs) for ischemic vascular events according to single nucleotide polymorphisms Gly16Arg, Gln27Glu, and Thr164Ile in the *ADRB2* gene among women in the Women' Health Study (n=25,224) assuming an additive mode

Polymorphisms	HR	95% CI	p-value
Myocardial infarction (n=274)			
Gly16Arg	1.06	0.89−1.27	0.50
Gln27Glu	1.08	0.91−1.28	0.39
Thr164Ile	0.92	0.44−1.92	0.83
Ischemic stroke (n=299)			
Gly16Arg	1.07	0.90−1.26	0.46
Gln27Glu	0.85	0.72−1.00	0.06
Thr164Ile	1.03	0.54−1.96	0.92
Death due to cardiovascular disease (n=159)			
Gly16Arg	0.93	0.74−1.18	0.55
Gln27Glu	1.02	0.82−1.28	0.86
Thr164Ile	1.11	0.47−2.62	0.82

* adjusting for: age, body mass index, history of hypertension, diabetes, physical activity, smoking categories, alcohol consumption, postmenopausal hormone use, oral contraceptive use, family history of myocardial infarction before age 60, and randomized aspirin assignment.

Table 3

Multivariable-adjusted* hazard ratios (HRs) and 95% confidence intervals (CIs) for haplotypes >1% in the *ADRB2* gene (considering the Gly16Arg, Gln27Glu, and Thr164Ile polymorphisms) among women with ischemic vascular events in the Women's Health Study (n=25,215†): Gly16-Glu27-Thr164 as the reference haplotype

Haplotypes	HR	95% CI	p-value
Myocardial infarction (n=274)			
Gly16-Glu27-Thr164	Reference	------	----
Gly16-Gln27-Thr164	0.80	0.63−1.02	0.07
Gly16-Gln27-Ile164	0.90	0.43−1.88	0.08
Arg16-Gln27-Thr164	0.99	0.82−1.20	0.95
Ischemic stroke (n=299)			
Gly16-Glu27-Thr164	Reference	------	----
Gly16-Gln27-Thr164	1.24	1.00−1.54	0.05
Gly16-Gln27-Ile164	1.14	0.60−2.19	0.69
Arg16-Gln27-Thr164	1.15	0.95−1.38	0.14
Death due to cardiovascular disease (n=159)			
Gly16-Glu27-Thr164	Reference	-----	----
Gly16-Gln27-Thr164	1.02	0.76−1.38	0.88
Gly16-Gln27-Ile164	1.09	0.46−2.62	0.84
Arg16-Gln27-Thr164	0.94	0.73−1.22	0.65

*adjusting for: age, body mass index, history of hypertension, diabetes, physical activity, smoking categories, alcohol consumption, postmenopausal hormone use, oral contraceptive use, family history of myocardial infarction before age 60, and randomized aspirin assignment.

†9 women were deleted from the analysis with a posterior probability ≤ 95% of the inferred haplotypes.

Table 4

Multivariable-adjusted* hazard ratios (HRs) and 95% confidence intervals (CIs) for ischemic vascular events according to single nucleotide polymorphisms Gly16Arg, Gln27Glu, and Thr164Ile in the *ADRB2* gene among Caucasian women in the Women' Health Study (n=23,814) assuming an additive mode

Polymorphisms	HR	95% CI	p-value
Myocardial infarction (n=253)			
Gly16Arg	1.06	0.88−1.28	0.52
Gln27Glu	1.11	0.93−1.32	0.26
Thr164Ile	0.98	0.47−2.04	0.96
Ischemic stroke (n=275)			
Gly16Arg	1.06	0.89−1.27	0.48
Gln27Glu	0.87	0.73−1.03	0.10
Thr164Ile	1.10	0.58−2.09	0.77
Death due to cardiovascular disease (n=150)			
Gly16Arg	0.91	0.71−1.16	0.44
Gln27Glu	1.03	0.82−1.30	0.78
Thr164Ile	1.17	0.49−2.78	0.72

* adjusting for: age, body mass index, history of hypertension, diabetes, physical activity, smoking categories, alcohol consumption, postmenopausal hormone use, oral contraceptive use, family history of myocardial infarction before age 60, and randomized aspirin assignment.

Table 5a

Multivariable adjusted* hazard ratios (HRs) and 95% confidence intervals (CIs) for haplotypes >1% in the *ADRB2* gene (considering the Gly16Arg, Gln27Glu, and Thr164Ile polymorphisms) among Caucasian women with ischemic vascular events in the Women's Health Study (n=23,805†): Gly16-Glu27-Thr164 as the reference haplotype

Haplotypes	HR	95% CI	p-value
Myocardial infarction (n=253)			
Gly16-Glu27-Thr164	Reference	------	----
Gly16-Gln27-Thr164	0.75	0.58−0.97	0.03
Gly16-Gln27-Ile164	0.95	0.45−1.98	0.88
Arg16-Gln27-Thr164	0.98	0.81−1.19	0.85
Ischemic stroke (n=275)			
Gly16-Glu27-Thr164	Reference	------	----
Gly16-Gln27-Thr164	1.20	0.96−1.51	0.11
Gly16-Gln27-Ile164	1.21	0.63−2.31	0.57
Arg16-Gln27-Thr164	1.14	0.94−1.38	0.20
Death due to cardiovascular disease (n=150)			
Gly16-Glu27-Thr164	Reference	-----	----
Gly16-Gln27-Thr164	1.02	0.75−1.39	0.89
Gly16-Gln27-Ile164	1.15	0.48−2.74	0.76
Arg16-Gln27-Thr164	0.92	0.71−1.20	0.54

*adjusting for: age, body mass index, history of hypertension, diabetes, physical activity, smoking categories, alcohol consumption, postmenopausal hormone use, oral contraceptive use, family history of myocardial infarction before age 60, and randomizedaspirin assignment.

†9 women were deleted from the analysis with a posterior probability ≤ 95% of the inferred haplotypes.

Table 5b

Multivariable-adjusted* hazard ratios (HRs) and 95% confidence intervals (CIs) for haplotypes >1% in the *ADRB2* gene (considering the Gly16Arg and Gln27Glu polymorphisms) among Caucasian women with ischemic vascular events in the Women's Health Study (n=23,814): Gly16-Glu27 as the reference haplotype

Haplotypes	HR	95% CI	p-value
Myocardial infarction (n=253)			
Gly16-Glu27	Reference	------	----
Gly16-Gln27	0.77	0.60−0.98	0.04
Arg16-Gln27	0.98	0.81−1.19	0.85
Ischemic stroke (n=275)			
Gly16-Glu27	Reference	------	----
Gly16-Gln27	1.21	0.97−1.50	0.10
Arg16-Gln27	1.14	0.94−1.38	0.20
Death due to cardiovascular disease (n=150)			
Gly16-Glu27	Reference	-----	----
Gly16-Gln27	1.03	0.77−1.39	0.84
Arg16-Gln27	0.92	0.71−1.20	0.54

* adjusting for: age, body mass index, history of hypertension, diabetes, physical activity, smoking categories, alcohol consumption, postmenopausal hormone use, oral contraceptive use, family history of myocardial infarction before age 60, and randomized aspirin assignment.