Pharmacogenetic Associations of β1-Adrenergic Receptor Polymorphisms With Cardiovascular Outcomes in the SPS3 Trial (Secondary Prevention of Small Subcortical Strokes)

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Background and Purpose—Functional polymorphisms (Ser49Gly and Arg389Gly) in *ADRB1* have been associated with cardiovascular and β-blocker response outcomes. Herein we examined associations of these polymorphisms with major adverse cardiovascular events (MACE), with and without stratification by β-blocker treatment in patients with a history of stroke.

Methods—Nine hundred and twenty-six participants of the SPS3 trial's (Secondary Prevention of Small Subcortical Strokes) genetic substudy with hypertension were included. MACE included stroke, myocardial infarction, and all-cause death. Kaplan–Meier and multivariable Cox regression analyses were used. Because the primary component of MACE was ischemic stroke, we tested the association of Ser49Gly with ischemic stroke among 41 475 individuals of European and African ancestry in the NINDS (National Institute of Neurological Disorders and Stroke) SiGN (Stroke Genetics Network).

Results—MACE was higher in carriers of the Gly49 allele than in those with the Ser49Ser genotype (10.5% versus 5.4%, log-rank P=0.005). Gly49 carrier status was associated with MACE (hazard ratio, 1.62; 95% confidence interval, 1.00–2.68) and ischemic stroke (hazard ratio, 1.81; 95% confidence interval, 1.01–3.23) in SPS3 and with small artery ischemic stroke (odds ratio, 1.14; 95% confidence interval, 1.03–1.26) in SiGN. In SPS3, β-blocker-treated Gly49 carriers had increased MACE versus non–β-blocker-treated individuals and noncarriers (hazard ratio, 2.03; 95% confidence interval, 1.20–3.45). No associations were observed with the Arg389Gly polymorphism.

Conclusion—Among individuals with previous small artery ischemic stroke, the *ADRB1* Gly49 polymorphism was associated with MACE, particularly small artery ischemic stroke, a risk that may be increased among β-blocker-treated individuals. Further research is needed to define β-blocker benefit among ischemic stroke patients by *ADRB1* genotype.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00059306.

(*Stroke*. 2017;48:1337-1343. DOI: 10.1161/STROKEAHA.116.015936.)

Key Words: *ADRB1* ■ β-blockers ■ cardiovascular disease ■ pharmacogenetics ■ Ser49Gly ■ stroke

Stroke is the second leading cause of death worldwide after ischemic heart disease.[1] Ischemic stroke, the most common stroke, is classified by 3 major subtypes, namely cardioembolic, large artery, and small artery.[2,3] Many clinical risk factors for stroke, including hypertension, atrial fibrillation, dyslipidemia, and diabetes mellitus have been identified.[4] However, not all of the risk is explained by clinical factors. It is estimated that genetic factors may explain ≤30% to 40% of the remaining risk.[5]

Several meta-analyses of genome-wide association studies have identified convincing single nucleotide polymorphism (SNP) associations with overall ischemic,[6–9] as well as cerebral small vessel stroke or small vessel pathology.[8–10] Collectively, SNPs showing the strongest evidence for association with ischemic stroke have been subtype-specific, suggesting a potential role for targeted therapies for stroke subtypes.

Numerous cardiovascular pharmacogenetic studies with implications for stroke prevention and treatment have been conducted, with strongest evidence for anticoagulation and antiplatelet therapies.[11–13] Compelling pharmacogenetic data

Received November 4, 2016; final revision received February 5, 2017; accepted February 9, 2017.

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Presented in part at the Annual Meeting of the American Society of Clinical Pharmacology and Therapeutics, San Diego, CA, March 8–12, 2016.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.116.015936/-/DC1.

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also exist for antihypertensive agents, particularly β-blockers with *ADRB1* and thiazide diuretics with *NEDD4L*.[14]

There has been longstanding interest in genetic variants in *ADRB1*, the gene encoding the β_1 adrenergic receptor (β_1AR), and their role in cardiovascular disease and treatment response. Several studies have tested these variants with various cardiovascular phenotypes, including blood pressure (BP),[15] heart rate,[16] dilated cardiomyopathy,[17] and composite cardiovascular outcomes.[18] However, less is known about the relation of *ADRB1* variants to the pathology and pharmacogenetics of ischemic stroke. The 2 most common and well-studied SNPs in *ADRB1* are missense SNPs rs1801252 (A>G; Ser49Gly) and rs1801253 (G>C; Arg389Gly). Pharmacogenetic studies have extensively evaluated these SNPs with different cardiovascular response phenotypes.[14,19] Among hypertensive individuals, homozygous carriers of the wild-type alleles with serine at codon 49 (Ser49) and arginine at codon 389 (Arg389) may experience greater BP lowering from β-blocker therapy than carriers of the minor allele (Gly49, Gly389), with effect size larger for codon 389 than codon 49.[19] To our knowledge, 1 study has examined the associations of Ser49Gly and Arg389Gly with ischemic stroke,[20] and no study has examined the pharmacogenetic associations of either SNP with ischemic stroke.

The previous literature has identified associations with ischemic stroke for the minor alleles Gly49 and Gly389.[20] In vitro studies indicate Gly49 has greater agonist-induced receptor downregulation, and Gly389 has reduced β_1AR coupling with Gs-protein.[19] Based on these findings, and the fact that in our study major adverse cardiovascular events (MACE) was primarily composed of recurrent ischemic stroke, we hypothesized that carriers of the minor allele at both loci will have a greater risk of MACE and reduced response to β-blocker therapy as reflected by a higher incidence of MACE among β-blocker-treated carriers compared with noncarriers.

Using data from the SPS3 trial (Secondary Prevention of Small Subcortical Strokes), we investigated associations of Ser49Gly and Arg389Gly with MACE, particularly ischemic stroke, among participants with recent history of small artery ischemic stroke. We also tested pharmacogenetic associations among β-blocker-treated participants. To validate the association with MACE, we tested the association of Ser49Gly with ischemic stroke using consortium data from the SiGN (Stroke Genetics Network).[21]

Methods

Study Population

The data for this study come from SPS3, an international multicenter randomized controlled clinical trial that evaluated the effect of 2 different antihypertensive and antiplatelet therapy regimens on the rate of recurrent stroke (ClinicalTrials.gov identifier NCT00059306). SPS3 has been described elsewhere.[22] Briefly, 3020 participants ≥30 years old with recent symptomatic small artery ischemic stroke (within 180 days) were recruited and randomized in a 2×2 factorial design to an antiplatelet regimen (aspirin 325 mg daily plus clopidogrel 75 mg daily versus aspirin 325 mg daily plus placebo) and a BP target (lower [also referred to as intensive]: systolic BP (SBP) <130 mm Hg versus higher [also referred to as usual]: SBP 130–149 mm Hg). The primary outcome was recurrent stroke (ischemic or hemorrhagic). Secondary outcomes were rate of cognitive decline and major vascular events, including transient ischemic attack, acute myocardial infarction, noncentral nervous system thromboembolism, and all-cause death. The mean length of follow-up was 3.9±1.9 years (median, 3.8 years). In the SPS3-GENES (SPS3 Genetic Substudy), DNA was collected from saliva samples using OG-500 Oragene DNA self-collection kits (DNA Genotek Inc) on 1139 participants.

The NINDS (National Institute of Neurological Disorders and Stroke) SiGN consortium provided data for the validation, which was a meta-analysis study of the association with presence of ischemic stroke.[21] Data on a total of 41 475 individuals of European and African ancestry in SiGN, excluding SPS3, were analyzed.

Institutional review boards at participating centers for SPS3 and SiGN approved the studies. All participants provided written informed consent.

Genotyping and Imputation

DNA samples collected from SPS3-GENES participants were isolated using the prepIT-L2P kit (PT-LP2-45; DNA Genotek Inc). Genotyping for Ser49Gly (rs1801252) and Arg389Gly (rs1801253) was performed using a TaqMan assay–based QuantStudio polymerase chain reaction system (Life Technologies, Carlsbad, CA). Genotyping call rate was 99% for both Ser49Gly and Arg389Gly. In total, 1126 samples were successfully genotyped. Genotype concordance between genotyped and 1000 Genomes (phase I) imputed data, which became available later, was 99% for Ser49Gly and 96% for Arg389Gly. We tested deviation from Hardy–Weinberg Equilibrium in each ancestry group using the χ^2 test. In SiGN, Ser49Gly and Arg389Gly were imputed from a reference panel that included 1000 Genomes (phase I) and Genome of the Netherlands. Imputation quality for Ser49Gly was 0.984 to 0.998 and for Arg389Gly was 0.982 to 0.993.

Statistical Analysis

Descriptive statistics of baseline and follow-up characteristics on SPS3-GENES participants were compared between participants with and without MACE by the end of the trial. Categorical variables are presented in frequencies, and differences were tested between groups (MACE versus no MACE) using Fisher's exact or χ^2 test, as appropriate. Continuous variables are presented as mean±standard deviation, and differences were tested between groups using Student's *t* test. Principal components analysis was conducted on high-quality, linkage disequilibrium-pruned genome-wide SNP data using the EIGENSTRAT software.[23] Genome-wide genotyping was performed on the Illumina Omni 5M array. The top 2 principal components separated self-identified Whites, African Americans/Blacks, and Hispanics.

In SPS3-GENES, associations between Ser49Gly and MACE, with and without β-blocker treatment, were tested using Kaplan–Meier survival analysis. MACE comprised stroke (ischemic or hemorrhagic), acute myocardial infarction, and all-cause death. Because 75% of MACE was ischemic stroke, the secondary analysis was of ischemic stroke. Participants were classified as β-blocker-treated if they used a β-blocker at any time during the study. In multivariable Cox proportional hazards regression analyses, covariates included age, sex, ancestry-specific principal components 1 to 2, history of myocardial infarction, history of diabetes mellitus, on-treatment SBP, and β-blocker treatment (except for the model stratified on β-blocker treatment). We selected covariates based on associations with time-to-event in univariate Cox regression analyses with $P<0.20$; however, age, sex, history of diabetes mellitus, and on-treatment SBP were forced into the model. Because of prior genetic associations of Ser49Gly and Arg389Gly with ischemic stroke,[20] we considered 2-sided $P<0.05$ as statistically significant for the main effects analyses. Ancestry groups were combined in all analyses given the well-documented functional evidence for these SNPs. Because no prior studies have examined the pharmacogenetic associations of Ser49Gly and Arg389Gly with ischemic stroke, we used a *P* value threshold of 0.025 (0.05/2 SNPs tested) for the pharmacogenetic analyses. A dominant model of inheritance was assumed, consistent

with methodology used in previous studies.[18,20] We similarly examined ischemic stroke alone. Analyses were performed using SAS 9.4 (Cary, NC) and GraphPad Prism 5.01 (San Diego, CA).

This analysis only included participants with hypertension at baseline, defined as SBP ≥140 mm Hg or diastolic BP ≥90 mm Hg or taking antihypertensive medication(s) at the time of enrollment because we anticipated β-blockers would not be expected to be prescribed in this population in the absence of hypertension. In total, 926 participants were included in the analyses.

Although there was no data set available for validation of MACE because the majority of events were ischemic stroke, and specifically small artery ischemic stroke, we validated our findings using SiGN consortium data. Specifically, meta-analysis of 36 studies in SiGN was conducted for association with presence of ischemic stroke. The SiGN sample (with exclusion of the SPS3 stratum) included 13 449 ischemic stroke cases and 28 026 nonstroke controls. The association was tested under a dominant model by logistic regression, adjusted for sex and ancestry principal components. Stroke subtypes were classified using TOAST criteria (Trial of Org 10172 in Acute Stroke Treatment).[3] We are aware of no stroke data set in which we can validate the pharmacogenetic finding.

Results

Description of Study Participants

Overall, background characteristics of SPS3-GENES participants did not differ significantly by MACE or no MACE status at the time of event or censoring (Table 1). There were more men (60.6%), and the average age was 62.2±10.3 years. Treatment with thiazide diuretics, calcium-channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers did not differ by MACE status. However, β-blocker use was significantly higher among those with MACE at follow-up (*P*=0.002).

The frequency of MACE was 7.2% in the overall sample (67/926). The majority of first events at follow-up was recurrent ischemic stroke (75%), of which 86% was small artery stroke. The remaining events included acute myocardial infarction (n=12), central nervous system hemorrhage (n=4), and death (n=1).

The minor allele frequencies for Ser49Gly were 12%, 26%, and 25% and for Arg389Gly were 29%, 45%, and 15% in whites, African Americans/blacks, and Hispanics, respectively. For both polymorphisms, these are consistent with reference population values in the 1000 Genomes database. Both variants were in Hardy–Weinberg Equilibrium.

SiGN participants have been described elsewhere.[21,24] Briefly, their mean age was 67 years, half (49%) were women, and White participants comprised 79%. Twenty-five percent of the participants had diabetes mellitus, 68% had hypertension, and 23% had coronary artery disease.[24]

ADRB1 SNP Associations With MACE

Overall, events were observed in 10.5% of Gly49 allele carriers versus 5.4% of those with the Ser49Ser genotype (*P*=0.004). Among Gly389 carriers versus noncarriers, event frequencies were 7.5% and 6.9%, respectively (*P*=0.707). Kaplan–Meier analysis showed that Gly49 allele carriers had a significantly higher incidence of MACE than noncarriers (log-rank *P*=0.005; Figure 1). In additional analyses, Gly49 allele was

Table 1. Demographic and Clinical Characteristics Among Hypertensive Participants in SPS3-GENES, N=926

Characteristics	Overall (N=926)	Outcome		
		MACE (N=67)	Non-MACE (N=859)	*P* Value
Age, y	62.2±10.3	61.4±10.5	62.3±10.3	0.49
Male	561 (60.6)	40 (59.7)	521 (60.6)	0.88
BMI, kg/m^2	29.1±5.9	29.8±6.0	28.0±5.86	0.33
Baseline SBP, mm Hg	145.3±18.0	147.1±17.2	145.1±18.1	0.36
Baseline DBP, mm Hg	79.5±10.5	80.2±11.0	79.5±10.5	0.64
Lower BP target	472 (51.0)	29 (43.3)	443 (51.6)	0.19
Current smoker	166 (17.9)	17 (25.4)	149 (17.3)	0.10
Disease history				
Myocardial infarction	38 (4.1)	6 (9.0)	32 (3.7)	0.05
Congestive heart failure	9 (1.0)	1 (1.5)	8 (1.0)	0.49
Diabetes mellitus	285 (30.8)	27 (40.3)	258 (30.0)	0.10
Drug exposure during study				
Thiazide diuretic	766 (82.7)	52 (77.6)	714 (83.1)	0.25
Calcium-channel blocker	528 (57.0)	42 (62.7)	486 (56.6)	0.33
β-Blocker	385 (41.6)	40 (59.7)	345 (40.2)	0.002
ACE inhibitor	668 (72.1)	51 (76.1)	617 (71.8)	0.45
ARB	432 (46.6)	28 (41.8)	404 (47.0)	0.41

Mean±SD or n (%) shown; *P* values derived from Student's *t* test and Fisher's exact or χ^2 tests. ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; MACE, major adverse cardiovascular events (stroke, myocardial infarction, all-cause death); SBP, systolic blood pressure; and SPS3-GENES, SPS3 Genetic Substudy.

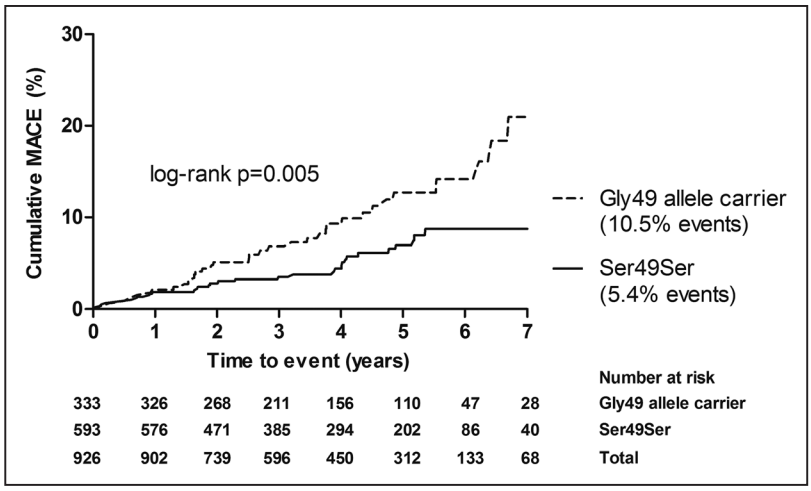

Figure 1. Cumulative incidence of MACE by Ser49Gly genotype among hypertensive participants in SPS3-GENES (SPS3 Genetic Substudy), N=926. MACE indicates major adverse cardiovascular events: stroke, myocardial infarction, all-cause death; 75% of events were ischemic stroke.

associated with increased risk of ischemic stroke (log-rank *P*=0.007) and small artery ischemic stroke (log-rank *P*=0.005; Figures I and II in the online-only Data Supplement).

In multivariable Cox regression analysis, which included adjustment for β-blocker use, Gly49 allele carriers compared with noncarriers had marginally significant associations with MACE (hazard ratio [HR], 1.62; 95% confidence interval [CI], 1.00–2.68) and ischemic stroke (HR, 1.81; 95% CI, 1.01–3.23). In stratified analysis among non–β-blocker users (n=541), HR of Gly49 with risk crossed 1.0 for both MACE (HR, 1.52; 95% CI, 0.69–3.38) and ischemic stroke (HR, 1.95; 95% CI, 0.79–4.85). Arg389Gly was not associated with MACE in Kaplan–Meier analysis (log-rank *P*=0.460) or with MACE (*P*=0.291) or ischemic stroke (*P*=0.537) in adjusted Cox regression analyses.

In the SiGN validation study (n=41 475), multivariable logistic regression analysis showed that Gly49 was significantly associated with presence of small artery ischemic stroke (odds ratio, 1.14; 95% CI, 1.03–1.26; *P*=0.012), but not other types of ischemic stroke.

Pharmacogenetic Associations

Gly49 allele carriers treated with β-blockers had a significantly higher incidence of MACE when compared with other groups (15.7% versus 5.8%; log-rank *P*=0.0004; Figure 2). Additionally, Kaplan–Meier analysis showed that among β-blocker-treated individuals, cumulative incidence of MACE was significantly higher among Gly49 allele carriers than among those with Ser49Ser genotype (15.7% versus 7.6%; log-rank *P*=0.018; Figure III in the online-only Data Supplement). Among individuals with the Ser49Ser genotype, event rates did not differ significantly by β-blocker treatment (log-rank *P*=0.107).

In stratified multivariable Cox regression analysis, with Ser49Ser and no β-blocker treatment as reference, Gly49 allele carriers treated with β-blockers had significantly increased risk of MACE and ischemic stroke, whereas there were no differences in these outcomes for Ser49Ser treated with β-blockers or Gly49 carriers not treated with β-blockers (Table 2). When compared with the 3 other groups, β-blocker-treated Gly49 carriers had increased risk of MACE and ischemic stroke (Table 2). In parallel analyses, we did not find significant pharmacogenetic associations between Arg389Gly and outcomes (Gly389 carrier+β-blocker-treated versus Arg389Arg+β-blocker-treated, *P*=0.506; and Gly389 carrier+β-blocker-treated versus other groups, *P*=0.408).

To assess whether achieved BP differed by Ser49Gly genotype and β-blocker use, we compared on-treatment BP, taken at the visit prior to MACE or censoring, between Gly49 allele carriers treated with β-blockers and Gly49 allele

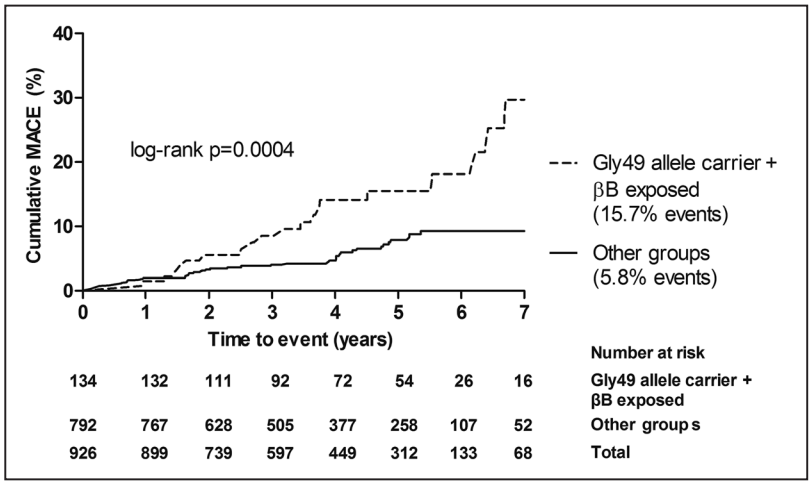

Figure 2. Cumulative incidence of MACE among Gly49 allele carriers treated with β-blockers compared with other groups* in SPS3-GENES (SPS3 Genetic Substudy), N=926. MACE indicates major adverse cardiovascular events: stroke, myocardial infarction, all-cause death; 75% of events were ischemic stroke. βB indicates β-blocker exposed participants. *Other groups included Gly49 allele carriers not treated with β-blockers and noncarriers (Ser49Ser), regardless of β-blocker treatment.

Table 2. Pharmacogenetic Associations of Ser49Gly With MACE and Ischemic Stroke Stratified by β-Blocker Treatment Among Hypertensive Participants in SPS3-GENES, N=926

	HR (95% CI)	*P* Value
I. MACE		
Ser49Ser+not β-blocker-treated	Ref.	
Gly49 carrier+β-blocker-treated	2.79 (1.37–5.70)	0.005
Gly49 carrier+not β-blocker-treated	1.51 (0.70–3.26)	0.30
Ser49Ser+β-blocker-treated	1.64 (0.80–3.36)	0.18
II. Ischemic stroke		
Ser49Ser+not β-blocker-treated	Ref.	
Gly49 carrier+β-blocker-treated	2.92 (1.24–6.84)	0.01
Gly49 carrier+not β-blocker-treated	1.93 (0.79–4.66)	0.15
Ser49Ser + β-blocker-treated	1.69 (0.72–3.97)	0.23
III. Gly49 allele carrier+β-blocker-treated vs other groups*		
MACE	2.03 (1.20–3.45)	0.01
Ischemic stroke	1.92 (1.04–3.58)	0.04

Hazard ratio and 95% confidence interval in parentheses; Cox regression models with adjustments for age, sex, principal components 1–2 for ancestry, history of myocardial infarction, history of diabetes mellitus, on-treatment systolic blood pressure. CI indicates confidence interval; HR, hazard ratio; MACE, major adverse cardiovascular events (stroke, myocardial infarction, all-cause death); and SPS3-GENES, SPS3 Genetic Substudy.

*Other groups included Gly49 allele carriers not treated with β-blockers and noncarriers (Ser49Ser), regardless of β-blocker treatment.

carriers treated with other antihypertensives. β-Blocker-treated Gly49 allele carriers had a higher mean on-treatment SBP, but not diastolic BP, compared with Gly49 carriers treated with other antihypertensives (SBP: 132.4 versus 128.4 mm Hg; *P*=0.023). Among noncarriers, on-treatment SBP did not differ by β-blocker treatment (*P*=0.180). Additionally, in spite of higher SBP, β-blocker-treated Gly49 carriers on average took more antihypertensive medications than Gly49 carriers treated with other antihypertensive drugs (4.2 versus 2.5; *P*<0.001). In parallel analyses, the difference in on-treatment SBP between Gly389 carriers treated with β-blockers and Gly389 carriers treated with other agents was nonsignificant (*P*=0.641). On average, Gly389 carriers treated with β-blockers were treated with a higher number of antihypertensive medication classes than Gly389 carriers treated with other agents (4.1 versus 2.7; *P*<0.001).

Discussion

Using data from a randomized controlled trial, we tested the association of β_1AR polymorphisms with cardiovascular outcomes, composed primarily of ischemic stroke, and evaluated pharmacogenetic associations in β-blocker-treated individuals. Our study population was relatively young (mean age, 62 years), which is comparable to that of similar stroke cohorts.[7] Among participants with a recent history of small artery ischemic stroke, Gly49 allele carriers had an increased risk of recurrent ischemic stroke, particularly small artery stroke, and this finding was corroborated by SiGN consortium meta-analysis that showed Gly49 allele carriers were more likely to have small artery ischemic stroke, but not other types of stroke. These results are consistent with those of a previous small case–control study that reported a higher risk of ischemic stroke among Gly49 allele carriers.[20] Additional analysis suggested that in SPS3-GENES, the association between Gly49 allele and cardiovascular risk may be increased among β-blocker-treated individuals.

The mechanism by which the Gly49 allele may confer risk for ischemic stroke, especially of small arteries, is unclear. It is well-established that β_2AR is important in mediating peripheral vasodilation; however, recent studies have indicated that this is true only for large conductance vessels, such as the aorta.[25] In smaller arteries, such as small mesenteric resistance arteries[25–27] and cerebral arteries,[28] vasodilation may primarily depend on β_1AR stimulation. Another study showed that agonist-induced vasodilation of cerebral arteries is blunted by β_1AR blockade.[29] If β_1AR plays a similar role in small subcortical arteries as it does in peripheral resistance arteries and cerebral arteries, it is possible that perturbing the normal functioning of β_1AR may attenuate its vasodilating effects in small subcortical arteries. The Gly49 variant of β_1AR might be one such perturbation that may attenuate or even reverse β1AR-induced vasodilation in small subcortical arteries. Evidence from recombinant cell studies have suggested that the Gly49 allele leads to greater agonist-induced β_1AR downregulation, which means the number of receptors on the cell surface available to bind catecholamines, and, thus, trigger a downstream response, is reduced.[19] In light of β_1AR's vasodilating effects, the Gly49 variant of β_1AR may be less responsive to adrenergic stimulation and, consequently, less able to mediate vasodilation in small arteries. Collectively, these studies may in part explain our main effect finding of an association of Gly49 with ischemic stroke of small arteries.

To our knowledge, this is the first study to examine β-blocker pharmacogenetic associations with ischemic stroke, and the data suggest that the main effect finding of risk of MACE and ischemic stroke based on the Gly49 polymorphism may be slightly increased with β-blocker treatment. Specifically, when considering Ser49Ser and no β-blocker treatment as the reference, Gly49 carriers treated with β-blockers had increased risk of adverse outcomes, whereas Ser49Ser participants treated with β-blockers did not. Additionally, Gly49 carriers treated with β-blockers had a 3-fold increased risk, while Gly49 carriers without β-blocker treatment had a 2-fold risk, which suggests that β-blocker treatment may have an amplifying effect on the Gly49 allele. Further, analysis of on-treatment BP and number of antihypertensive drugs suggests that Gly49 participants treated with β-blockers had higher on-treatment BPs, despite being treated with, on average, 1.7 more antihypertensive drugs. In contrast, noncarriers did not have different BPs, based on β-blocker treatment. Collectively, these data suggest Gly49 carriers may be less responsive to the antihypertensive effects of β-blockers, which is consistent with previous studies.[19] A recent Cochrane Review indicated that β-blockers have significantly reduced stroke risk compared with placebo, but have increased stroke risk when compared against other renin–angiotensin system inhibitors and calcium-channel blockers.[30] Our data suggest this increased risk might be particularly important in Gly49 carriers treated

with β-blockers. Further research is needed to provide greater clarity on the role of the Gly49 polymorphism on stroke outcomes in patients treated with β-blockers.

We did not find significant main effects or pharmacogenetic associations for Arg389Gly. While previous studies have found associations of Arg389Gly with cardiovascular and cardiovascular pharmacogenetic phenotypes,[19] Arg389Gly may play a minor role in ischemic stroke pathophysiology and pharmacogenetics when compared with Ser49Gly.

This study has several strengths. In a multiethnic population, Ser49Gly was associated with ischemic stroke in a direction consistent with the previous literature, and we validated this association specifically with small artery ischemic stroke in over 40 000 participants in the SiGN consortium. Furthermore, we may have identified novel pharmacogenetic associations between β-blocker treatment and the *ADRB1* Gly49 allele.

This study has limitations that are acknowledged. We were unable to conduct replication analyses of the pharmacogenetic findings because currently, to our knowledge, there are no other replication cohorts of patients with a history of stroke available. Future studies in participants with a previous history of small artery ischemic stroke with medication and genetic information are needed to validate the pharmacogenetic findings. Additionally, we were unable to detect significant associations for some multivariable regression analyses, but the reduced sample sizes and, thus, limited power in these secondary analyses should be considered when interpreting those analyses.

Conclusions

Our study provides evidence that the *ADRB1* Gly49 allele may increase cardiovascular risk, particularly for small artery stroke. Additionally, these data suggest that the association between the Gly49 allele and cardiovascular risk may be enhanced by β-blocker treatment. However, because stroke risk was higher in the study population overall, it is difficult to know with certainty if β-blockers in the population are a marker for increased stroke risk, if β-blockers are increasing risk overall and should be avoided, or if, indeed, β-blocker treatment enhances the apparent risk of carrying the *ADRB1* Gly49 allele. This highlights the critical importance of future studies to clarify this point because it may have important clinical implications for treatment of patients with small artery stroke.

Acknowledgments

We thank the site investigators and are grateful to the participants of SPS3, SPS3-GENES, and SiGN. We also thank Tushar Dave for assistance with SiGN data analysis.

Sources of Funding

This project was supported by funding from the National Institutes of Health's National Institute of Neurological Disorders and Stroke (Grants R01 NS073346, U01 NS038529, and U01 NS069208), National Institute for General Medical Sciences (U01 GM074492), and National Institute for Diabetes, Kidney, and Digestive Disorders (P30 DK072488).

Disclosures

Dr Shuldiner is employed by Regeneron Pharmaceuticals, Inc. The other authors report no conflicts.

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Pharmacogenetic Associations of β1-Adrenergic Receptor Polymorphisms With Cardiovascular Outcomes in the SPS3 Trial (Secondary Prevention of Small Subcortical Strokes)

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Stroke. 2017;48:1337-1343; originally published online March 28, 2017;
doi: 10.1161/STROKEAHA.116.015936
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628

ONLINE SUPPLEMENTS

Figure I. Cumulative incidence of ischemic stroke by Ser49Gly genotype among hypertensive participants in SPS3-GENES, N=926

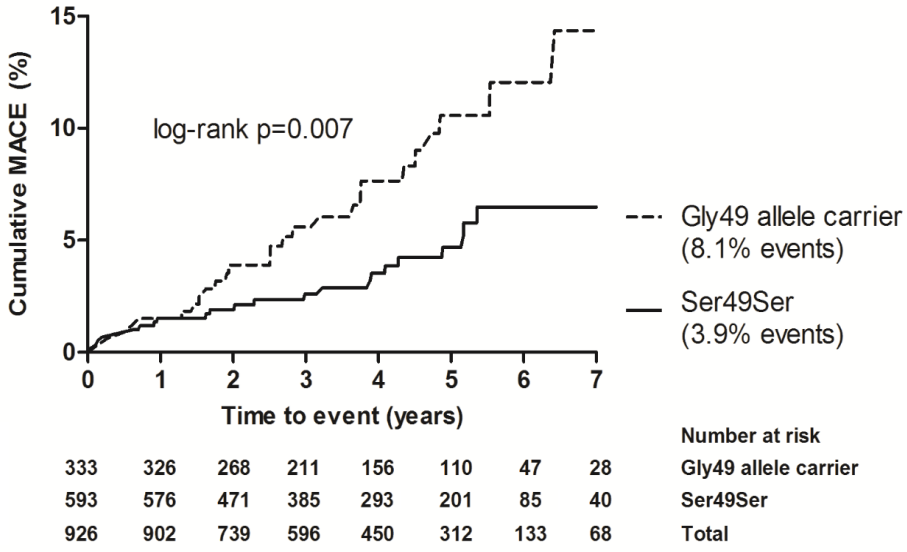


Figure II. Cumulative incidence of small artery ischemic stroke by Ser49Gly genotype among hypertensive participants in SPS3-GENES, N=926

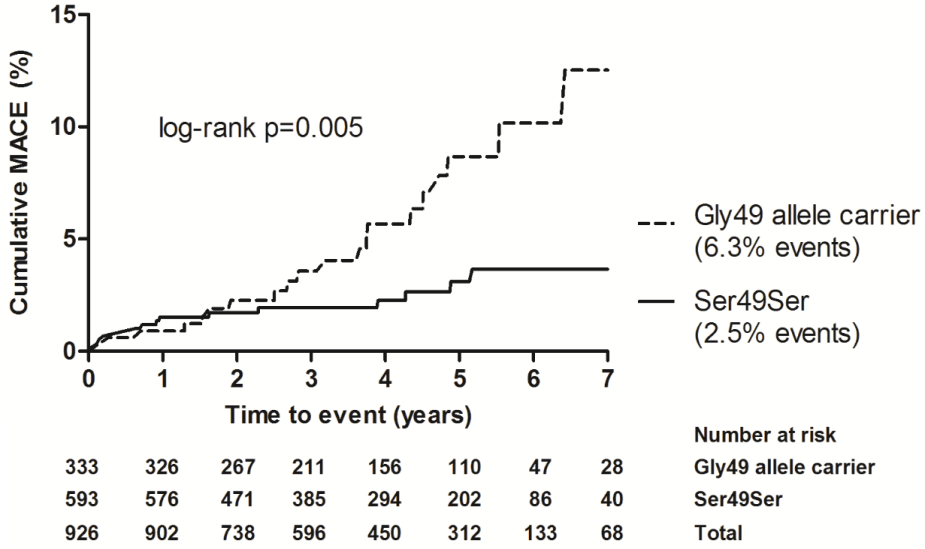
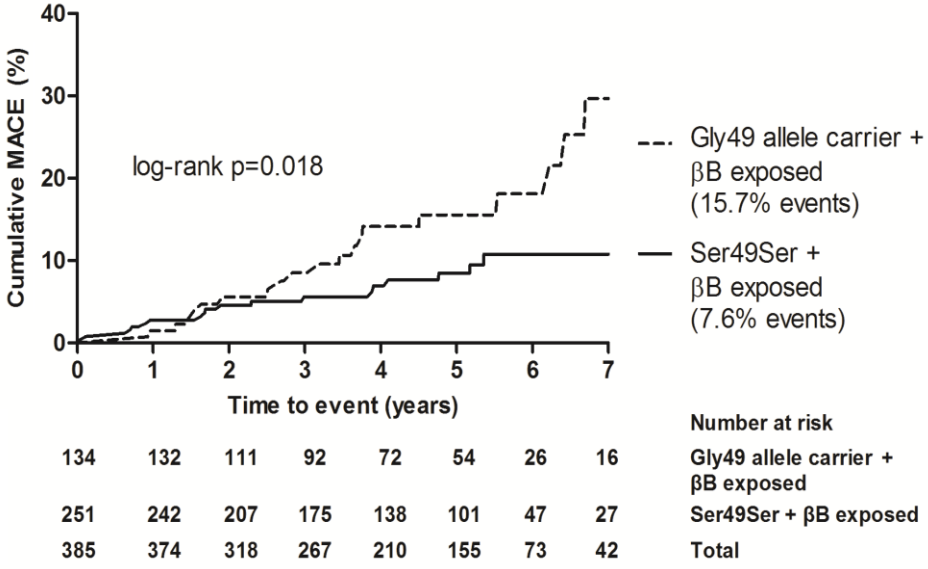


Figure III. Cumulative incidence of MACE stratified by Ser49Gly genotype among β-blocker treated participants in SPS3-GENES, N=385



MACE (Major Adverse Cardiovascular Events): stroke, myocardial infarction, all-cause death; 75% of events were ischemic stroke