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GOSR2 Lys67Arg is associated with hypertension in whites

TE Meyer[1], **D Shiffman**[2], **AC Morrison**[1], **CM Rowland**[2], **JZ Louie**[2], **LA Bare**[2], **DA Ross**[2], **AR Arellano**[2], **DI Chasman**[3], **PM Ridker**[3,4], **JS Pankow**[5], **J Coresh**[6], **MJ Malloy**[7], **JP Kane**[7], **SG Ellis**[8], **JJ Devlin**[2], and **E Boerwinkle**[1]

[1]Human Genetics Center and Division of Epidemiology, University of Texas Health Science Center at Houston, Houston, TX

[2]Celera, Alameda, CA

[3]Center for Cardiovascular Disease Prevention, Division of Preventive Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

[4]Center for Cardiovascular Disease Prevention, Division of Cardiology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

[5]Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN

[6]Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

[7]The Cardiovascular Research Institute, UCSF, San Francisco, CA

[8]The Cleveland Clinic Foundation, Department of Cardiovascular Medicine, Cleveland, OH

Abstract

BACKGROUND—Hypertension is a risk factor for coronary heart disease (CHD), but the causes of hypertension remain largely unknown. Genetic variation is thought to contribute to the etiology of hypertension. We tested a single nucleotide polymorphism (SNP) (Lys67Arg, rs197922) in the Golgi SNAP Receptor Complex Member 2 gene (*GOSR2*) for association with hypertension and blood pressure (BP). We chose this SNP because it was nominally associated with CHD in earlier studies. Further, *GOSR2* is located in a linkage region for hypertension and BP in human and animal studies.

METHODS—We used logistic and linear regression to test associations of the *GOSR2* SNP with hypertension and BP among 3,528 blacks and 9,861 whites from the Atherosclerosis Risk in Communities study. Race-specific regression models of hypertension were adjusted for age and gender. Regression models of BP were further adjusted for anti-hypertensive medication use.

Corresponding Author: Eric Boerwinkle, PhD, Human Genetics Center, School of Public Health, University of Texas Health Science Center at Houston, 1200 Herman Pressler, Suite RAS-E447 Houston, TX 77030, Phone: (713) 500-9816 Fax: 713-500-0900, Eric.Boerwinkle@uth.tmc.edu.

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DISCLOSURE

D.S., C.M.R., J.Z.L., L.A.B, D.A.R., A.R.A, and J.J.D. have employment or ownership interests in Celera.

RESULTS—The *GOSR2* Lys67 allele was associated with hypertension in whites (odds ratio [OR]=1.09, *P*=0.01) but not blacks (OR=0.96, *P*=0.47). The Lys67 allele was associated with increased systolic BP in both races (0.87 mmHg, *P*<0.001 among whites and 1.05 mmHg, *P*=0.05 among blacks). A similar association in whites was observed for the *GOSR2* SNP and systolic BP in the Women's Genome Health Study (WGHS) (OR=1.03, *P*=0.04). The OR remained unchanged after adjustment for anti-hypertensive medication use (OR=1.03, *P*=0.11), though it was no longer statistically significant.

CONCLUSIONS—We found evidence that a SNP in *GOSR2* is modestly associated with hypertension in whites from the ARIC study and the WGHS.

INTRODUCTION

Hypertension is an important, modifiable risk factor for both heart disease and stroke; two of the top three causes of mortality in the United States.[1, 2] The prevalence of hypertension in US adults is estimated to be 29%[3] and is expected to increase in the future.[1, 4] While about 5% of hypertension has known causes (classified as secondary hypertension), the majority of hypertension is due to unknown causes (classified as essential hypertension).[5] Genetic variation is thought to contribute to between 30–60% of inter-individual blood pressure (BP) variation[6] but the identity and nature of the contributing loci are largely unknown. As part of a search for single nucleotide polymorphisms (SNPs) associated with coronary heart disease (CHD), we identified a SNP in the Golgi SNAP Receptor Complex Member 2 gene (*GOSR2*) that was nominally associated with CHD. *GOSR2* is a Golgi-associated soluble N-ethylmaleimide-sensitive factor (NSF) attachment receptor (SNARE) protein involved in intra-Golgi protein trafficking that is expressed in multiple tissues.[7] Furthermore, *GOSR2* is located under a hypertension linkage peak on human chromosome 17,[8–10] as well as the syntenic rat chromosome 10, and murine chromosome 11.[11] Therefore, we investigated the association between the *GOSR2* SNP (Lys67Arg, rs197922) and essential hypertension in the Atherosclerosis Risk in Communities (ARIC) cohort and sought evidence that this effect might generalize to women participating in the Women's Genome Health Study (WGHS).

METHODS

Atherosclerosis Risk in Communities Study (ARIC)

Study Population—ARIC is a longitudinal cohort study of atherosclerosis and CHD. The population and study methods have been described in detail elsewhere.[12] Briefly, from 1987 to 1989, 15,792 participants aged 45–64 were sampled from four communities in the United States: Forsyth County, NC; Jackson, MS; suburban Minneapolis, MN; and Washington County, MD. At baseline and in three-year intervals following the baseline visit (1990–1992, 1993–1995, and 1996–1998) participants were interviewed and underwent clinical examinations. The study was approved by institutional review boards from each field center and written informed consent was obtained from all participants.

We excluded individuals from races other than white or black (*N*=48) or blacks from MN and MD (*N*=55) due to small numbers. We also excluded those who refused participation in genetic studies (*N*=44). Because incident CHD was the outcome in the primary study in

which *GOSR2* was selected as a gene of interest, participants were excluded for prevalent CHD (*N*=762), missing CHD (*N*=337), or prevalent stroke (*N*=331) at baseline leaving 14,215 participants (10,401 whites, and 3,814 blacks; 6,146 males and 8,069 females). During 196,069 person-years of follow up (mean 13.8 years), 1,747 (12%) of the 14,215 ARIC participants had an incident CHD event. In total, 13,389 participants (9,861 whites and 3,528 blacks; 5,787 males and 7,602 females) had complete information on *GOSR2* genotype. At baseline, 4,416 of the 13,389 participants (33%) had prevalent hypertension.

Measurements—Systolic BP (SBP) and diastolic BP (DBP) were measured after resting for 5 minutes in the seated position using a random-zero sphygmomanometer. Second and third readings were averaged to derive the BP measures used here. Hypertension was defined as SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or use of BP lowering medications in the past two weeks. Forty-one percent of blacks (*N*=1,454) and 23% of whites (*N*=2,242) reported using BP-lowering medication in the past two weeks. Incident hypertension was defined at visit four using the same definition as for baseline hypertension. Participants with hypertension at baseline were excluded from the incident measure. Baseline weight and height was measured with participants wearing scrub suits without shoes. BMI was calculated from weight and height as kg/m^2. High density lipoprotein cholesterol (HDL) was measured using standard methods.[13] Heart rate was measured using a 12-lead resting electrocardiogram. Education, smoking status and usual alcohol use were collected by structured interview. Prevalent stroke was defined by a history of stroke symptoms at baseline. Prevalent CHD included documented myocardial infarction (MI), a self-reported history of MI, or a self-reported history of cardiac surgical procedures at baseline. Incident CHD was defined by documented MI, unstable angina, sudden coronary death, or non-elective cardiovascular surgical procedures. Incident CHD events were determined through 2003. Follow-up time for CHD was calculated from the baseline visit date to the date of the first CHD event or until either December 31, 2003 or the last date of contact for those without CHD events. Genotypes were determined by an oligonucleotide ligation procedure that combined PCR amplification of target sequences from 3 ng of genomic DNA with subsequent allele-specific oligonucleotide ligation.[14] The ligation products of the two alleles were separated by hybridization to product-specific oligonucleotides, each coupled to spectrally distinct Luminex100 xMAP microspheres (Luminex, Austin, TX). The captured products were fluorescently labeled with streptavidin R-phycoerythrin (Prozyme, San Leandro, CA), sorted on the basis of microsphere spectrum, and detected by a Luminex100 instrument.

SNP Selection—We identified SNPs associated with CHD in two antecedent case-control studies of MI. Briefly, 20,009 SNPs (in 9,874 Entrez or Ensembl genes) were tested in 475 cases of MI and 619 non-MI controls. The 1,568 SNPs nominally associated with MI in this first study ($P<0.1$), were then tested in a second study of 793 MI cases and 1,000 healthy controls. Further details of the antecedent case-control studies can be found elsewhere.[15–17] Seventy-seven SNPs were nominally associated with MI ($P<0.1$) and had the same risk alleles in both studies (see online Supplementary Table S1). The risk alleles of 72 of these 77 SNPs were then tested in association with incident CHD in ARIC using Cox proportional hazards models, where each SNP was modeled in an additive genetic manner (i.e., 0, 1, or 2

risk alleles) along with gender and age. Five of the 77 SNPs were not tested in ARIC because we were unable to make good multiplex assays for them. One of the SNPs nominally associated with CHD in ARIC and the antecedent case-control studies was in *GOSR2*. We hypothesized that this SNP (Lys67Arg, rs197922) would be associated with essential hypertension since hypertension is a risk factor for CHD and *GOSR2* is located under a known hypertension linkage peak.[8–10] CHD results in ARIC for 34 of the 72 SNPs are reported in Morrison et al.[17] and Bare et al.[16] Results for the remaining 38 SNPs are reported in Supplementary Table S2.

Statistics—Means and standard deviations or frequencies and percents were calculated for continuous and categorical variables, respectively. Differences in means or frequencies by genotype were determined using the F-test or chi-square test as appropriate. Linear regression was used to analyze the association between the *GOSR2* SNP and continuous variables. Logistic regression was used to analyze the association between the *GOSR2* SNP and prevalent or incident hypertension. Minimally-adjusted regression models included the *GOSR2* SNP coded in an additive genetic manner (i.e., 0, 1, or 2 risk alleles), age and gender. Fully-adjusted models included age (years), high school education, BMI (kg/m^2), alcohol use (g/wk), current smoking, heart rate (beats/min), and HDL cholesterol (mmol/L). Regression models for BP additionally included anti-hypertensive medication use. Additive models have been shown to perform well even when the underlying inheritance model is recessive or dominant,[18, 19] so we report estimates for the additive model. Differences in results by gender, age and race were evaluated using likelihood ratio tests. No significant interactions by gender or age were detected; however, results differed by race for prevalent hypertension and BP ($P<0.1$). Therefore, results were stratified by race. Power to detect an association between the *GOSR2* SNP and hypertension with OR=1.1 was 79% among ARIC whites and 45% among blacks. The power to detect an association between genotype and a one-mmHg increase in SBP was 96% for whites and 53% for blacks.

Women's Genome Health Study (WGHS)—The WGHS includes 28,345 women initially enrolled in the Women's Health Study (WHS) who provided consent and sufficient blood samples for subsequent DNA analysis.[20] The WHS is a randomized trial designed to evaluate the benefits of aspirin and vitamin E therapy in preventing cardiovascular and cancer outcomes. Women who enrolled in the WHS from 1992–1995 were mostly white, initially healthy health professionals aged ≥45.[21] The WHS and WGHS were approved by the IRB of Brigham and Women's Hospital, Boston, MA. Baseline BP was self-reported within categories. Hypertension was defined as use of anti-hypertensive treatment at baseline, SBP≥140 mmHg, or DBP≥90 mmHg. *GOSR2* genotype was determined as described for the ARIC study. Since the BP data was collected in categories, ordinal logistic regression of 9 SBP categories (<110, 110–119, 120–129, 130–139, 140–149, 150–159, 160–169, 170–179, ≥180) or 7 DBP categories (<65, 66–74, 75–84, 85–89, 90–94, 95–104, ≥105) was used to determine associations between the *GOSR2* SNP and BP after adjusting for age and anti-hypertensive treatment. Binomial logistic regression was used to determine the association between the *GOSR2* SNP and hypertension adjusted for age. Analyses in the WGHS sample were restricted to whites with known *GOSR2* genotypes (*N*=25,372).

RESULTS

GOSR2 genotype frequencies differed by race (*P*<0.001; ARIC whites: ArgArg 43.2%, LysArg 44.6%, LysLys 12.2%; ARIC blacks: ArgArg 48.1%, LysArg 42.4%, LysLys 9.5%) but were consistent with Hardy-Weinberg expectations for both whites (chi-square=1.57; *P*=0.21) and blacks (chi-square=0.08; *P*=0.78).

Means and percentages for selected baseline variables are presented in Table 1 by race and genotype. Mean SBP (*P*<0.001) and DBP (*P*=0.01) differed significantly by genotype among whites. Among blacks, only mean SBP differed significantly by genotype (*P*=0.02). Prevalent hypertension differed by genotype among whites (*P*<0.01) but not blacks (*P*=0.66).

We assessed the association between the *GOSR2* SNP and hypertension after adjustment for traditional risk factors (Table 2). Among whites, the Lys67 allele was significantly associated with baseline hypertension (OR=1.09; *P*=0.01) as well as incident hypertension (OR=1.16, *P*<0.01 after adjustment for age and gender). Similar results were observed in fully adjusted models. Among blacks, the Lys67 allele was not associated with prevalent or incident hypertension after adjustment. In a genotypic assessment of the *GOSR2* variant and hypertension (Table 2), the OR for LysArg compared with ArgArg and LysLys compared with ArgArg were similar in magnitude after adjustment for age and gender for both baseline and incident hypertension among whites (baseline: LysArg OR=1.18; *P*<0.01, LysLys OR=1.13; *P*=0.11; incident: LysArg OR=1.25; *P*<0.01, LysLys OR=1.28; *P*=0.01) consistent with a dominant inheritance model.

Next, we assessed the association of the SNP with BP using linear regression (Table 3). The Lys67 allele was significantly associated with increased SBP in whites (0.87 mmHg per allele, *P*<0.001) and blacks (1.05 mmHg per allele, *P*=0.05) after adjusting for age, gender, and anti-hypertensive treatment. The Lys67 allele was significantly associated with DBP among whites (0.37 mmHg per allele, *P*=0.01) but not blacks (0.44 mmHg per allele, *P*=0.14) after adjustment. In fully-adjusted models, the estimates for increased BP per allele were similar in whites. However, in blacks, the association was weaker in the fully-adjusted model. Allele frequencies of Lys67Arg in whites from the WGHS were similar to those of whites in ARIC (data not shown). We observed a modest effect for the Lys67 allele of *GOSR2* in this WGHS population on increased SBP (OR=1.03, *P*=0.04, in an ordinal logistic regression of nine SBP categories after adjusting for age). However, this association was no longer significant after additionally adjusting for use of anti-hypertensive medications (OR=1.03, *P*=0.11). There was no association with DBP or other measures of hypertension in the WGHS (data not shown).

DISCUSSION

In this study, the Lys67 allele of rs197922 was significantly associated with hypertension, SBP and DBP in white participants from the large, bi-racial ARIC study. The Lys67 allele had been nominally associated with increased risk of CHD in multiple antecedent studies (see Supplementary Tables). The impetus for investigating the association between the

GOSR2 Lys67 allele and hypertension was that hypertension is a known risk factor for CHD and the *GOSR2* gene lies under a known linkage peak for hypertension.[8–10] Whether or not this *GOSR2* polymorphism accounts for the linkage peak cannot be determined from this study of unrelated individuals. The *GOSR2* Lys67 allele was associated with hypertension in ARIC whites at baseline (OR=1.09; *P*=0.01). This Lys67 allele was also associated with baseline quantitative traits such as SBP and DBP among whites in ARIC. In blacks, the only significant association was with SBP. Interestingly, associations between the SNP and incident hypertension in ARIC were strengthened as compared to estimates for prevalent hypertension.

To address the generalizability of these data, the *GOSR2* SNP was evaluated in the WGHS. In this cohort, an association was observed between the Lys67 allele and increased SBP after adjusting for age (OR=1.03, *P*=0.04). The effect was similar in magnitude and direction after adjustment for anti-hypertensive medications (OR=1.03, *P*=0.11). The more modest effect seen in the WGHS as compared to the ARIC study may be explained by differences in the distribution of potential modifying factors (such as smoking) between the two studies since ARIC participants were from a population-based sample of four communities while WGHS participants were initially healthy women in health professions.

The Wellcome Trust Case Control Consortium (WTCCC) reported significant associations (*P*<0.02 for a one-parameter model) between hypertension and four SNPs in linkage disequilibrium (LD) (r^2>0.8) with the Lys67Arg SNP.[22] These four SNPs span a 37 kb region encompassing the *GOSR2* gene. One of the four SNPs (rs1867237) is located in an intronic region of *GOSR2*, but the other three SNPs are not in known genes (rs9911967, rs197912, rs1662594). Of the four SNPs, rs197912 is most highly correlated with the Lys67Arg *GOSR2* SNP (r^2=0.92 in the HapMap CEU population).[23] The association between rs197912 and hypertension in the WTCCC was similar in magnitude and direction to the association between hypertension and the *GOSR2* SNP reported here for ARIC whites (OR=1.11 for heterozygotes compared with major homozygotes and OR=1.21 for minor compared with major homozygotes). The combined evidence suggests that the contribution of *GOSR2* gene variation to the etiology of hypertension is modest but deserves further consideration.

GOSR2 maps to chromosome 17q21,[24] the same location at which Julier et al.[10] had previously reported linkage for familial essential hypertension, making *GOSR2* an obvious positional candidate gene. Since then, hypertension and quantitative BP traits have been linked to this region.[11] The strongest evidence for linkage to 17q21 to date is from a genome-wide linkage study of long-term SBP by Levy et al.[8] A LOD>4 at 17q12-21 was reported in a sample of 1,585 subjects with complete SBP measurements over time from 332 large families from the Framingham Heart Study. Evidence for linkage with hypertension has also been reported for orthologous chromosomal regions in mice and rats.[11] Despite the evidence for the region's contribution to BP and risk of hypertension, we found no previous reports of association between *GOSR2* variation and hypertension. Other genes in this region may be considered hypertension candidates, but of these only *MYL4* (myosin light polypeptide 4) is in a LD region with *GOSR2* rs197922. Using data from the International

HapMap Project,[23] one SNP (rs16941671) from *MYL4* was in slight LD with *GOSR2* rs197922 (r^2=0.19) in whites.

The mechanism by which *GOSR2* may contribute to BP traits is not clear. *GOSR2* codes for a vesicular N-ethylmaleimide sensitive factor attachment protein receptor (v-SNARE) that is involved in intra-Golgi trafficking of vesicles.[25] v-SNAREs such as GOSR2 interact with target-localized SNAREs (t-SNAREs) to allow directed movement of macromolecules, such as insulin, leptin, and angiotensinogen, between Golgi compartments.[26–28] Unfortunately, as of this writing, there is no report describing a phenotype, if any, of a *GOSR2* knock-out mouse. Further studies of this variant's contribution to BP in animal models would be informative.

Our study has several limitations to consider when interpreting results. First, despite sufficient power to detect associations in whites, power was limited in blacks. Evaluation of this association in a larger black study is warranted. Second, in this study, only one SNP in *GOSR2* was tested in association with BP traits. Therefore we cannot rule out the possibility that our results are due to LD with other SNPs. However, we found no other exonic SNPs in LD with Lys67Arg (rs197922) within *GOSR2* or other nearby candidate genes. Third, we did not find strong replication of the *GOSR2* variant and BP traits in the WGHS since results were not statistically significant after adjusting for anti-hypertensive medication. However, the WGHS results approached statistical significance and were similar in magnitude and direction to results in ARIC. Furthermore, several SNPs in LD with Lys67Arg were significantly associated with hypertension in the WTCCC data. Fourth, since hypertension is a strong risk factor for CHD, it's possible that the SNP-hypertension result is explained by underlying CHD. Nevertheless, hypertension and BP linkage studies provide support for our conclusions.

In summary, we report modest associations between variation at Lys67Arg (rs197922) in *GOSR2* with BP and essential hypertension in ARIC whites that have not been previously reported. Results for SBP in ARIC blacks and in whites from the WGHS were marginally significant and consistent in direction with those of ARIC whites. SNPs in LD with the Lys67Arg SNP were significantly associated with hypertension in the WTCCC study with similar magnitude to the association in ARIC. While effect sizes were modest, limiting utility as a clinical diagnostic, these findings provide further documentation of the polygenic nature of hypertension and the complex regulatory mechanisms controlling blood pressure levels. *GOSR2* codes for a vesicular membrane protein involved in intra-Golgi protein trafficking. Therefore, we can speculate that altered regulation of protein trafficking within the Golgi may contribute to determination of BP and essential hypertension. Future studies to validate our findings and to understand the mechanism through which intra-Golgi protein processing may influence BP and essential hypertension are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline descriptive information by *GOSR2* genotype

	Whites (*N*=9,861)				Blacks (*N*=3,528)			
	ArgArg (*N*=4,264)	**LysArg (*N*=4,398)**	**LysLys (*N*=1,199)**	***P*[a]**	**ArgArg (*N*=1,698)**	**LysArg (*N*=1,494)**	**LysLys (*N*=336)**	***P*[a]**
Characteristic (mean±s.d.)								
SBP (mm Hg)[b]	117.4±16.5	118.9±17.0	119.0±17.3	< 0.001	127.9±20.6	128.3±20.9	131.4±22.2	0.02
DBP (mm Hg)[b]	71.3±9.8	71.9±10.1	72.0±10.4	0.01	79.6±11.7	79.5±12.1	80.7±12.2	0.36
Age, y	54±5.7	54±5.7	54±5.8	0.26	53±5.7	53±5.9	54±5.8	0.01
BMI (kg/m^2)	26.8±4.8	27.1±4.9	26.8±4.9	0.13	29.6±6.2	29.6±6.0	29.6±6.0	0.83
Usual Alcohol Use (g/wk)	46.8±93.9	44.2±92.2	44.6±87.0	0.27	31.0±93.7	31.9±89.0	41.1±142.9	0.18
Heart Rate (beats/min)	66.6±9.8	66.7±10.1	66.5±10.0	0.83	66.9±11.1	66.8±10.8	66.6±10.7	0.53
HDL cholesterol (mmol/L)	1.3±0.4	1.3±0.4	1.3±0.4	0.96	1.4±0.4	1.4±0.5	1.4±0.5	0.13
No. (%)								
Prevalent hypertension	1,012 (24)	1,181 (27)	314 (26)	<0.01	926 (55)	797 (53)	186 (55)	0.66
Male	1,952 (46)	1,983 (45)	551 (46)	0.77	643 (38)	542 (36)	116 (35)	0.42
High school education	3,636 (85)	3,621 (82)	1,012 (84)	<0.01	999 (59)	906 (61)	211 (63)	0.31
Current smoker	1,051 (25)	1,076 (24)	284 (24)	0.80	520 (31)	405 (27)	103 (31)	0.07

s.d., standard deviation;

[a] *P*-values are for the differences between genotypes (F-test for continuous variables or X^2 test for categorical variables).

[b] Adjusted for use of anti-hypertensive treatment.

Table 2

Association between *GOSR2* (Lys67Arg, rs197922) and hypertension (HYT)

	Whites (N=9,822)					Blacks (*N*=3,513)				
	n	Minimally adjusted		Fully-adjusted		n	Minimally adjusted		Fully-adjusted	
		OR (95% CI)[a]	*P*[b]	OR (95% CI)[c]	*P*[b]		OR (95% CI)[a]	*P*[b]	OR (95% CI)[c]	*P*[b]
Prevalent HYT										
Additive Model	2,507	1.09 (1.02–1.17)	0.01	1.09 (1.02–1.17)	0.02	1,909	0.96 (0.87–1.07)	0.47	0.97 (0.87–1.08)	0.62
Genotypic Model										
ArgArg	1,012	Ref		Ref		926	Ref		Ref	
LysArg	1,181	1.18 (1.06–1.30)	<0.01	1.14 (1.03–1.27)	0.01	797	0.93 (0.81–1.08)	0.34	0.94 (0.81–1.09)	0.39
LysLys	314	1.13 (0.97–1.31)	0.11	1.15 (0.98–1.34)	0.08	186	0.96 (0.76–1.23)	0.77	0.99 (0.77–1.28)	0.95
Incident HYT										
Additive Model	1,506	1.16 (1.07–1.27)	<0.01	1.16 (1.06–1.27)	<0.01	458	1.04 (0.87–1.26)	0.65	1.03 (0.85–1.25)	0.76
Genotypic Model										
ArgArg	604	Ref		Ref		212	Ref		Ref	
LysArg	703	1.25 (1.10–1.42)	<0.01	1.23 (1.08–1.40)	<0.01	207	1.17 (0.91–1.50)	0.23	1.20 (0.93–1.57)	0.16
LysLys	199	1.28 (1.06–1.55)	0.01	1.29 (1.06–1.56)	<0.01	39	0.94 (0.61–1.46)	0.78	0.86 (0.54–1.37)	0.52

n, number of affected; OR, odds ratio; CI, confidence interval;

[a] Adjusted for baseline gender and age.

[b] *P* is Wald test *P*-value.

[c] Adjusted for baseline gender, age (years), high school education, BMI (kg/m^2), alcohol use (g/wk), current smoking, heart rate (beats/min), and HDL cholesterol (mmol/L).

Table 3

Association between *GOSR2* (Lys67Arg, rs197922) and measures of blood pressure

	Whites (N=9,822)				**Blacks (*N*=3,513)**			
	Minimally adjusted		**Fully-adjusted**		**Minimally adjusted**		**Fully-adjusted**	
	β (95% CI)[a,c]	***P*[b]**	**β (95% CI)[c,d]**	***P*[b]**	**β (95% CI)[a,c]**	***P*[b]**	**β (95% CI)[c,d]**	***P*[b]**
SBP (mmHg)	0.87 (0.41–1.33)	< 0.001	0.83 (0.39–1.27)	<0.001	1.05 (0.03–2.08)	0.05	0.96 (−0.07–1.98)	0.07
DBP (mmHg)	0.37 (0.09–0.65)	0.01	0.36 (0.09–0.63)	0.01	0.44 (−0.14–1.03)	0.14	0.34 (−0.24– 0.93)	0.25

CI, confidence interval; SBP, systolic blood pressure; DBP, diastolic blood pressure;

[a] Adjusted for baseline gender and age.

[b] *P* is Wald test *P*-value.

[c] Further adjusted for use of anti-hypertensive treatment.

[d] Adjusted for baseline gender, age (years), high school education, BMI (kg/m^2), alcohol use (g/wk), current smoking, heart rate (beats/min), and HDL cholesterol (mmol/L).