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## Gene Variants Associated With Ischemic Stroke:

### The Cardiovascular Health Study

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### Abstract

**Background and Purpose**—The purpose of this study was to determine whether 74 single nucleotide polymorphisms (SNPs), which had been associated with coronary heart disease, are associated with incident ischemic stroke.

**Methods**—Based on antecedent studies of coronary heart disease, we prespecified the risk allele for each of the 74 SNPs. We used Cox proportional hazards models that adjusted for traditional risk factors to estimate the associations of these SNPs with incident ischemic stroke during 14 years of follow-up in a population-based study of older adults: the Cardiovascular Health Study (CHS).

**Results**—In white CHS participants, the prespecified risk alleles of 7 of the 74 SNPs (in *HPS1*, *ITGAE*, *ABCG2*, *MYH15*, *FSTL4*, *CALM1*, and *BAT2*) were nominally associated with increased risk of stroke (one-sided  $P < 0.05$ , false discovery rate=0.42). In black participants, the prespecified risk alleles of 5 SNPs (in *KRT4*, *LY6G5B*, *EDG1*, *DMXL2*, and *ABCG2*) were nominally associated with stroke (one-sided  $P < 0.05$ , false discovery rate=0.55). The Val12Met SNP in *ABCG2* was associated with stroke in both white (hazard ratio, 1.46; 90% CI, 1.05 to 2.03) and black (hazard ratio, 3.59; 90% CI, 1.11 to 11.6) participants of CHS. Kaplan-Meier estimates of the 10-year cumulative incidence of stroke were greater among Val allele homozygotes than among Met allele carriers in both white (10% versus 6%) and black (12% versus 3%) participants of CHS.

**Conclusions**—The Val12Met SNP in *ABCG2* (encoding a transporter of sterols and xenobiotics) was associated with incident ischemic stroke in white and black participants of CHS.

### Keywords

brain infarction; cerebrovascular accident; epidemiology; genetics; prevention; risk factors

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### Disclosures

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Multiple reports based on twin studies<sup>1,2</sup> and family studies<sup>3,4</sup> have shown that genetics contributes to risk of stroke independently of traditional risk factors. Identifying gene variants that are associated with risk of ischemic stroke could shed light on the mechanisms of stroke pathology and potentially lead to new methods for treatment or prevention of this serious and complex disease.

Ischemic stroke and coronary heart disease (CHD) are vascular diseases that, although affecting different vascular beds, share many traditional risk factors such as age, sex, smoking, diabetes, and hypertension.<sup>5</sup> Ischemic stroke and CHD also share major pathological processes such as atherosclerosis and thrombosis. We hypothesized that some gene variants associated with CHD would also be associated with ischemic stroke. We selected 74 single nucleotide polymorphisms (SNPs) that had been previously shown to be associated with CHD in at least one antecedent association study<sup>6</sup> and tested whether the risk alleles of these 74 SNPs were associated with increased risk of incident ischemic stroke in a prospective population-based study of older adults, the Cardiovascular Health Study (CHS).<sup>7,8</sup>

## Materials and Methods

### Cardiovascular Health Study

CHS is a prospective population-based study of risk factors for cardiovascular disease, including CHD and stroke, in older adults. Men and women aged  $\geq 65$  years were recruited from random samples of individuals on Medicare eligibility lists in 4 US communities (Sacramento County, Calif; Washington County, Md; Forsyth County, NC; and Pittsburgh, Allegheny County, Pa) and from age-eligible members of the same households. Potential participants were excluded if they were institutionalized, not ambulatory at home, under hospice care, receiving radiation or chemotherapy for cancer, not expected to remain in the area for at least 3 years, or unable to be interviewed. CHS enrolled 5201 participants in 1989 to 1990. An additional 687 black participants entered the cohort in 1992 to 1993. Participants who did not donate DNA or who did not consent to the use of their DNA for studies by private companies ( $n=514$ ) as well as participants for whom the amount of DNA samples were insufficient ( $n=130$ ) were excluded, leaving 5244 participants available for a genetic study. The Institutional Review Board at each site approved the study methods, and all participants gave written informed consent. Details of CHS design<sup>7</sup> and recruitment<sup>8</sup> have been reported.

Participants completed a baseline clinic examination that included a medical history interview, physical examination, and blood draw.<sup>9</sup> Baseline self-reported myocardial infarction (MI) or stroke was confirmed by information from the clinic examination or by review of medical records or physician questionnaires.<sup>10</sup> Cardiovascular events during follow-up were identified at semiannual contacts, which alternated between clinic visits and telephone calls. Suspected events were adjudicated according to standard criteria by a physician review panel using information from medical records, brain imaging studies,<sup>11</sup> and, in some cases, interviews with the physician, participant, or a proxy informant.<sup>12</sup> Medicare use files were searched to ascertain events that may have been missed.<sup>13</sup>

At baseline, 722 of the 5244 participants available for a genetic study had a history of stroke or MI. Because the risk of incident ischemic stroke might be influenced by whether a patient had a prior stroke or MI, these 722 participants were excluded from the analysis, leaving 4522 (3849 white and 673 black) participants in this genetic study of first incident ischemic stroke. Baseline characteristics of these 4522 participants are presented in Table 1. During follow-up, 642 participants had an incident nonprocedure-related stroke, and 47 of these 642 had an MI before their stroke, leaving 595 stroke events. Of these 595 stroke events, 72 (12%) were hemorrhagic, 46 (8%) were not classified for type, and the remaining 477 stroke events were classified as ischemic stroke events, the end point for this analysis.

## Covariates

Risk estimates for ischemic stroke were adjusted for the following traditional risk factors: diabetes mellitus (defined by fasting serum glucose levels of at least 126 mg/dL or the use of either insulin or oral hypoglycemic medications), impaired fasting glucose (defined as a fasting glucose levels of 110 to 125 mg/dL<sup>14</sup>), hypertension (defined by systolic blood pressure of at least 140 mm Hg, diastolic blood pressure of at least 90 mm Hg, or a physician's diagnosis of hypertension plus the use of antihypertensive medications<sup>10</sup>), current smoking, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and body mass index. Other covariates included atrial fibrillation, carotid intima media thickness, and genotypes. Atrial fibrillation was identified on the basis of 12-lead resting electrocardiograms performed at the baseline examination. Tracings were read for atrial fibrillation or flutter at the CHS Electrocardiography Reading Center.<sup>15</sup> Ultrasonography of the common and internal carotid arteries was also performed at baseline. The intima media thickness was defined as the mean of the maximum intima media thicknesses of the near and far walls of the left and right carotid arteries.<sup>16</sup> Genotypes of the CHS participants were determined by a multiplex method that combines polymerase chain reaction, allele-specific oligonucleotide ligation assays, and hybridization to oligonucleotides coupled to Luminex 100TM xMAP microspheres (Luminex, Austin, Texas) followed by detection of the spectrally distinct microsphere on a Luminex 100 instrument (Supplemental text in reference <sup>6</sup>).

## Prespecification of Risk Alleles for 74 Single Nucleotide Polymorphisms Investigated in CHS

For each of the 74 SNPs that were genotyped in CHS, we prespecified a risk allele based on antecedent data (Supplemental Table I in reference <sup>6</sup>). For 14 of the 74 SNPs, genetic associations with CHD have been previously published.<sup>17–21</sup> The remaining 60 SNPs were associated with MI in one or more antecedent studies of MI as described (Supplemental Text and Table II in reference <sup>6</sup>).

## Statistics

Because the risk estimate for gene variants can differ between whites and blacks, we investigated the association of SNPs with incident ischemic stroke in CHS in each race separately. We conducted analyses of time to primary end point. Follow-up began at CHS enrollment and ended on the date of incident stroke of any type, incident MI, death, loss to follow-up, or June 30, 2004, whichever occurred first. The median follow-up time was 11.2 years (11.9 years for the 1989 to 1990 cohort and 10.7 years for the black cohort).

Cox regression models were used to estimate hazard ratios of each SNP. In Model 1, Cox models were adjusted for baseline age (continuous) and sex. In Model 2, Cox models were adjusted for baseline age (continuous), sex, body mass index (continuous), current smoking, diabetes, impaired fasting glucose, hypertension, LDL cholesterol (continuous), and HDL cholesterol (continuous). Risk estimates were also further adjusted for 2 additional risk factors of ischemic stroke: atrial fibrillation and carotid intima media thickness. The SNP variable in the Cox models was coded as 0 for the nonrisk homozygote, 1 for those who carried one copy of the risk allele, and 2 for those who carried 2 copies of the risk allele. Thus, the hazard ratios represent the log-additive increase in risk for each additional copy of the risk allele a subject carried compared with the nonrisk homozygotes. Because we were testing the hypotheses that the allele associated with increased risk of CHD would also be associated with increased risk of ischemic stroke, we used a one-sided probability value to test the significance of the Cox model coefficients. Correspondingly, we estimated 90% CIs for the hazard ratios (for hazard ratios greater than one, there is 95% confidence that a true risk estimate is greater than the lower bound of a 90% CI). In white participants, this study had 80% or more power to detect associations between SNPs and incident ischemic stroke for SNPs that have relative risks of 1.3 and 1.5 (in an additive model) and risk allele frequencies of 0.13 and 0.05, respectively,

assuming an alpha level of 0.05 and a one-sided test. In black participants, this study had 80% or more power to detect associations between SNPs and incident ischemic stroke for SNPs that have relative risks of 1.6 and 1.8 (in an additive model) and risk allele frequencies of 0.3 and 0.14, respectively. The cumulative incidence of stroke was estimated by the method of Kaplan and Meier. Data were analyzed using Stata Statistical Software.<sup>22</sup>

The influence of multiple testing was evaluated using the false discovery rate (FDR)<sup>23</sup> to estimate the expected fraction of false-positives in a group of SNPs with probability values below a given threshold. An FDR of 1 would indicate that all the nominally associated results are expected to be false discoveries, and an FDR of 0 would indicate that none of the nominally associated results are expected to be false discoveries. FDR calculations were performed with R Statistical Software.<sup>24</sup>

## Results

The baseline characteristics of the 3849 white and 673 black participants of CHS in this genetic study of ischemic stroke are presented in Table 1. There were 407 first incident ischemic stroke events in the white participants and 70 in the black participants during follow-up (median of 11.2 years). We investigated the association between incident ischemic stroke and 74 SNPs that had previously been found to be associated with CHD in one or more antecedent studies.<sup>6</sup> Specifically, for each SNP, we asked if the allele that had been associated with increased risk of CHD (the risk allele) was also associated with increased risk of stroke.

In white participants of CHS, we found that the risk alleles of 7 of these 74 SNPs were nominally associated ( $P < 0.05$ ) with increased risk of stroke after adjusting for traditional risk factors (age, sex, body mass index, smoking, diabetes, impaired fasting glucose, hypertension, LDL cholesterol, and HDL cholesterol). These 7 SNPs were in *HPS1*, *ITGAE*, *ABCG2*, *MYH15*, *FSTL4*, *CALM1*, and *BAT2*. The additive (per allele) hazard ratios for stroke ranged from 1.15 to 1.49 (Table 2). In black participants of CHS, we found that the risk alleles of 5 SNPs (in *KRT4*, *LY6G5B*, *EDG1*, *DMXL2*, and *ABCG2*) were nominally associated ( $P < 0.05$ ) with increased risk of stroke after adjusting for traditional risk factors. The hazard ratios for these 5 SNPs ranged from 1.40 to 3.59 (Table 3). The risk estimates for the 11 SNPs that were associated with stroke in either whites or blacks (Tables 2 and 3) were essentially unchanged when further adjusted for atrial fibrillation and internal carotid artery intima media thickness (data not shown). The associations between incident ischemic stroke and all 74 SNPs are shown in Supplemental Table, available online at <http://stroke.ahajournals.org>.

To account for multiple comparisons, we estimated the FDR<sup>23</sup> for the set of SNPs found to be nominally associated with incident ischemic stroke in CHS participants. These FDRs were 0.42 for the 7 SNPs in white and 0.55 for the 5 SNPs in black participants of CHS.

*ABCG2* Val12Met (rs2231137) was the only SNP associated with incident ischemic stroke in both white and black participants of CHS. The risk of ischemic stroke was higher in Val allele homozygotes than in Met allele carriers. The adjusted hazard ratio for Val allele homozygotes, compared with Met allele carriers, was 1.50 (90% CI, 1.06 to 2.12) in whites and 3.62 (90% CI, 1.11 to 11.9) in black participants (Table 4). The 10-year cumulative incidence of ischemic stroke was greater in Val allele homozygotes than in Met allele carriers in both the white (10% versus 6%) and black (12% versus 3%; Figure) participants of CHS.

## Discussion

Among 74 genetic variants tested in CHS, we found that 7 were nominally associated with incident ischemic stroke in white participants and 5 were nominally associated with incident ischemic stroke in black participants. The FDRs of 0.42 (for the set of 7 associated SNPs in

whites) and 0.55 (for the set of 5 associated SNPs in blacks) suggest that some of these nominally associated SNPs are true-positives. The most notable finding, consistent in both whites and blacks, was the association between the Val allele of *ABCG2* Val12Met and increased risk of incident ischemic stroke.

Three of the 11 gene variants nominally associated with incident ischemic stroke in CHS had particularly notable associations with CHD in the antecedent studies. The first of these 3 gene variants was the Val allele of *ABCG2* Val12Met (rs2231137). This gene variant had previously been found to be associated with angiographically defined severe coronary artery disease in 2 case-control studies.<sup>20</sup>

*ABCG2* encodes the ATP-binding cassette, subfamily G, member 2, which is a protein that belongs to a large family of transporters. It is expressed on the cell surface of stem cells in bone marrow and skeletal muscle,<sup>25</sup> progenitor endothelial cells that are capable of vasculogenesis in adipose tissue,<sup>26</sup> and endothelial cells in blood vessels of the heart<sup>27</sup> and brain.<sup>28</sup> The *ABCG2* protein has been reported recently to transport sterols.<sup>29,30</sup> It is interesting to note that the related ATP-binding cassette proteins ABCA1,<sup>31</sup> ABCG5, and ABCG8<sup>32</sup> are transporters of lipids; variants of these transporters have been shown to cause lipid disorders such as Tangier disease<sup>31</sup> and sitosterolemia.<sup>32</sup> However, a well-known function of the *ABCG2* protein is to act as a multidrug transporter of anticancer drugs, and the *ABCG2* protein is overexpressed in drug-resistant cancer cells.<sup>33</sup> The Met variant of *ABCG2* has been reported to confer lower drug resistance and have altered pattern of localization when compared with the Val variant.<sup>34</sup> We speculate that the Met variant of the *ABCG2* protein may function in the vascular endothelium and have an altered function as a transporter. Homozygotes of the Val allele of *ABCG2* (88% of whites and 88% of blacks) were at higher risk of stroke than carriers of the Met allele in CHS. Because there were only 16 homozygotes of the Met allele, the Met homozygotes were pooled with heterozygotes and used as the reference group. The Met allele could also be considered to be a protective allele in that the Met allele carriers had a lower risk of incident ischemic stroke than the Val allele homozygotes.

The second of the 3 gene variants with notable findings in antecedent studies is the Ala allele of *MYH15* Thr1125Ala (rs3900940). In addition to being associated with MI in 2 antecedent association studies,<sup>6</sup> it was associated with increased risk of incident CHD in the white participants of the Atherosclerosis Risk in Communities Study.<sup>21</sup> *MYH15* encodes myosin heavy polypeptide 15 and the Thr1125Ala SNP is located in the tail domain of the MYH15 protein.<sup>35</sup> It is not clear how MYH15 might be involved in vascular biology or how the Ala substitution in MYH15 affects the risk of vascular disease.

The third gene variant with notable findings in antecedent studies is the G allele of rs3814843 in the 3' untranslated region in *CALM1*. This SNP was associated with angiographically defined severe coronary artery disease in 2 case-control studies.<sup>20</sup> *CALM1* encodes calmodulin 1, which binds calcium and functions in diverse signaling pathways, including those involved in cell division,<sup>36</sup> membrane trafficking,<sup>37</sup> and platelet aggregation.<sup>38</sup> The functional consequence of the rs3814843 SNP in the *CALM1* gene remains to be investigated.

Potential limitations of this study include the advanced age of CHS participants, who were  $\geq 65$  at enrollment; genetic association results obtained in the CHS cohort may have been affected by survival bias. Additionally, because CHD was the end point in the antecedent studies, variants that are associated with stroke but not with CHD would not have been tested in this study. Although the 74 SNPs investigated in this study had been found to be associated with CHD in antecedent studies and 16 of these 74 SNPs had also been found to be associated with MI in either the white or black participants of CHS,<sup>6</sup> none of these 16 SNPs were associated with stroke in CHS. Although the failure to identify SNPs that were associated with both MI



and stroke in CHS could be a reflection of genetic risk factor that differ between MI and stroke, it could also result from low power to detect associations in CHS. Finally, a fraction of the 74 SNPs we tested may have been false-positives in the antecedent studies of CHD.

## Summary

In conclusion, we found that a subset of gene variants previously associated with CHD in antecedent studies were also associated with incident ischemic stroke in CHS; however, in CHS, none of these SNPs were associated with both MI and stroke. Notably, the Val allele of the Val12Met SNP in *ABCG2* (which encodes a transporter of sterols and anticancer drugs) was associated with increased risk of incident ischemic stroke in both white and black participants of CHS. Nevertheless, results from this study should be further validated in other populations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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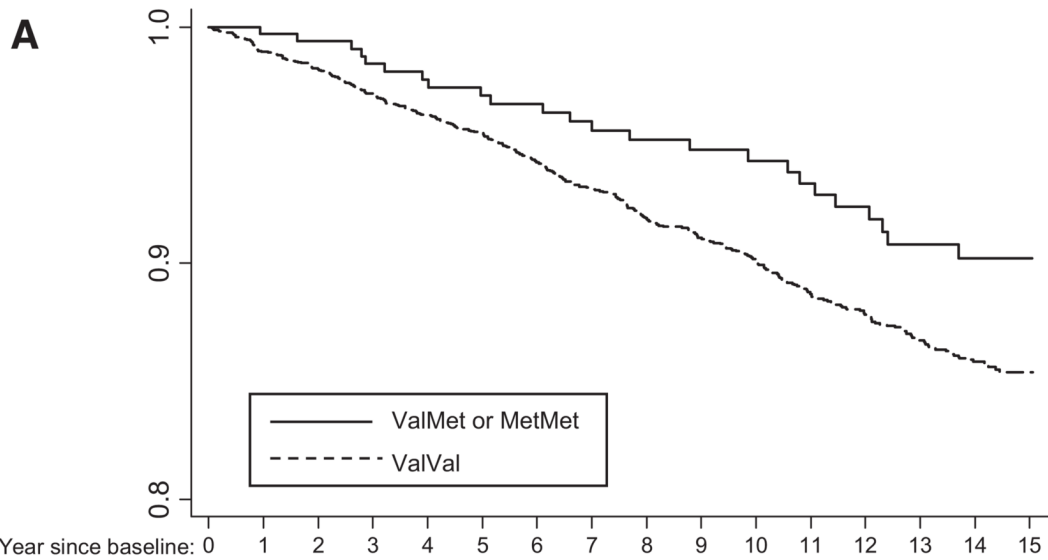
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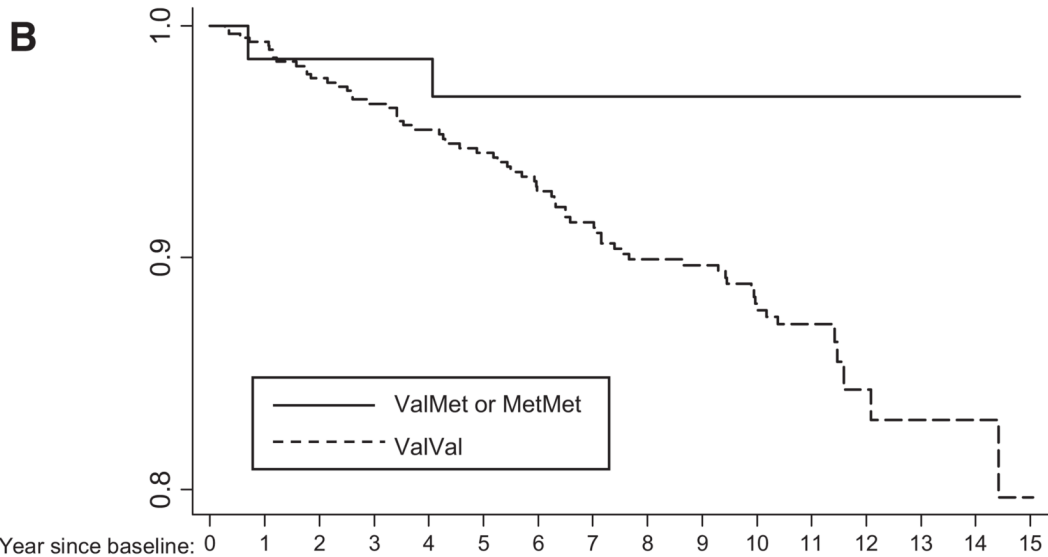
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Number at risk

ValMet or MetMet	335	331	321	307	292	280	262	248	236	220	207	189	179	164	145	4
ValVal	3398	3286	3185	3056	2940	2778	2625	2448	2280	2123	1972	1817	1668	1528	1344	51



Number at risk

ValMet or MetMet	70	68	65	62	60	53	51	50	48	45	43	40	9	9	9	0
ValVal	592	570	546	523	496	464	434	403	375	345	309	267	64	59	53	1

**Figure.**  
 Comparison of Kaplan-Meier estimates of the cumulative incidence of ischemic stroke among Val allele homozygotes of the *ABCG2* Val12Met and among Met allele carriers in white (A) and in black (B) participants of CHS.

**Table 1**

## Baseline Characteristics of CHS Participants in This Ischemic Stroke Study

Characteristic	Whites	Blacks
No. of individuals in this analysis	3849	673
Male	1575 (41)	243 (36)
Age, mean (SD), years	72.7 (5.6)	72.9 (5.7)
Body mass index, mean (SD), kg/m <sup>2</sup>	26.3 (4.5)	28.5 (5.6)
Smoking, current	423 (11)	113 (17)
Diabetes	511 (13)	151 (23)
Impaired fasting glucose	522 (14)	92 (14)
Hypertension	2110 (55)	490 (73)
LDL cholesterol, mean (SD), mg/dL	130 (36)	129 (36)
HDL cholesterol, mean (SD), mg/dL	54 (16)	58 (15)
Total cholesterol, mean (SD), mg/dL	212 (39)	210 (39)

Data presented as number of participants (%) unless otherwise indicated.

**Table 2**  
Gene Variants Associated With Incident Ischemic Stroke in White Participants of CHS

Gene	dbSNP ID	Risk Allele*	Allele Frequency <sup>†</sup>	HR <sup>‡</sup> (90% CI)	P
<i>HPS1</i>	rs1804689	T	0.30	1.23 (1.09–1.40)	0.003
<i>ITGAE</i>	rs220479	C	0.82	1.26 (1.08–1.48)	0.008
<i>ABCG2</i>	rs2231137	C	0.95	1.46 (1.05–2.03)	0.03
<i>MYH15</i>	rs3900940	C	0.29	1.15 (1.02–1.31)	0.03
<i>FSTL4</i>	rs13183672	A	0.76	1.17 (1.01–1.35)	0.04
<i>CALM1</i>	rs3814843	G	0.05	1.31 (1.02–1.68)	0.04
<i>BAT2</i>	rs11538264	G	0.97	1.49 (1.02–2.16)	0.04

\* Risk allele was defined to be the CHD risk allele as described in Supplemental Table 1 in reference 6.

<sup>†</sup> Frequency of the risk allele in white participants of CHS.

<sup>‡</sup> Hazard ratios (HRs) are adjusted for baseline age, sex, body mass index, current smoking, diabetes, impaired fasting glucose, hypertension, LDL cholesterol, and HDL cholesterol at baseline. HRs are per copy of the risk allele.

**Table 3**  
Gene Variants Associated With Incident Ischemic Stroke in Black Participants of CHS

Gene	dbSNP ID	Risk Allele*	Allele Frequency <sup>†</sup>	HR <sup>‡</sup> (90% CI)	P
<i>KRT4</i>	rs89962	T	0.11	2.08 (1.48–2.94)	<0.001
<i>LY6G5B</i>	rs11758242	C	0.89	2.28 (1.20–4.33)	0.02
<i>EDG1</i>	rs2038366	G	0.74	1.59 (1.08–2.35)	0.02
<i>DMXL2</i>	rs12102203	G	0.47	1.40 (1.03–1.90)	0.04
<i>ABCG2</i>	rs2231137	C	0.95	3.59 (1.11–11.7)	0.04

\* Risk allele was defined to be the CHD risk allele as described in Supplemental Table 1 in reference 6.

<sup>†</sup> Frequency of the risk allele in black participants of CHS.

<sup>‡</sup> Hazard ratios (HRs) are adjusted for baseline age, sex, body mass index, current smoking, diabetes, impaired fasting glucose, hypertension, LDL cholesterol, and HDL cholesterol at baseline. HRs are per copy of the risk allele.

The Val Allele Homozygotes of *ABCG2* Val12Met, Compared With the Met Allele Carriers Are Associated With Increased Risk of Incident Ischemic Stroke in Both White and Black Participants of CHS

**Table 4**

<i>ABCG2</i> Genotype	Events, n	Total, n	Model 1*		Model 2*	
			HR (90% CI)	P	HR (90% CI)	P
White						
ValVal	370	3398	1.58 (1.12–2.23)	0.02	1.50 (1.06–2.12)	0.03
ValMet+MetMet	24	335	1 (Reference)		1 (Reference)	
ValMet	23	321				
MetMet	1	14				
Black						
ValVal	66	592	3.80 (1.16–12.4)	0.03	3.62 (1.11–11.9)	0.04
ValMet+MetMet	2	70	1 (Reference)		1 (Reference)	
ValMet	2	69				
MetMet	0	1				

\* Model 1 was adjusted for baseline age and sex. Model 2 was adjusted for baseline age, sex, body mass index, current smoking, diabetes, impaired fasting glucose, hypertension, LDL cholesterol, and HDL cholesterol.