Published in final edited form as:
Hypertension. 2011 January ; 57(1): 116–122. doi:10.1161/HYPERTENSIONAHA.110.161695.

GENETIC VARIATION IN SOLUBLE EPOXIDE HYDROLASE (*EPHX2*) IS ASSOCIATED WITH FOREARM VASODILATOR RESPONSES IN HUMANS

Craig R. Lee, Pharm.D., Ph.D., **Mias Pretorius, M.D.**, **Robert N. Schuck, Pharm.D.**, **Lauranell H. Burch, Ph.D.**, **Jackie Bartlett, M.S.**, **Scott M. Williams, Ph.D.**, **Darryl C. Zeldin, M.D.**, and **Nancy J. Brown, M.D.**
Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC (C.R.L., R.N.S.); Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC (L.H.B., D.C.Z.); Department of Anesthesiology (M.P.), Center for Human Genetics Research, Department of Molecular Physiology and Biophysics (J.B., S.M.W.), and the Division of Clinical Pharmacology, Departments of Medicine and Pharmacology (N.J.B.), Vanderbilt University Medical Center, Nashville, TN

Abstract

Cytochrome P450-derived epoxyeicosatrienoic acids are potent vasodilators in preclinical models and are hydrolyzed by soluble epoxide hydrolase (*EPHX2*). Associations between the *EPHX2* Lys55Arg and Arg287Gln polymorphisms and cardiovascular disease risk have been reported; however, their impact on vascular function in humans has not been investigated. In 265 volunteers (198 white, 67 black American), forearm blood flow was measured by strain-gauge venous occlusion plethysmography at baseline and in response to bradykinin, methacholine and sodium nitroprusside. Forearm vascular resistance was calculated as mean arterial pressure/forearm blood flow. In white Americans, Lys55Arg genotype was associated with vasodilator response to bradykinin, such that forearm blood flow was significantly lower (P=0.043) and forearm vascular resistance was significantly higher (P=0.013) in Arg55 variant allele carriers compared to wild-type individuals. Significant associations were also observed with methacholine and sodium nitroprusside. In contrast, no relationship was observed in black Americans. In black Americans, Arg287Gln genotype was associated with vasodilator response to bradykinin. Although the difference in forearm blood flow did not reach statistical significance (P=0.058), forearm vascular resistance was significantly lower (P=0.037) in Gln287 variant allele carriers compared to wild-type individuals. Significant associations were also observed with methacholine and sodium nitroprusside. In white Americans, Gln287 variant allele carriers did not exhibit significantly higher forearm blood flow (P=0.128) or lower forearm vascular resistance (P=0.080). Genetic variation in *EPHX2* is associated with forearm vasodilator responses in a bradykinin receptor- and endothelium-independent manner, suggesting an important role for soluble epoxide hydrolase in the regulation of vascular function in humans.

Correspondence to: Craig R. Lee, Pharm.D., Ph.D., CB# 7569, Kerr Hall, Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, Chapel Hill, NC 27599-7569; Phone: 919-843-7673; Fax: 919-962-0644; craig_lee@unc.edu.

CONFLICT OF INTEREST DISCLOSURES

Dr. Zeldin is a co-inventor on U.S. Patent No. 6,531,506 B1 (issued March 11, 2003) titled “Inhibition of Epoxide Hydrolases for the Treatment of Hypertension,” U.S. Patent No. 6,693,130 B2 (issued February 17, 2004) titled “Inhibition of Epoxide Hydrolases for the Treatment of Hypertension,” and U.S. Patent No.6,916,843 B1 (issued July 12, 2005) titled “Anti-inflammatory Actions of Cytochrome P450 Epoxygenase-Derived Eicosanoids.” No other authors have conflicts of interest to disclose.

Keywords

Soluble epoxide hydrolase; *EPHX2*; polymorphism; bradykinin; endothelium-derived factors

INTRODUCTION

Cytochrome P450 (CYP) epoxygenase enzymes from the CYP2J and CYP2C subfamilies catalyze the oxidative metabolism of arachidonic acid to epoxyeicosatrienoic acids (EETs).[1] EETs are synthesized in the endothelium and cause vasodilation in numerous vascular beds via activation of calcium-sensitive potassium channels (BK_{Ca}) and smooth muscle cell hyperpolarization.[2,3] Consequently, CYP-derived EETs are regarded as one of the primary endothelium-derived hyperpolarizing factors (EDHFs).[2] Soluble epoxide hydrolase (sEH) rapidly hydrolyzes EETs to less biologically active dihydroxyeicosatrienoic acid (DHET) metabolites.[4] Pharmacological inhibition of sEH increases cellular and circulating EET levels, potentiates EET-mediated vasodilation, and lowers blood pressure in preclinical models.[4–6] Importantly, sEH inhibitors are currently in clinical development for the treatment of hypertension.

Associations between polymorphisms in the gene encoding sEH (*EPHX2*) and cardiovascular and cerebrovascular disease risk in humans have been recently reported.[7–13] Most notably, the nonsynonymous Arg287Gln and Lys55Arg polymorphisms exhibit lower and higher sEH metabolic activity *in vitro*, respectively.[14–16] The Gln287 variant allele has been associated with a lower prevalence of ischemic stroke in a Chinese population,[8] although the presence and strength of this relationship has been inconsistent across studies and racial groups.[9,10,17] The Arg55 variant allele has been associated with significantly higher risk of developing coronary artery disease and ischemic stroke events in populations of European ancestry.[11,12] Although multiple reports demonstrate presence of a significant relationship between genetic variation in *EPHX2* and cardiovascular and cerebrovascular disease risk in humans,[8–13] the role of sEH in the regulation of vascular function in humans remains poorly understood. Due to their effects on sEH metabolic function *in vitro*, we hypothesized that the Arg55 and Gln287 variant alleles, respectively, predispose individuals to higher and lower vascular resistance *in vivo*. Consequently, we characterized the relationship between genetic variation in *EPHX2* and forearm vasodilator responses.

METHODS

Please see http://hyper.ahajournals.org for an expanded description of the methods.

Subjects

DNA was extracted from 265 healthy adults who participated in studies that evaluated forearm vasodilator responses.[18,19] All subjects gave written informed consent. The protocol was approved by the Vanderbilt University Institutional Review Board and conducted according to the Declaration of Helsinki.

Experimental Protocol

Forearm blood flow (FBF) was measured using strain-gauge venous occlusion plethysmography (D.E. Hokanson, Bellevue, WA) at baseline and in response to incremental doses of bradykinin (100, 200, and 400 ng/min; Clinalfa AG, Läufelfingen, Switzerland), methacholine (3.2, 6.4, and 12.8 μg/min; Pharmaceutical Compounding Center, Nashville, TN) and sodium nitroprusside (1.6, 3.2, and 6.4 μg/min; Gensia Siccor Pharmaceuticals,

Irvine, CA), as described previously.[18–21] Forearm vascular resistance (FVR) was calculated as the ratio of mean arterial pressure/FBF.

Polymorphism Selection and Genotyping

In addition to the nonsynonymous Lys55Arg and Arg287Gln polymorphisms, eight polymorphisms in *EPHX2* with >5% frequency and r^2 <0.80 were also genotyped for haplotype construction (Table S1).

Statistical analysis

Due to previously reported racial differences in forearm vasodilator responses,[18,21] all analyses were stratified by race. Differences in FBF and FVR were determined using general linear model repeated-measures ANOVA in which the within-subject variable was vasoactive agent dose and the between-subject variables were genotype/haplotype group, cigarette smoking status (yes/no), and body mass index (quartile).[18,19] A post-hoc Scheffe's test was performed to identify between-group differences. Data are presented as least-squares mean ± standard error (SE) unless otherwise indicated. To minimize the impact of the multiple statistical tests conducted, we estimated the false discovery rate (FDR) *q*-value for each comparison.[11,22] Only *q*-values for statistically significant findings (P<0.05) are presented.

RESULTS

EPHX2 Genotype and Subject Characteristics

Study subject characteristics and genotype distributions for the *EPHX2* Lys55Arg and Arg287Gln polymorphisms are presented in Table 1 according to race. No significant differences in age, gender, cigarette smoking status, body mass index, or resting blood pressure were observed across either genotype in either racial group (Table S2).

EPHX2 Genotype and Forearm Vasodilator Responses to Bradykinin

Bradykinin infusion increased FBF and decreased FVR in a dose-dependent fashion (Figures 1, 2 and 3), but did not significantly affect mean arterial pressure or heart rate (P=NS). Race, cigarette smoking status and body mass index (ANOVA P<0.05), but not gender, were each significant predictors of bradykinin-stimulated changes in FBF and FVR.

There was no relationship between Lys55Arg genotype and either resting FBF (P=0.932 and P=0.876) or FVR (P=0.663 and P=0.752) in white and black American subjects, respectively (Table S2). In white Americans, vasodilator response to bradykinin was significantly associated with Lys55Arg genotype, such that FBF was significantly lower (Figure 1A, P=0.043, *q*=0.078) and FVR was significantly higher (Figure 1C, P=0.013, *q*=0.047) in Arg55 variant allele carriers (Lys/Arg or Arg/Arg) relative to wild-type (Lys/Lys) individuals. In contrast, genotype differences were not observed in black Americans (Figure 1B and 1D).

Resting FVR was significantly lower in black American Gln287 variant allele carriers (Arg/Gln or Gln/Gln) compared to wild-type (Arg/Arg) individuals (P=0.040, Table S2). Although resting FBF also appeared to be higher in white and black Americans (P=0.086 and P=0.078, respectively), and FVR appeared to be lower in white Americans (P=0.053), these differences were not statistically significant (Table S2). Vasodilator response to bradykinin was associated with Arg287Gln genotype in black Americans. Although the difference in FBF did not reach statistical significance (Figure 2B, P=0.058), FVR was significantly lower (Figure 2D, P=0.037, *q*=0.072) in Gln287 variant allele carriers relative to wild-type individuals. In white Americans, the observed differences in FBF (Figure 2A,

P=0.128) and FVR (Figure 2C, P=0.080) were less substantial than those observed in black Americans and were not statistically significant.

Inclusion of both Lys55Arg and Arg287Gln genotype in the model did not alter the observed relationships between each individual polymorphism and FVR in either white (Lys55Arg: P=0.017; Arg287Gln: P=0.171) or black Americans (Lys55Arg: P=0.731; Arg287Gln: P=0.037). An exploratory analysis evaluating the combination of both genotypes yielded consistent results (Figure S1).

EPHX2 Genotype and Forearm Vasodilator Responses to Methacholine and Sodium Nitroprusside

Vasodilator responses to methacholine (P=0.021, *q*=0.063) and sodium nitroprusside (P=0.006, *q*=0.039) were also significantly associated with Lys55Arg genotype in white Americans (Table 2). As observed with bradykinin, FVR was significantly higher in response to each agent in Arg55 variant allele carriers (Lys/Arg or Arg/Arg) relative to wild-type (Lys/Lys) individuals. Significant genotype differences were not observed in black Americans.

Arg287Gln genotype was associated with vasodilator responses to methacholine (P=0.033, *q*=0.072; and P=0.025, *q*=0.063) and sodium nitroprusside (P=0.125; and P=0.006, *q*=0.039) in both white and black Americans, respectively (Table 2). As observed with bradykinin, FVR was lower at baseline and in response to each agent in Gln287 variant allele carriers (Arg/Gln or Gln/Gln) relative to wild-type (Arg/Arg) individuals.

EPHX2 Haplotypes and Forearm Vasodilator Responses

Nine *EPHX2* polymorphisms were included in haplotype construction, which identified six common haplotypes (>5%) in white American subjects (Figure S2). Only one common haplotype was tagged by the Lys55Arg polymorphism variant *G* allele, which had a frequency of 13.7%. No differences in either resting FBF (P=0.471) or FVR (P=0.301) were observed in subjects carrying the variant Arg55-tagged haplotype. Vasodilator response to bradykinin, however, was significantly associated with this haplotype, such that FBF was significantly lower (Figure 3A, P=0.016, *q*=0.053) and FVR was significantly higher (Figure 3B, P=0.004, *q*=0.039) in subjects carrying the Arg55-tagged haplotype. Similar results were observed with Arg55-tagged haplotypes constructed using the sliding-window approach (Figure S3). In response to incremental doses of methacholine and sodium nitroprusside, respectively, FBF was significantly lower (P=0.025, *q*=0.063; and P=0.001, *q*=0.016) and FVR was significantly higher (P=0.009, *q*=0.047; and P=0.001, *q*=0.016) in subjects carrying the Arg55-tagged haplotype.

A distinct haplotype tagged by the Arg287Gln polymorphism variant *A* allele had a frequency of 11.1%. Although carriers of the Gln287-tagged haplotype appeared to exhibit higher resting FBF (P=0.127) and lower FVR (P=0.083), these differences were not statistically significant. Similar results were observed with Gln287-tagged haplotypes constructed using the sliding-window approach (Figure S3). No statistically significant associations between the Gln287-tagged haplotype and vasodilator responses to incremental doses of bradykinin (Figure 3C and 3D), methacholine (FBF: P=0.184, FVR: P=0.107) or sodium nitroprusside (FBF: P=0.254, FVR: P=0.233) were observed.

DISCUSSION

Cytochrome P450 (CYP) epoxygenases catalyze endothelial EET biosynthesis. These potent vasodilators are rapidly hydrolyzed by sEH, and pharmacological inhibition of sEH potentiates the effect of EETs.[1,2,4] This study identified a significant association between

functional genetic variants in *EPHX2*, the gene which encodes sEH, and vascular function in humans. Specifically, the Arg55 variant allele was associated with significantly lower FBF and higher FVR in response to vasodilators in white Americans, while the Gln287 variant allele was associated with higher FBF and lower FVR at baseline and in response to vasodilators in black Americans. Similar genotype-phenotype relationships were observed in response to bradykinin, methacholine and sodium nitroprusside, suggesting that the mechanism underlying the functional link between sEH and forearm vascular responsiveness in humans is both bradykinin receptor-and endothelium-independent. Collectively, these data demonstrate a potentially important role for sEH in the regulation of vascular function in humans, and offer important mechanistic insight into previously reported associations between the *EPHX2* Lys55Arg and Arg287Gln polymorphisms and cardiovascular and cerebrovascular disease risk.

The nonsynonymous Lys55Arg polymorphism exhibits higher sEH metabolic activity *in vitro*[14,15] and *in vivo*,[11] and has been associated with higher risk of coronary artery disease and ischemic stroke events in individuals of European ancestry.[11,12] Consistent with higher sEH metabolic activity and EET hydrolysis, white American carriers of the Arg55 variant allele exhibited lower vasodilation responses compared to wild-type individuals. Lower vasodilation responses were also observed in carriers of the Arg55-tagged haplotype, suggesting that Lys55Arg is the functional allele driving these associations. We cannot rule out, however, that Lys55Arg is simply a marker in linkage disequilibrium with the true causative locus. In contrast, there was no association between Lys55Arg genotype and either resting or agonist-stimulated changes in FBF or FVR in black American subjects. Although the mechanism underlying the racial differences in this genotype-phenotype relationship is not known, these findings are consistent with the prior observation that Lys55Arg genotype was associated with sEH metabolic activity *in vivo* and cardiovascular disease risk in white, but not black, Americans enrolled in the Atherosclerosis Risk in Communities (ARIC) study.[11]

Multiple studies have demonstrated that the Arg287Gln polymorphism exhibits significantly lower sEH metabolic activity and EET hydrolysis *in vitro*,[14–16] although its functional impact on sEH metabolic activity *in vivo* remains unknown. Epidemiological studies provide inconsistent data regarding the presence and direction of a significant relationship between the Arg287Gln polymorphism and risk of cardiovascular and cerebrovascular events.[8–13,17] Consistent with the vascular protective effects associated with sEH inhibition in preclinical models,[4] the Gln287 variant allele was recently associated with a significantly lower risk of ischemic stroke in a Chinese population.[8] In contrast, the Gln287 variant allele was associated with a higher prevalence and extent of coronary artery calcification in black Americans[9] and insulin resistance in Japanese type 2 diabetics.[23] Similarly, in contrast to the neuronal protective effects conferred by the Gln287 variant allele *in vitro*,[15] an association with significantly higher risk of ischemic stroke was reported in a white European population.[10] No association with coronary artery disease and ischemic stroke risk has been observed in other white or black American[11,13] and European populations.[12,17] Collectively, these conflicting data demonstrate that the functional relevance of the Arg287Gln polymorphism in humans remains unclear and requires further study.

Consistent with the hypothesis that decreased endogenous sEH metabolic activity enhances the vasodilator effects of EETs, Gln287 variant allele carriers demonstrated higher FBF and lower FVR under basal and agonist-stimulated conditions compared to wild-type individuals in the current investigation. These data are also consistent with the observation that administration of pharmacological inhibitors of sEH cause vasodilation in preclinical models and isolated human vessels.[4–6] The observed relationship between the Arg287Gln polymorphism and vasodilator responses was more pronounced in black American subjects

compared to the white American subjects studied. Although the current study was underpowered to investigate genotype-by-genotype interactions in each racial group, an interaction between the Lys55Arg and Arg287Gln polymorphisms appears unlikely to account for the observed racial differences.

The Lys55Arg and Arg287Gln polymorphisms were associated with altered vasodilation responses to both bradykinin and methacholine. These endothelium-dependent vasodilators cause vascular relaxation in part by stimulating the endothelial formation of EETs, which subsequently hyperpolarize vascular smooth muscle.[2] Somewhat unexpectedly, these functional variants in *EPHX2* also affected the endothelium-independent vasodilator response to sodium nitroprusside. Although it is well-established that endothelial-derived EETs act as paracrine mediators of vasodilation,[2] *EPHX2* deletion and pharmacological sEH inhibition also attenuate vascular remodeling in preclinical models by potentiating the anti-proliferative and anti-inflammatory effects of EETs.[24,25] Our findings suggest that chronic inter-individual differences in EET exposure based on Lys55Arg and Arg287Gln genotype could contribute to physiological differences in forearm vascular responsiveness via an effect on vascular remodeling. An important limitation of our work, however, is the lack of functional data demonstrating that Arg55 and Gln287 variant allele carriers, respectively, exhibit higher and lower EET hydrolysis *in vivo*. Further studies will be necessary to characterize the direct effects of exogenous EET administration and sEH inhibition on vasodilation and vascular remodeling in humans, and define the underlying mechanisms. Moreover, although *in vitro* studies have demonstrated that the Arg55 and Gln287 variant alleles elicit lower and higher sEH phosphatase activity, respectively,[26,27] the potential contribution of sEH phosphatase activity to the association between *EPHX2* polymorphisms, vascular function, and cardiovascular and cerebrovascular risk remains unknown and requires further investigation.

This study examined the vascular effects of *EPHX2* polymorphisms in one of the largest cohorts that directly measured vasodilation responses to infused agonists. Nevertheless, the relatively small sample size represents a limitation since we may have been underpowered to detect an association, particularly in the black Americans studied. Moreover, due to the very low number of homozygous Arg55 and Gln287 carriers, we were only powered to characterize these relationships using a dominant genetic model of inheritance. In the bradykinin studies, there was excellent power to detect a 30% difference in FVR across the Lys55Arg (β=0.97) and Arg287Gln (β=0.94) genotype groups in the white American subset. The power to detect these differences in the black American subset was considerably less (Lys55Arg: β=0.67; Arg287Gln: β=0.52). We also made multiple comparisons and there is a possibility of false positive associations. In order to account for this possibility, we calculated a FDR *q*-value for each comparison. Although no gold-standard *q*-value threshold has been established to identify “true” associations, incorporation of this statistical approach into candidate gene association studies has become an increasingly recognized method to account for multiple comparisons and enhance confidence in observed associations.[28] Since all *q*-values were estimated to be <0.08, we have a higher level of confidence in our reported findings. Importantly, validation in a well-powered, independent population will be ultimately necessary to confirm presence of a functional relationship between genetic variation in *EPHX2* and vasodilator responses in humans.

PERSPECTIVES

Cytochrome P450 epoxygenases catalyze the biosynthesis of EETs, which are potent vasodilators that undergo rapid hydrolysis by sEH. Although associations between polymorphisms in *EPHX2* and cardiovascular and cerebrovascular disease risk have been recently reported, their impact on vascular function has remained unknown. The current

study demonstrates that genetic variation in *EPHX2* is significantly associated with vasodilator responses in humans. Specifically, carriers of the increased function Arg55 and decreased function Gln287 variant alleles were predisposed to attenuated and enhanced forearm vasodilator responses, respectively. Collectively, these findings suggest that sEH has an important role in the regulation of vascular resistance and blood pressure in humans, and offer important mechanistic insight into previously reported associations between the *EPHX2* Lys55Arg and Arg287Gln polymorphisms and cardiovascular and cerebrovascular disease risk. Pharmacological inhibition of sEH is currently under clinical development as a novel therapeutic strategy for the treatment of hypertension. These findings may ultimately help identify specific subsets of the population who may be more (Arg55 carriers) or less (Gln287 carriers) likely to respond to the anti-hypertensive effects of sEH inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge Delia Woods, RN for her nursing assistance and Jeff Petro, BS for his technical assistance.

SOURCES OF FUNDING

This publication was made possible by a Beginning Grant-in-Aid from the American Heart Association to Dr. Lee, grant HL085740 to Dr. Pretorius, funds from the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences to Dr. Zeldin (Z01 ES025034), and grants 2P01 DK38226 and 5R01 HL065193 to Dr. Brown. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NHLBI or NIH.

References

1. Zeldin DC. Epoxygenase pathways of arachidonic acid metabolism. J Biol Chem 2001;276:36059–36062. [PubMed: 11451964]
2. Campbell WB, Falck JR. Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. Hypertension 2007;49:590–596. [PubMed: 17200437]
3. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ Res 1996;78:415–423. [PubMed: 8593700]
4. Imig JD, Hammock BD. Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases. Nat Rev Drug Discov 2009;8:794–805. [PubMed: 19794443]
5. Imig JD, Zhao X, Capdevila JH, Morisseau C, Hammock BD. Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. Hypertension 2002;39:690–694. [PubMed: 11882632]
6. Larsen BT, Miura H, Hatoum OA, Campbell WB, Hammock BD, Zeldin DC, Falck JR, Gutterman DD. Epoxyeicosatrienoic and dihydroxyeicosatrienoic acids dilate human coronary arterioles via BK(Ca) channels: implications for soluble epoxide hydrolase inhibition. Am J Physiol Heart Circ Physiol 2006;290:H491–499. [PubMed: 16258029]
7. Theken KN, Lee CR. Genetic variation in the cytochrome P450 epoxygenase pathway and cardiovascular disease risk. Pharmacogenomics 2007;8:1369–1383. [PubMed: 17979511]
8. Zhang L, Ding H, Yan J, Hui R, Wang W, Kissling GE, Zeldin DC, Wang DW. Genetic variation in cytochrome P450 2J2 and soluble epoxide hydrolase and risk of ischemic stroke in a Chinese population. Pharmacogenet Genomics 2008;18:45–51. [PubMed: 18216721]
9. Fornage M, Boerwinkle E, Doris PA, Jacobs D, Liu K, Wong ND. Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African-American subjects: The Coronary Artery Risk Development in Young Adults (CARDIA) study. Circulation 2004;109:335–339. [PubMed: 14732757]

10. Gschwendtner A, Ripke S, Freilinger T, Lichtner P, Muller-Myhsok B, Wichmann HE, Meitinger T, Dichgans M. Genetic variation in soluble epoxide hydrolase (EPHX2) is associated with an increased risk of ischemic stroke in white Europeans. Stroke 2008;39:1593–1596. [PubMed: 18323494]
11. Lee CR, North KE, Bray MS, Fornage M, Seubert JM, Newman JW, Hammock BD, Couper DJ, Heiss G, Zeldin DC. Genetic variation in soluble epoxide hydrolase (EPHX2) and risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. Hum Mol Genet 2006;15:1640–1649. [PubMed: 16595607]
12. Fava C, Montagnana M, Danese E, Almgren P, Hedblad B, Engstrom G, Berglund G, Minuz P, Melander O. Homozygosity for the EPHX2 K55R polymorphism increases the long-term risk of ischemic stroke in men: a study in Swedes. Pharmacogenet Genomics 2010;20:94–103. [PubMed: 20065888]
13. Fornage M, Lee CR, Doris PA, Bray MS, Heiss G, Zeldin DC, Boerwinkle E. The soluble epoxide hydrolase gene harbors sequence variation associated with susceptibility to and protection from incident ischemic stroke. Hum Mol Genet 2005;14:2829–2837. [PubMed: 16115816]
14. Przybyla-Zawislak BD, Srivastava PK, Vazquez-Matias J, Mohrenweiser HW, Maxwell JE, Hammock BD, Bradbury JA, Enayetallah AE, Zeldin DC, Grant DF. Polymorphisms in human soluble epoxide hydrolase. Mol Pharmacol 2003;64:482–490. [PubMed: 12869654]
15. Koerner IP, Jacks R, DeBarber AE, Koop D, Mao P, Grant DF, Alkayed NJ. Polymorphisms in the human soluble epoxide hydrolase gene EPHX2 linked to neuronal survival after ischemic injury. J Neurosci 2007;27:4642–4649. [PubMed: 17460077]
16. Merkel MJ, Liu L, Cao Z, Packwood W, Young J, Alkayed NJ, Van Winkle DM. Inhibition of soluble epoxide hydrolase preserves cardiomyocytes: role of STAT3 signaling. Am J Physiol Heart Circ Physiol 2010;298:H679–687. [PubMed: 20008276]
17. Lee J, Dahl M, Grande P, Tybjaerg-Hansen A, Nordestgaard BG. Genetically reduced soluble epoxide hydrolase activity and risk of stroke and other cardiovascular disease. Stroke 2010;41:27–33. [PubMed: 19940276]
18. Pretorius MM, Gainer JV, Van Guilder GP, Coelho EB, Luther JM, Fong P, Rosenbaum DD, Malave HA, Yu C, Ritchie MD, Vaughan DE, Brown NJ. The bradykinin type 2 receptor BE1 polymorphism and ethnicity influence systolic blood pressure and vascular resistance. Clin Pharmacol Ther 2008;83:122–129. [PubMed: 17522594]
19. Van Guilder GP, Pretorius M, Luther JM, Byrd JB, Hill K, Gainer JV, Brown NJ. Bradykinin type 2 receptor BE1 genotype influences bradykinin-dependent vasodilation during angiotensin-converting enzyme inhibition. Hypertension 2008;51:454–459. [PubMed: 18180402]
20. Brown NJ, Gainer JV, Murphey LJ, Vaughan DE. Bradykinin stimulates tissue plasminogen activator release from human forearm vasculature through B(2) receptor-dependent, NO synthase-independent, and cyclooxygenase-independent pathway. Circulation 2000;102:2190–2196. [PubMed: 11056091]
21. Rosenbaum DA, Pretorius M, Gainer JV, Byrne D, Murphey LJ, Painter CA, Vaughan DE, Brown NJ. Ethnicity affects vasodilation, but not endothelial tissue plasminogen activator release, in response to bradykinin. Arterioscler Thromb Vasc Biol 2002;22:1023–1028. [PubMed: 12067915]
22. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A 2003;100:9440–9445. [PubMed: 12883005]
23. Ohtoshi K, Kaneto H, Node K, Nakamura Y, Shiraiwa T, Matsuhisa M, Yamasaki Y. Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients. Biochem Biophys Res Commun 2005;331:347–350. [PubMed: 15845398]
24. Revermann M, Schloss M, Barbosa-Sicard E, Mieth A, Liebner S, Morisseau C, Geisslinger G, Schermuly RT, Fleming I, Hammock BD, Brandes RP. Soluble epoxide hydrolase deficiency attenuates neointima formation in the femoral cuff model of hyperlipidemic mice. Arterioscler Thromb Vasc Biol 2010;30:909–914. [PubMed: 20224052]
25. Simpkins AN, Rudic RD, Roy S, Tsai HJ, Hammock BD, Imig JD. Soluble epoxide hydrolase inhibition modulates vascular remodeling. Am J Physiol Heart Circ Physiol 2010;298:H795–806. [PubMed: 20035028]

26. Srivastava PK, Sharma VK, Kalonia DS, Grant DF. Polymorphisms in human soluble epoxide hydrolase: effects on enzyme activity, enzyme stability, and quaternary structure. Arch Biochem Biophys 2004;427:164–169. [PubMed: 15196990]

27. Enayetallah AE, Grant DF. Effects of human soluble epoxide hydrolase polymorphisms on isoprenoid phosphate hydrolysis. Biochem Biophys Res Commun 2006;341:254–260. [PubMed: 16414022]

28. Smith NL, Hindorff LA, Heckbert SR, Lemaitre RN, Marciante KD, Rice K, Lumley T, Bis JC, Wiggins KL, Rosendaal FR, Psaty BM. Association of genetic variations with nonfatal venous thrombosis in postmenopausal women. JAMA 2007;297:489–498. [PubMed: 17284699]

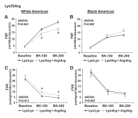

Figure 1. *EPHX2* Lys55Arg polymorphism and vasodilator responses to bradykinin

Least-squares mean ± SE FBF (A, B) and FVR (C, D) at baseline and in response to incremental doses of bradykinin (BK, 100 and 200 ng/min) among *EPHX2* Lys55Arg genotype groups in white (A, C) and black (B, D) Americans. The repeated-measures ANOVA P-value for the genotype group comparison is provided. *Post-hoc P<0.05 versus wild-type (Lys/Lys).

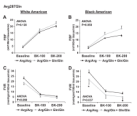

Figure 2. *EPHX2* Arg287Gln polymorphism and vasodilator responses to bradykinin

Least-squares mean ± SE FBF (A, B) and FVR (C, D) at baseline and in response to incremental doses of bradykinin (BK, 100 and 200 ng/min) among *EPHX2* Arg287Gln genotype groups in white (A, C) and black (B, D) Americans. The repeated-measures ANOVA P-value for the genotype group comparison is provided. *Post-hoc P<0.05 versus wild-type (Arg/Arg).

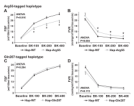

Figure 3. *EPHX2* haplotypes and vasodilator responses to bradykinin in white Americans

Least-squares mean ± SE FBF (A, C) and FVR (B, D) at baseline and in response to incremental doses of bradykinin (BK, 100, 200 and 400 ng/min) among those carrying the Arg55-tagged (C, D) and Gln287-tagged (E, F) haplotypes compared to all other *EPHX2* haplotypes (Hap-WT). The repeated-measures ANOVA P-value for the haplotype group comparison is provided. *Post-hoc P<0.05 versus all other haplotype groups (Hap-WT).

Table 1

Subject characteristics and *EPHX2* genotype by race.

Characteristic*	White American	Black American
N	198	67
Age (years)	31.6 ± 10.5	32.7 ± 10.8
Gender (% female)	88 (44.4%)	41 (61.2%) †
Smoker (% yes)	19 (9.6%)	4 (6.0%) †
BMI (kg/m^2)	24.5 ± 3.4	26.0 ± 4.0 †
MAP (mmHg)	83.4 ± 8.3	86.9 ± 11.2 †
FBF (mL/100mL tissue/min)	4.2 ± 2.0	3.9 ± 1.7
FVR (mmHg/mL/100mL tissue/min)	24.2 ± 10.6	27.5 ± 13.5 †
Lys55Arg		
A/A (Lys/Lys)	135 (70.0%)	42 (62.7%)
A/G (Lys/Arg)	56 (29.0%)	24 (35.8%)
G/G (Arg/Arg)	2 (1.0%)	1 (1.5%)
Arg287Gln		
G/G (Arg/Arg)	152 (78.4%)	53 (81.5%)
G/A (Arg/Gln)	39 (20.1%)	10 (15.4%)
A/A (Gln/Gln)	3 (1.6%)	2 (3.1%)

* Data presented as mean ± standard deviation or count (proportion).

† P<0.05 versus white American. There were no statistically significant differences in genotype distribution or allelic frequency between racial groups for either polymorphism (P>0.30 for each comparison).

BMI=body mass index, MAP=mean arterial pressure, FBF=baseline forearm blood flow, FVR=baseline forearm vascular resistance.

Table 2

Forearm vascular resistance in response to methacholine and sodium nitroprusside according to *EPHX2* genotype.

Genotype	Forearm Vascular Resistance (mmHg/mL/100 mL tissue/min)									
	Methacholine					Sodium Nitroprusside				
	N	Baseline	3.2 (μg/min)	6.4 (μg/min)	12.8 (μg/min)	N	Baseline	1.6 (μg/min)	3.2 (μg/min)	6.4 (μg/min)
Lys55Arg										
White American		ANOVA P=0.021					ANOVA P=0.006			
Lys/Lys	94	20.6 ± 1.5	4.4 ± 0.5	3.1 ± 0.3	2.5 ± 0.2	124	21.2 ± 1.4	8.4 ± 1.0	5.6 ± 0.6	4.4 ± 0.4
Lys/Arg + Arg/Arg	49	24.6 ± 2.0	5.7 ± 0.6	4.3 ± 0.3*	3.1 ± 0.2	56	27.0 ± 1.9*	11.5 ± 1.3*	7.5 ± 0.7*	5.7 ± 0.5*
Black American		ANOVA P=0.249					ANOVA P=0.852			
Lys/Lys	26	23.0 ± 1.8	7.2 ± 1.0	5.1 ± 0.4	4.7 ± 0.5	41	28.0 ± 2.5	15.0 ± 1.4	8.3 ± 0.9	6.6 ± 0.8
Lys/Arg + Arg/Arg	18	21.7 ± 2.1	5.9 ± 1.2	4.0 ± 0.5	3.3 ± 0.6	22	27.2 ± 3.2	13.0 ± 1.9	9.3 ± 1.2	6.9 ± 1.0
Arg287Gln										
White American		ANOVA P=0.033					ANOVA P=0.125			
Arg/Arg	114	23.2 ± 1.2	4.9 ± 0.4	3.7 ± 0.2	2.8 ± 0.2	144	24.2 ± 1.2	9.5 ± 0.8	6.5 ± 0.5	5.1 ± 0.3
Arg/Gln + Gln/Gln	30	17.6 ± 2.0*	4.9 ± 0.7	3.0 ± 0.4	2.4 ± 0.3	37	20.6 ± 1.9	9.6 ± 1.3	5.3 ± 0.7	4.1 ± 0.5
Black American		ANOVA P=0.025					ANOVA P=0.006			
Arg/Arg	35	23.7 ± 1.5	6.9 ± 0.8	4.9 ± 0.4	4.4 ± 0.4	49	30.3 ± 2.0	15.2 ± 1.3	9.4 ± 0.8	7.2 ± 0.7
Arg/Gln + Gln/Gln	8	17.8 ± 3.1	5.5 ± 1.7	3.2 ± 0.8*	2.8 ± 0.9*	12	19.0 ± 4.2*	9.7 ± 2.6*	4.8 ± 1.6*	4.3 ± 1.4

Data are presented as least-squares mean ± SE. The repeated-measures ANOVA P-value for the genotype group comparison is provided.

*Post-hoc P<0.05 versus wild-type (Lys/Lys, Arg/Arg).