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Genetic variation in cytochrome P450 2J2 and soluble epoxide hydrolase and risk of ischemic stroke in a Chinese population

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Abstract

Objective—Epoxyeicosatrienoic acids have been recognized for their protective effects on the cardiovascular system. This study investigated whether two common polymorphisms in genes believed to be influential in regulating circulating levels of epoxyeicosatrienoic acids, namely cytochrome P450 2J2 (*CYP2J2*) G-50T and soluble epoxide hydrolase (*EPHX2*) G860A, were associated with ischemic stroke risk in a Chinese population.

Methods and results—Screening of 200 patients with ischemic stroke and 350 control participants revealed that *CYP2J2*–50T allele frequency was not significantly different in ischemic stroke cases versus controls. In contrast, *EPHX2* 860A allele frequency was 16.8% in ischemic stroke cases versus 21.7% in controls ($P = 0.047$), and the presence of this variant allele was associated with a significantly lower risk of ischemic stroke after adjustment for sex, age and multiple cardiovascular risk factors (adjusted odds ratio = 0.50, 95% confidence interval 0.29–0.86). Moreover, there was a significant interaction between the *EPHX2* G860A polymorphism, smoking and ischemic stroke risk such that nonsmokers carrying the *EPHX2* G860A variant allele were at the lowest risk of ischemic stroke (odds ratio = 0.33, 95% confidence interval, 0.17–0.67, $P = 0.002$), whereas no significant association was observed in smokers.

Conclusions—Collectively, these data indicate a protective influence of the G860A polymorphism of *EPHX2* on ischemic stroke in Chinese nonsmokers.

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Keywords

CYP2J2; cytochrome P450; EPHX2; genetics; ischemic stroke; polymorphism

Introduction

Stroke is a major cause of morbidity and mortality worldwide [1]. In China, 2.6 million people are estimated to experience a first-ever stroke event every year, and ischemic stroke was recently estimated to account for 43.7–78.9% of all the strokes in this country [2]. The

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development of stroke is influenced by a variety of cardiovascular risk factors including hypertension, smoking and diabetes mellitus, as well as by certain genetic predispositions. Acute cardiovascular events and stroke are thought to be ultimately caused largely by an inflammation-mediated destabilization and rupture of atherosclerotic lesions [3,4].

Arachidonic acid is metabolized in endothelial cells by cytochrome P450 (CYP) epoxygenases into four regioisomeric epoxyeicosatrienoic acids (EETs; 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET) which display many characteristics of the putative endothelium-derived hyperpolarizing factor [5,6]. Accumulating evidence indicates that CYP epoxygenase-derived EETs exert diverse cardiovascular protective effects including upregulation of endothelial nitric oxide synthase (eNOS) expression and activity, antiapoptotic effects in endothelial cells and anti-inflammatory and antiangiogenic effects, all of which suggest a potential antiatherosclerotic effect of CYP epoxygenase-derived EETs that may be beneficial against stroke [7–12].

CYP2J2 is a human CYP epoxygenase expressed predominantly in vascular endothelial cells and heart tissue that metabolizes arachidonic acid into all four EET regioisomers [13]. Recently, the G-50T promoter polymorphism in the *CYP2J2* gene was found to be independently associated with an increased risk of coronary artery disease, and plasma concentrations of EETs were shown to be significantly lower in individuals with the *CYP2J2* –50TT genotype than in those with the *CYP2J2*–50GG genotype [14]. Soluble epoxide hydrolase, encoded by the *EPHX2* gene, metabolizes EETs to less biologically active dihydroxyeicosatrienoic acids [15,16]. A number of single nucleotide polymorphisms have been identified in *EPHX2* [17], some of which appear to influence, either positively or negatively, the incidence of ischemic stroke [18]. One of these, G860A (Arg287Gln), results in decreased enzyme activity but was not found to be associated with an altered risk of ischemic stroke in a cohort of Americans of African or Caucasian descent [17,18]. Animal studies have demonstrated that experimental focal ischemic stroke is reduced by *EPHX2* deletion or by pharmacological inhibition of soluble epoxide hydrolase [19], whereas sequence variation in *EPHX2* does not appear to be a primary determinant of blood pressure in spontaneously hypertensive rats [20].

The available evidence suggests that CYP2J2 and soluble epoxide hydrolase have important roles in regulating circulating EET levels and that variation in their gene sequences may lead to altered enzyme activity and subsequent effects on cardiovascular homeostasis and disease outcomes. The present study was undertaken to determine whether specific genetic variations in *CYP2J2* and *EPHX2* are associated with risk of ischemic stroke events in a Chinese population.

Materials and methods

Study population and data collection

This was a multicenter study for assessment of risk factors for stroke sponsored by the Ministry of Science and Technology of China. The study protocol was approved by the review board of the Ministry of Public Health, Ministry of Science and Technology of China and the ethics committees at all participating hospitals. Informed consent was obtained from all individuals.

All 200 ischemic stroke patients and 350 control individuals were recruited between November 2000 and June 2002 from five hospitals in Wuhan, China. Two subtypes of ischemic stroke, cerebral thrombosis and lacunar infarction, were included. Other types of strokes including subarachnoid hemorrhage, embolic brain infarction, brain tumors and cerebrovascular malformation, and severe systemic diseases such as pulmonary fibrosis, endocrine and metabolic disease (except diabetes mellitus), severe inflammatory diseases, autoimmune

disease, tumors and serious chronic diseases (e.g. hepatic cirrhosis, renal failure) were criteria for exclusion. Diagnosis of stroke was based on the results of neurological examination, CT or MRI according to the International Classification of Diseases, ninth revision. Controls were selected from inpatients with minor illnesses from the Departments of Ophthalmology, Gastroenterology, Otorhinolaryngology and Orthopedics, and from community-based inhabitants free of neurological diseases following the same exclusion criteria as cases. All the participants received detailed medical history and a physical examination of cardiovascular and neurological systems, including evaluation of body mass index.

Blood sample collection and genomic DNA extraction

Ten milliliters of blood was drawn from an arm vein into a sterile tube containing Ethylenediamine tetraacetic acid. Plasma was isolated and genomic DNA was extracted using a standard phenol-chloroform extraction method [21] and stored at -80°C until genotype analysis was performed.

Genotyping of *CYP2J2* G-50T and *EPHX2* G860A variants

Genotyping was performed by a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. A 373-bp fragment of *CYP2J2* G-50T (Genbank sequence AF272142, nucleotides 5856–6228, rs89029) was amplified with the primer pairs: 5'-TTTCTGAGACCGGTGCGTG-3' and 5'-CAGGTGCGACTGCTCGAAG-3'. The reaction was carried out in a 25- μl volume that included 100 ng of genomic DNA, 200 $\mu\text{mol/l}$ dNTP mixture, 0.2 $\mu\text{mol/l}$ of each primer, $2 \times$ GC buffer and 1 U of TaKaRa LA Taq DNA Polymerase (TaKaRa Biomedicals, Dalian, Liaoning, China). PCR was performed in an Applied Biosystems (Foster City, California, USA) 2720 Thermocycler with an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s and extension at 72°C for 30 s. A final extension at 72°C for 5 min was performed. The PCR products were digested with *Alu* I (Fermentas, Burlington, Ontario, Canada) at 37°C for 2–3 h and digested products were separated on 1.5% agarose gels containing ethidium bromide. The PCR primers for *EPHX2* G860A (197-bp fragment, Genbank sequence X97030, nucleotides 605–801, rs751141) were 5'-CCCTGTGGCTCTTGGTTTG-3' and 5'-ACCGGGAGGAGCAGATGAC-3'. PCR was performed as described above except that the annealing temperature was 58°C . The PCR products were digested with *Msp* I (Fermentas) and separated on 2.5% agarose gels containing ethidium bromide.

To independently validate the PCR-RFLP methods, we analyzed 136 randomly selected DNA samples (24.7% of the total samples) using the TaqMan 5' nuclease assay on a Rotor-Gene 6000 real-time quantitative thermal cycler (Corbett Research, Sydney, Australia). The concurrence rate of these two methods was 99.3% ($k = 0.982$; $P = 0.0001$), suggesting that the PCR-RFLP method was reliable. The following primers and probes were used in this assay: forward primer EPX2-F, 5'-GCTGGAGTGTGCCTGTTTGTT-3'; reverse primer EPX2-R, 5'-CCGGGAGGAGCAGATGACT-3'; probe 1 EPX2_G860-FAM, FAM-TTACCgGGTCCTAGCMGB; probe 2 EPX2_A860-TET, TET-CAGGTTACCaGGTCC-MGB. To validate the genotyping results for the *CYP2J2* G-50T polymorphism, we analyzed 96 randomly selected DNA samples by direct sequencing. The results were 100% concordant. In both cases, re-genotyping was performed by investigators who were masked to case or control status, and a 10% random sample was tested in duplicate by different investigators.

Data analysis

Summary statistics are expressed as the mean $\pm$ standard error of the mean (SE) or as percentages. All statistical analyses were performed with SPSS 10.0 software (SPSS Science). The χ^2 test was used to analyze the deviation of Hardy–Weinberg equilibrium for genotype

frequencies and to compare genotype and allele frequencies. Continuous variables were compared between cases of ischemic stroke and controls using Student's *t*-test; discrete variables were compared using χ^2 tests. The effects of sex, age and classic cardiovascular risk factors on the potential association of ischemic stroke with *CYP2J2* G-50T and *EPHX2* G860A polymorphisms were evaluated with multiple unconditional logistic regression analysis. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were computed. Assuming a case-control design, type I error $\alpha = 0.05$ and either 5% (*CYP2J2* G-50T) or 20% (*EPHX2* G860A) variant allele frequency, we had approximately 54 and 94% power to detect an OR of 2.0, respectively. Interaction testing was completed on a multiplicative scale between genotypes and between genotype and smoking status (yes/no) using a Wald χ^2 test for significance of the estimated β -coefficients for the interaction term. As interaction hypothesis testing on a multiplicative scale is underpowered, the critical value for statistical significance was set to $\alpha = 0.15$, two sided. For all other analyses, statistical significance was defined as $P < 0.05$.

Results

Characteristics of the study population

Table 1 presents the characteristics of the study population. Ages of the study population were 61.7 ± 0.8 (mean $\pm$ SE) years in ischemic stroke studies and 63.2 ± 0.4 years in control individuals ($P = 0.092$). Men accounted for 67% of cases and 36% of controls ($P < 0.001$). As expected, cases had a higher prevalence of conventional risk factors for cardiovascular disease, including cigarette smoking, hypertension, coronary heart disease and diabetes, but they had lower plasma total cholesterol and low density lipoprotein levels compared with control individuals.

CYP2J2 G-50T and *EPHX2* G860A genotype determination

The *CYP2J2*-50T allele has an *Alu* I cleavage site, whereas the wild type *CYP2J2*-50G allele does not. As such, the homozygous GG genotype was detected by the presence of a single 373-bp fragment whereas the heterozygous GT genotype yielded three digested fragments that were 99, 274 and 373 bp in length (Fig. 1a). No homozygous TT genotypes were observed in the study cohort. The wild type *EPHX2* 860G allele has a *Msp* I cleavage site such that the GG genotype was detected as two digested fragments that were 54 and 143 bp in length (Fig. 1b). The 860A allele does not contain the *Msp* I cleavage site; as such, the heterozygous GA genotype yielded fragments that were 54, 143 and 197 bp in length whereas the homozygous AA genotype was detected as a single undigested fragment that was 197 bp long (Fig. 1b).

CYP2J2 G-50T and *EPHX2* G860A genotypes and ischemic stroke risk

Genotype distributions for *CYP2J2* G-50T and *EPHX2* G860A were in accordance with Hardy-Weinberg equilibrium. Genotype distributions and allele frequencies for ischemic stroke cases and controls are shown in Table 2. No significant differences were observed in genotype distributions or allele frequencies for *CYP2J2* G-50T with regard to ischemic stroke. *EPHX2* genotype distributions also did not differ between ischemic stroke case studies and control patients. The frequency of the A allele at position 860 of *EPHX2* was, however, significantly lower in ischemic stroke case studies than in control individuals (16.8 vs. 21.7%, $P < 0.05$) (Table 2). Moreover, the presence of at least one variant A allele was associated with a significantly lower risk of ischemic stroke after adjustment for sex, age and multiple cardiovascular risk factors [adjusted OR = 0.50, 95% CI, 0.29–0.86; $P < 0.05$] (Table 3). These data suggest a protective influence of the *EPHX2* G860A polymorphism on ischemic stroke.

To determine whether an interaction between effects of *CYP2J2* G-50T and *EPHX2* G860A polymorphisms on stroke might exist, an allied genotype analysis was performed. Distributions

of the genotype combinations observed in the study population are shown in Table 4. In comparison with the reference combination of *CYP2J2* GG and *EPHX2* GG wild type genotypes, the combination of the *CYP2J2* GG genotype together with either the *EPHX2* GA or AA genotype (i.e. the presence of at least one A allele) was found to be significantly different in ischemic stroke cases vs. control individuals ($P < 0.05$; Table 4). The distribution of other genotype combinations did not differ from the wild type reference. The combination of the *CYP2J2* GG genotype with either *EPHX2* GA or AA genotypes was associated with significantly lower risk of ischemic stroke after adjustment for sex, age, and multiple cardiovascular risk factors (OR = 0.45, 95% CI, 0.26–0.80; $P < 0.05$) (Table 5). By comparison, the combination of the *CYP2J2* GT genotype with either the *EPHX2* GA or AA genotypes was not significantly associated with ischemic stroke risk (Table 5); however, the CIs were wide owing to the small number of individuals with this combination. We also performed gene–gene interaction testing on a multiplicative scale between the *CYP2J2* G-50T and *EPHX2* G860A polymorphisms but found no significant interaction (P value for interaction = 0.33).

The interaction between *EPHX2* G860A polymorphism, smoking and ischemic stroke risk was statistically significant (P value for interaction = 0.045). When stratified by smoking status, the *EPHX2* G860A variant allele was significantly less common among nonsmoking stroke cases compared with control patients (24 vs. 39%, respectively, $P < 0.05$) (Table 6). Significant differences were not observed in smokers (33 vs. 31%, respectively, $P = 0.36$). Nonsmokers carrying the *EPHX2* G860A variant allele were at lower risk of ischemic stroke than nonsmokers without this polymorphism (OR = 0.33, 95% CI, 0.17–0.67, $P = 0.002$) (Table 6). No significant association was observed in smokers (OR = 1.04, 95% CI, 0.41–2.65, $P = 0.34$). Smoking status did not modify the risk of ischemic stroke for the *CYP2J2* G-50T polymorphism (P value for interaction = 0.32).

Discussion

The purpose of this study was to examine potential associations between specific polymorphisms in *CYP2J2* and *EPHX2* and ischemic stroke in a Chinese population. Results indicate that the *CYP2J2* G-50T polymorphism was not independently associated with an altered risk for ischemic stroke in this study population, whereas the *EPHX2* G860A polymorphism was. The presence of at least one A allele at position 860 of *EPHX2* was associated with an adjusted OR of 0.50 for ischemic stroke, and with an adjusted OR of 0.45 when present in combination with the wild type *CYP2J2* GG genotype. Thus, the *EPHX2* G860A polymorphism appears to be an independent protective factor against ischemic stroke in this Chinese population.

To our knowledge, this is the first study of ischemic stroke in which potential associations with both the *CYP2J2* G-50T and *EPHX2* G860A polymorphisms have been investigated. The *CYP2J2* G-50T variant results in loss of one of the four Sp1 binding sites in the promoter of the *CYP2J2* gene and decreased promoter activity [14,22], and was recently shown to be associated with an increased risk of coronary heart disease and decreased circulating EET metabolite levels [14,22]. Although reduced enzyme activity and EET production resulting from the presence of the *CYP2J2* G-50T polymorphism might be expected to negatively impact cardiovascular homeostasis and increase the risk of stroke, no significant association between this polymorphism and stroke risk was observed in the present study. In agreement with our results, Lee *et al.* [23] recently reported no significant association between the *CYP2J2* G-50T polymorphism and coronary heart disease risk in Caucasians enrolled in the Atherosclerosis Risk in Communities Study. Interestingly, the *CYP2J2* G-50T polymorphism was associated with significantly lower risk of incident coronary heart disease in African–Americans in that study [23]. Hence, the influence of this polymorphism on cardiovascular disease risk may vary significantly depending on the population demographics and disease outcome examined.

In contrast to *CYP2J2* G-50T, the *EPHX2* G860A polymorphism was associated with ischemic stroke risk in the present study. The presence of at least one A allele at position 860 of *EPHX2* was observed more frequently in control individuals than in ischemic stroke cases, suggestive of a protective effect of this polymorphism against stroke. Indeed, carriers of at least one 860A allele had an adjusted OR of 0.50 (95% CI, 0.29–0.86) for ischemic stroke. The G860A polymorphism results in an amino acid substitution (R287Q) that alters enzyme stability and reduces enzyme activity [17,24]; this in turn leads to increased circulating EET levels due to the decreased hydrolysis to dihydroxyeicosatrienoic acids [25,26]. Consistent with our observations, a recent study has shown that in-vitro toxicity induced by oxygen and glucose deprivation is reduced in rat neurons expressing a human soluble epoxide hydrolase with the R287Q amino acid substitution [27]. Pharmacological inhibitors of soluble epoxide hydrolase have been shown to decrease angiotensin II-induced hypertension in rats [28], inhibit vascular smooth muscle cell proliferation *in vitro* [10], exert potent systemic anti-inflammatory effects [29] and inhibit experimental ischemic brain injury in mice [30]. All of these effects are thought to be due, at least in part, to increased availability of EETs as a result of soluble epoxide hydrolase inhibition. Although circulating EETs were not measured in the present study, it is reasonable to predict that the reduced risk of ischemic stroke in carriers of the *EPHX2* G860A polymorphism may have been due, at least in part, to increased availability and activity of these bioactive eicosanoids.

One interesting observation in our study was that individuals with the *CYP2J2*–50GG genotype (i.e. wild type) who possessed at least one *EPHX2* 860A allele had the lowest risk of ischemic stroke. This is likely due to the fact that the combination of normal EET production via wild type *CYP2J2* and decreased EET inactivation by the mutated soluble epoxide hydrolase would provide these individuals with greater circulating EET levels than those not possessing this particular combination. Increased circulating EETs would be expected to exert a protective effect in the vasculature via several mechanisms including inhibition of cellular adhesion molecule expression [9] and inhibition of vascular smooth muscle cell migration and proliferation [10,31]. The contribution of EETs to maintenance of endothelial cell integrity via these and other mechanisms would be expected to depress the development of atherogenesis and in turn result in continued production of EETs from these cells, thereby contributing to a cyclical antiatherosclerotic loop [32]. Another possibility is that high EET levels increase tissue-type plasminogen activator protein expression and elevate tissue-type plasminogen activator fibrinolytic activity [33]. As such, persistently high levels of EETs may play an important role in regulating the fibrinolytic balance in ischemic stroke and acute coronary heart disease events. Finally, high levels of EETs might contribute to lowering hypertension via their vasodilator effects [34–37], and lower the risk of ischemic stroke as a result.

Another interesting finding in our study is the significant interaction between the *EPHX2* genotype, smoking and ischemic stroke risk. In a stratified analysis, the protective effect of the *EPHX2* G860A polymorphism occurred only in nonsmokers. Smoking substantially impairs endothelial function and has been shown to modify the association between eNOS polymorphisms and cardiovascular disease [38]. More recently, we reported an interaction between the *EPHX2* K55R variant allele, smoking and risk of coronary heart disease in Caucasians enrolled in the Atherosclerosis Risk in Communities Study [39]. Future studies in larger populations will be required to better characterize this potential gene–environment interaction and the mechanistic contribution of *EPHX2* to the vascular effects of smoking in humans.

The precise mechanism for the protective effect of the G860A polymorphism in *EPHX2* against ischemic stroke is unknown. Substantial evidence in the literature that ischemic stroke is closely associated with subclinical atherosclerosis in intracranial or carotid arteries, and with unstable plaque [2,3,40], however, exists. EETs have been shown to possess anti-inflammatory

properties [9], inhibit the migration of smooth muscle cells [31] and modulate platelet function during hemostasis [11]. We also found that EETs upregulate eNOS [41] and protect endothelial cells from apoptosis [42]. Together, these activities suggest a potential underlying mechanism for the antiatherosclerotic and protective effect of the *EPHX2* polymorphism on ischemic stroke risk. It should be noted, however, that there is currently no direct experimental evidence to demonstrate that EETs inhibit atherosclerosis.

Important limitations to this study that must be acknowledged exist. As mentioned previously, circulating EET levels were not measured and so correlations between *CYP2J2* and *EPHX2* polymorphisms, potential alterations in EET levels and stroke incidence could not be established in this study population. Additionally, only two common polymorphisms, one in *CYP2J2* and one in *EPHX2*, were examined for their potential association with ischemic stroke. Other polymorphisms in these genes, some of which are rare and/or believed to be functionally relevant, may also contribute to the risk of ischemic stroke and other cardiovascular events [17,22,24]. Indeed, recent studies have shown that common *EPHX2* haplotypes appear to influence the incidence of ischemic stroke and coronary heart disease differently in different racial groups [18,39], and future studies will in part focus on the contribution of these and other genetic variants to the risk of ischemic stroke in the Chinese population studied here. Finally, the number of individuals examined in the present study was relatively small, and this in turn prevented statistical analysis of some potentially interesting interactions among the genotypes observed. For example, although it is speculated that persons with a combined *CYP2J2*–50 GG genotype and an *EPHX2* 860 AA genotype might have the lowest risk for ischemic stroke, the fact that there were only nine of 200 ischemic stroke cases and 20 of 350 control individuals with the *EPHX2* 860 AA genotype meant that there was insufficient statistical power to determine whether this was in fact true.

In summary, potential associations between *CYP2J2* G-50T and *EPHX2* G860A polymorphisms and ischemic stroke risk were investigated in a Chinese population. Our data indicate that the *EPHX2* G860A polymorphism is independently associated with a decreased risk of ischemic stroke whereas the *CYP2J2* G-50T polymorphism does not alter stroke risk in this population. Future studies will expand on these findings and investigate the involvement of other polymorphisms in these genes that are known or thought to alter enzyme activity and EET pathway-mediated regulation of cardiovascular homeostasis.

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References

1. Bonow RO, Smaha LA, Smith SC Jr, Mensah GA, Lenfant C. World Heart Day 2002: the international burden of cardiovascular disease: responding to the emerging global epidemic. *Circulation* 2002;106:1602–1605. [PubMed: 12270848]
2. Jiang B, Wang W-Z, Chen H, Hong Z, Yang Q-D, Wu S-P, et al. Incidence and trends of stroke and its subtypes in China: results from three large cities. *Stroke* 2006;37:63–65. [PubMed: 16306469]
3. Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med* 1999;340:115–126. [PubMed: 9887164]
4. Stoll G, Bendszus M. Inflammation and atherosclerosis: novel insights into plaque formation and destabilization. *Stroke* 2006;37:1923–1932. [PubMed: 16741184]
5. 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]
6. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 1999;401:493–497. [PubMed: 10519554]

7. Jiang JG, Chen RJ, Xiao B, Yang S, Wang JN, Wang Y, et al. Regulation of endothelial nitric-oxide synthase activity through phosphorylation in response to epoxyeicosatrienoic acids. *Prostaglandins Lipid Mediat* 2007;82:162–174.
8. Wang Y, Wei X, Xiao X, Hui R, Card JW, Carey MA, et al. Arachidonic acid epoxygenase metabolites stimulate endothelial cell growth and angiogenesis via mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt signaling pathways. *J Pharmacol Exp Ther* 2005;314:522–532. [PubMed: 15840765]
9. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, et al. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 1999;285:1276–1279. [PubMed: 10455056]
10. Davis BB, Thompson DA, Howard LL, Morisseau C, Hammock BD, Weiss RH. Inhibitors of soluble epoxide hydrolase attenuate vascular smooth muscle cell proliferation. *PNAS* 2002;99:2222–2227. [PubMed: 11842228]
11. Heizer ML, McKinney JS, Ellis EF. 14,15-Epoxyeicosatrienoic acid inhibits platelet aggregation in mouse cerebral arterioles. *Stroke* 1991;22:1389–1393. [PubMed: 1750047]
12. Larsen BT, Gutterman DD, Hatoum OA. Emerging role of epoxyeicosatrienoic acids in coronary vascular function. *Eur J Clin Invest* 2006;36:293–300. [PubMed: 16634832]
13. Wu S, Moomaw CR, Tomer KB, Falck JR, Zeldin DC. Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. *J Biol Chem* 1996;271:3460–3468. [PubMed: 8631948]
14. Spiecker M, Darius H, Hankeln T, Soufi M, Sattler AM, Schaefer JR, et al. Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2. *Circulation* 2004;110:2132–2136. [PubMed: 15466638]
15. Capdevila JH, Falck JR, Estabrook RW. Cytochrome P450 and the arachidonate cascade. *FASEB J* 1992;6:731–736. [PubMed: 1537463]
16. Zeldin DC. Epoxygenase pathways of arachidonic acid metabolism. *J Biol Chem* 2001;276:36059–36062. [PubMed: 11451964]
17. Przybyla-Zawislak BD, Srivastava PK, Vazquez-Matias J, Mohrenweiser HW, Maxwell JE, Hammock BD, et al. Polymorphisms in human soluble epoxide hydrolase. *Mol Pharmacol* 2003;64:482–490. [PubMed: 12869654]
18. Fornage M, Lee CR, Doris PA, Bray MS, Heiss G, Zeldin DC, et al. 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]
19. Otsuka T, Sugo N, Koehler RC, Hurn PD, Traystman RJ, Alkayed NJ. Soluble epoxide hydrolase gene deletion is protective against experimental cerebral ischemia. *Stroke* 2003;34:301.
20. Fornage M, Hinojos CA, Nurowska BW, Boerwinkle E, Hammock BD, Morisseau CH, Doris PA. Polymorphism in soluble epoxide hydrolase and blood pressure in spontaneously hypertensive rats. *Hypertension* 2002;40:485–490. [PubMed: 12364351]
21. Blin N, Stafford DW. A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res* 1976;3:2303–2308. [PubMed: 987581]
22. King LM, Ma J, Srettabunjong S, Graves J, Bradbury JA, Li L, et al. Cloning of CYP2J2 gene and identification of functional polymorphisms. *Mol Pharmacol* 2002;61:840–852. [PubMed: 11901223]
23. Lee CR, North KE, Bray MS, Couper DJ, Heiss G, Zeldin DC. CYP2J2 and CYP2C8 polymorphisms and coronary heart disease risk: the Atherosclerosis Risk in Communities (ARIC) study. *Pharmacogenet Genom* 2007;17:349–358.
24. Sandberg M, Hassett C, Adman ET, Meijer J, Omiecinski CJ. Identification and functional characterization of human soluble epoxide hydrolase genetic polymorphisms. *J Biol Chem* 2000;275:28873–28881. [PubMed: 10862610]
25. Fang X, Weintraub NL, McCaw RB, Hu S, Harmon SD, Rice JB, et al. Effect of soluble epoxide hydrolase inhibition on epoxyeicosatrienoic acid metabolism in human blood vessels. *Am J Physiol Heart Circ Physiol* 2004;287:H2412–H2420. [PubMed: 15284062]
26. Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, et al. Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ Res* 2000;87:992–998. [PubMed: 11090543]

27. 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]
28. 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]
29. Schmelzer KR, Kubala L, Newman JW, Kim I-H, Eiserich JP, Hammock BD. Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *PNAS* 2005;102:9772–9777. [PubMed: 15994227]
30. Zhang W, Koerner IP, Noppens R, Grafe M, Tsai H-J, Morisseau C, et al. Soluble epoxide hydrolase: a novel therapeutic target in stroke. *J Cereb Blood Flow Metab.* 2007Epub ahead of print
31. Sun J, Sui X, Bradbury JA, Zeldin DC, Conte MS, Liao JK. Inhibition of vascular smooth muscle cell migration by cytochrome P450 epoxygenase-derived eicosanoids. *Circ Res* 2002;90:1020–1027. [PubMed: 12016269]
32. Libby P, Aikawa M, Jain MK. Vascular endothelium and atherosclerosis. *Handb Exp Pharmacol* 2006;■:285–306. [PubMed: 16999230]
33. Node K, Ruan X-L, Dai J, Yang S-X, Graham L, Zeldin DC, Liao JK. Activation of G alpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J Biol Chem* 2001;276:15983–15989. [PubMed: 11279071]
34. Roman RJ. P-450 Metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev* 2002;82:131–185. [PubMed: 11773611]
35. Capdevila JH, Falck JR, Harris RC. Cytochrome P450 and arachidonic acid bioactivation: molecular and functional properties of the arachidonate monooxygenase. *J Lipid Res* 2000;41:163–181. [PubMed: 10681399]
36. McGiff JC. Cytochrome P-450 metabolism of arachidonic acid. *Annu Rev Pharmacol Toxicol* 1991;31:339–369. [PubMed: 1905908]
37. Sinal CJ, Miyata M, Tohkin M, Nagata K, Bend JR, Gonzalez FJ. Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J Biol Chem* 2000;275:40504–40510. [PubMed: 11001943]
38. Leeson CPM, Hingorani AD, Mullen MJ, Jeerooburkhan N, Kattenhorn M, Cole TJ, et al. Glu298Asp endothelial nitric oxide synthase gene polymorphism interacts with environmental and dietary factors to influence endothelial function. *Circ Res* 2002;90:1153–1158. [PubMed: 12065317]
39. Lee CR, North KE, Bray MS, Fornage M, Seubert JM, Newman JW, et al. 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]
40. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007;115:459–467. [PubMed: 17242284]
41. Wang H, Lin L, Jiang J, Wang Y, Lu ZY, Bradbury JA, et al. Up-regulation of endothelial nitric-oxide synthase by endothelium-derived hyperpolarizing factor involves mitogen-activated protein kinase and protein kinase C signaling pathways. *J Pharmacol Exp Ther* 2003;307:753–764. [PubMed: 12975498]
42. Yang S, Lin L, Chen J-X, Lee CR, Seubert JM, Wang Y, et al. Cytochrome P-450 epoxygenases protect endothelial cells from apoptosis induced by tumor necrosis factor-alpha via MAPK and PI3K/Akt signaling pathways. *Am J Physiol Heart Circ Physiol* 2007;293:H142–H151. [PubMed: 17322420]

Fig. 1. (a) Representative PCR-RFLP analysis of the *CYP2J2* G-50T polymorphism. Lanes 1 and 2, homozygous GG genotype; Lanes 3 and 4, heterozygous GT genotype. No homozygous TT genotypes were observed in the study cohort. (b) Representative PCR-RFLP analysis of the *EPHX2* G860A polymorphism. Lanes 1 and 2, homozygous GG genotype; lanes 3 and 4, heterozygous GA genotype; lanes 5 and 6, homozygous AA genotype. PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism

Table 1

Characteristics of the study population

	Ischemic stroke cases (<i>n</i> =200 ^a)	Control individuals (<i>n</i> =350 ^a)	<i>P</i> value
Sex (% male)	67	36	<0.001
Smoking (% yes)	41.0	20.3	<0.001
DM (%)	15.5	6.8	0.002
Hypertension (%)	60.4	41.0	<0.001
CHD (%)	25.0	12.5	<0.001
Age (years)	61.7 ± 0.8	63.2 ± 0.4	0.092
BMI (kg/m ²)	24.0 ± 0.3	23.8 ± 0.2	0.504
TC (mmol/l)	4.91 ± 0.10	5.25 ± 0.06	0.005
LDL (mmol/l)	2.37 ± 0.12	2.97 ± 0.07	<0.001

BMI, body mass index; CHD, coronary heart disease; DM, diabetes mellitus; LDL, low density lipoprotein; TC, total cholesterol.

^a Sample sizes for some characteristics were less because of missing data.

Table 2
Genotype distributions and allele frequencies for *CYP2J2* G-50T and *EPHX2* G860A polymorphisms

<i>CYP2J2</i>	Genotype distribution			Allele frequency		<i>P</i> value
	GG	GT		G	T	
Ischemic stroke	179	21		0.947	0.053	
Control	320	30		0.957	0.043	
<i>EPHX2</i>	GG	GA	AA	G	A	0.46
Ischemic stroke	142	49	9	0.832	0.168	
Control	218	112	20	0.783	0.217	0.047

Genotype data are presented as number of patients.

Table 3
Analysis of multiple logistic regression model for *EPHX2* G860A allele frequency

Variable	Adjusted OR	95% CI	P value
Sex (F vs. M)	0.34	0.18–0.62	<0.001
Hypertension	2.15	1.28–3.61	0.004
CHD	2.92	1.58–5.42	<0.001
DM	2.91	1.38–6.15	0.005
LDL	0.71	0.57–0.88	0.002
GA or AA genotype	0.50	0.29–0.86	0.013

Variables included in the model were sex, age, hypertension, CHD, DM, BMI, smoking, total cholesterol and LDL cholesterol. The *P* values for sex, smoking, DM, hypertension, CHD, age, BMI, total cholesterol and LDL cholesterol were, respectively, 0.847, 0.657, 0.804, 0.060, 0.749, 0.825, 0.382, 0.509, 0.890 using χ^2 tests for categorical demographics and *t* tests for continuous variables. BMI, body mass index; CHD, coronary heart disease; DM, diabetes mellitus; LDL, low density lipoprotein; TC, total cholesterol.

Table 4
Distribution of combined *CYP2J2* and *EPHX2* genotypes

Genotype <i>CYP2J2</i>	<i>EPHX2</i>	Ischemic stroke cases (<i>n</i> =200)	Control individuals (<i>n</i> =350)	<i>P</i> value
GG	GG	127	197	Reference
GG	GA or AA	52	123	0.035
GT	GG	15	21	0.774
GT	GA or AA	6	9	0.950

Table 5

Analysis of multiple logistic regression model for synergism between the *CYP2J2*–50 GG and GT genotypes and *EPHX2* 860 GA or AA genotypes

Variable	Adjusted OR	95% CI	P value
Sex (F vs. M)	0.33	0.18–0.61	<0.001
Hypertension	2.15	1.28–3.61	0.004
CHD	2.90	1.56–5.40	<0.001
DM	2.96	1.40–6.25	0.005
LDL	0.71	0.57–0.88	0.002
<i>CYP2J2</i> –50 GG genotype combined with <i>EPHX2</i> 860 GA or AA genotypes	0.45	0.26–0.80	0.007
<i>CYP2J2</i> –50 GT genotype combined with <i>EPHX2</i> 860 GA or AA genotypes	1.19	0.28–5.04	0.809

Variables included in the model were sex, age, hypertension, CHD, DM, BMI, smoking, total cholesterol, and low-density lipoprotein. Variables not shown in the table were not significant ($P>0.05$).

BMI, body mass index; CHD, coronary heart disease; DM, diabetes mellitus; LDL, low density lipoprotein.

Table 6
EPHX2 G860A by smoking interaction and risk of ischemic stroke

	Non-smokers		Smokers	
	Stroke	Control	Stroke	Control
Frequencies				
GG	85 (76%)	169 (61%)	52 (67%)	49 (69%)
GA + AA	27 (24%)	109 (39%)	26 (33%)	22 (31%)
Total	112	278	78	71
Adjusted ORs (95% CI)				
GG	1.0 (reference)		0.96 (0.46, 1.97) <i>P</i> =0.36	
GA + AA	0.33 (0.17, 0.67) <i>P</i> =0.002		1.04 (0.41, 2.65) <i>P</i> =0.34	

Variables included in the model were sex, age, hypertension, CHD, DM, BMI, smoking, total cholesterol, and low-density lipoprotein.

BMI, body mass index; CHD, coronary heart disease; DM, diabetes mellitus; OR, odds ratio.