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HYPERTENSION, CARDIOVASCULAR RISK AND POLYMORPHISMS IN GENES CONTROLLING THE CYTOCHROME P450 PATHWAY OF ARACHIDONIC ACID: A SEX-SPECIFIC RELATION?

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Abstract

Hypertension is a multifactorial disease in which the interplay of genetic and environmental factors that maintain blood pressure stable throughout life is altered. Cytochrome P450 (CYP)-derived metabolites of arachidonic acid such as epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE), active on vascular tone, endothelial function and renal sodium reapportion, have been identified as candidate mediators in the development of hypertension in several animal models, with remarkable sex-specific effect. Several SNPs, some recognized as functional, in human genes implicated in EETs/20-HETE biosynthesis and metabolism, such as CYP2J2 and CYP4A11, have been tested for association with blood pressure, hypertension and its long-term cardiovascular consequences in different populations, with conflicting results. A sex-specific effect, related to CYP4F2 polymorphisms and expression, has been observed in association studies. This finding indicates that altered 20-HETE bioactivity underlay the excess of hypertension and associated vascular events observed in men with respect to women and is consistent with the results from experimental models. Further epidemiological and mechanistic studies are required to confirm the effect of lipid mediators on blood pressure in humans and define the mechanisms of a putative sex-specific effect.

Key Words

Epoxyeicosatrienoic acids, 20-hydroxyeicosatetraenoic acid, genetic polymorphism, hypertension, sex, cytochrome P450
Introduction

Hypertension and its long term cardiovascular consequences are major determinants of morbidity and mortality worldwide[1-3]. Both genetic and environmental factors are implicated in the homeostasis of blood pressure and the development of hypertension[4,5]. Despite large heritability of these traits, only few genes have been unequivocally associated to blood pressure phenotype, with common single nucleotide polymorphisms (SNPs) conferring individual risk of a very limited increase in mean blood pressure (a few mmHg) and rare variants responsible for Mendelian forms of hypertension or hypotension having a larger effect on blood pressure, but a negligible prevalence in the general population[6,7]. Also the Genome Wide Association Studies (GWAS) identified only a minority of genetic loci and candidate genes potentially associated with blood pressure and the development of hypertension[8-10]. The notion that the prevalence of hypertension and cardiovascular disease differ between men and women is well established, being women protected from cardiovascular events until menopause, with a rapid increase in their risk profile beyond that age[11-13]. Responsibility for the observed differences has been attributed to sex hormones, although hormone replacement therapy failed to decrease coronary events in post-menopausal women and had negligible effect on blood pressure[14-17]. Gene-specific effects have been indicated as responsible, but most of genetic studies did not include sex as a variable in data analysis and a recent meta-analysis pooling thousands of subjects of previous GWASs did not identify any sex-specific effect of genes associated to blood pressure/hypertension[18]. Despite these drawbacks, the evidence that sex-related differences exist in cardiovascular risk is strong, the challenge is to find out which genes are actually implicated and though which pathway they act.

The cytochrome P450 (CYP) is a complex enzyme system in which different isoforms share significant sequence homology and act on myriad of endogenous and exogenous substrates, representing one of the major systems implicated in drugs metabolism. The CYP enzymatic system may have a role in the development of hypertension as well as cardio- and cerebrovascular events[19,20], being implicated in steroidogenesis, including androgens and estrogens biosynthesis
and metabolism[21]. Arachidonic acid is oxidized by the CYP mono-oxygenase to produce hydroxy- and epoxy-arachidonic acid derivatives with vasoactive and natriuretic properties: namely 20-hydroxyeicosatetraenoic acid (20-HETE) and 5,6-, 8,9-, 11,12- and 14,15 epoxyeicosatrienoic acids (EETs), which are metabolized to the correspondent dihydroxyeicosatrienoic acids (DHETs), mostly inactive compounds, by soluble Epoxide Hydrolase (sEH). Thus, CYP isoforms, by producing vasoactive and natriuretic compounds, and interacting with sex hormones, could represent a cross-road in sex-related risk of cardiovascular disease.

In the present review, we describe the available evidences linking specific CYPs involved in 20-HETE and EETs metabolism, along with EPHX2, the gene codifying for sEH, with the development of hypertension and discuss possible sex-specific effects in the light of the results from experimental, mechanistic, genetic and epidemiological studies.

**CYP450 isoforms, 20-HETE and EETs formation and action.**

CYP2C/CYP2J and CYP4A/CYP4F subfamilies are the predominant and functionally relevant vascular and renal arachidonic acid epoxygenases and ω-hydroxylases, in humans, catalyzing the production of EETs and 20-HETE[19;20]. EETs bioactivity is terminated by the conversion, catalyzed by sEH, into the corresponding DHETs, almost inactive compounds.

20-HETE and EETs play critical roles in the regulation of renal, pulmonary, and cardiac function and vascular tone. In particular, EETs hyperpolarize vascular smooth muscle cells by increasing the open-state probability of the calcium-activated potassium (K\textsubscript{Ca}) channels, whereas 20-HETE exerts opposite effects[19;20]. The vasoconstrictors angiotensin II, vasopressin, and norepinephrine, activate phospholipases in vascular smooth muscle cells (VSMCs) and increase the release of arachidonic acid, thus triggering the formation of 20-HETE[19;20]. CYP-derived metabolites of arachidonic acid play an important role also in the modulation of renal reabsorption of sodium: acting on different channels at tubular level, both 20-HETE and EETs exert natriuretic effects. [19,20] Thus, by acting at vascular level, EETs and 20-HETE exert antihypertensive and pro-
hypertensive effects, respectively, whereas both compounds have antihypertensive properties through their effects at tubular level[19,20].

Beside vasoactive properties, EETs also display potent anti-inflammatory, antiplatelet and antithrombotic activities[22]. EETs increase the rate of growth in endothelial cells, stimulate angiogenesis and inhibit the proliferation of human VSMCs. Therefore, EETs have been proposed to have a dual vasoprotective effect by promoting neovascularisation in ischemic tissues and by inhibiting atherosclerosis[23]. EETs have been reported to be cardioprotective by virtue of their anti-inflammatory activity and their capacity of modulating several cardiac ion channels. 20-HETE has been described as harmful having pro-inflammatory and pro-oxidative effects, that lead to damage of vascular endothelium[20].

**Studies using animal models to explore the patophysiological role of 20-HETE and EETs**

A role in altered sodium handling and control of vascular tone has been attributed to altered 20-HETE and EETs bioactivity in animal models of hypertension. These include DOCA salt hypertensive rats, Lyon rats, spontaneously hypertensive rats (SHR), Dahl Salt sensitive (Dahl S) rats and two models of hypertension induced by the intravenous infusion of angiotensin II and by constructing a transgenic rats with two functionally active renin genes. Increased biosynthesis of 20-HETE has been recognized to be responsible for the increased reactivity to constrictor agonists in the renal vasculature of SHR, whereas decreased generation of 20-HETE in the renal tubuli may contribute to the shift of the pressure-natriuresis relation, responsible for salt-sensitive hypertension in Dahl S rats[19,24,25].

Interestingly, the results of several studies on different rat strains, using knockout models or specific pharmacological tools to alter the CYPs system, show a clear dimorphism between male and female animals: only male animals have an increased susceptibility to hypertension and either castration or the administration of androgen inhibitors revert the hypertensive phenotype[26-31].
Sex specificity in animal models

Male mice knockout for the *CYP4A14* gene develop androgen-dependent hypertension which is attenuated by castration and restored by androgen replacement[26]. Treatment of normotensive rats with 5alpha-dihydrotestosterone (DHT) is associated with an increase in systolic blood pressure both in males and females[27;29]. The ratio of 20-HETE to EETs in renal interlobar arteries from male rats is twofold higher than from female rats. Moreover, DHT treatment eliminates any difference between males and females in the 20-HETE to EETs ratio[29]. The effect of DHT administration on blood pressure is accompanied by the up-regulation of CYP isoforms responsible for the synthesis of 20-HETE (CYP4A12 in mice, CYP4A8 in rats) in the renal vasculature and down-regulation of those responsible for the synthesis of EETs (CYP2C23)[26;29]. In DHT-induced hypertension a selective CYP4A inhibitor, HET0016 [N-hydroxy-N-(4-butyl-2 methylphenyl)formamidine], decreases 20-HETE production, lowers blood pressure and restores endothelial function in the renal interlobar arteries of rats[30]. These data support the hypothesis of a causal role of CYP-derived eicosanoids in androgen-induced endothelial dysfunction and hypertension[30]. A more recent study, exploring the putative mechanism of hypertension in *CYP4A14* knockout mice, showed that increased oxidative stress, enhanced responses to vasoconstrictors, blunted response to vasodilators and a defect in the renal sodium excretory capacity is associated with increased 20-HETE biosynthesis[32]. Moreover, also in wild type mice, the expression of the CYP4A12, whose activity is driven by androgens, is several times higher in male with respect to female mice and correlates with the production of 20-HETE in renal microsomes[33].

The pro-hypertensive activity of 20-HETE was also tested in an experimental model of menopause: in spontaneously hypertensive female rats. Both a non-selective and a selective inhibitor of 20-HETE production reduced blood pressure in post-menopausal rats but not in fertile females and
increased mRNA and protein expression of renal CYP4A isoforms was observed in postmenopausal compared to fertile rats[34].

A reduction in the synthesis of 20-HETE and EETs in the renal medulla linked to male sex has been suggested to be implicated in altered renal function and increased blood pressure in obesity-induced hypertension. In fact, in male Sprague-Dawley rats fed with high fat diet, the expression of CYP4A and CYP2C23, catalyzing the synthesis of 20-HETE and EETs, is down-regulated in renal tubules[35]. A high fat diet reduces sodium excretion and blood pressure in male rats, confirming a sex-specific effect[31]. Clofibrate and fenofibrate increase tubular CYP4A1, CYP4A8 and CYP epoxygenase expression with subsequent improvement in renal and systemic haemodynamics[36,37]. The deletion of the ephx2 gene (ephx2-/-) in mice with subsequent increase in the EETs to diHETEs ratio was associated with transient reduction in blood pressure only in male mice[28].

**Human studies exploring the patophysiological role of 20-HETE and EETs in hypertension and vascular disease**

Several CYP are epoxygenases and metabolize arachidonic acid to EETs in humans: CYP1A, CYP2B, CYP2E and, primarily, CYP2C and CYP2J are the subfamilies responsible for most of EETs production in cardiovascular and renal tissues[20,38,39]. As for the ω-hydroxylase activity, CYP4A and CYP4F catalyze the ω-hydroxylation of arachidonic acid to 20-HETE especially in the liver and the kidney[40]. Also sEH is expressed in several human tissues, including the kidney and the brain and may therefore be implicated in altered EETs bioactivity[41-43]. Despite abundant literature concerning the role of 20-HETE and EETs in experimental hypertension, few studies addressed the pathophysiological role of these compounds in human hypertension. Laffer and colleagues, analyzed the urinary excretion of 20-HETE in response to salt loading, furosemide administration and insulin release. Despite similar increase in the urinary excretion of 20-HETE during salt repletion, sodium excretion correlates with 20-HETE excretion
in salt resistant, but not in salt sensitive subjects. This suggests that a disrupted relation between sodium and 20-HETE excretion in salt sensitive patients may result in the dependence of salt excretion on blood pressure\cite{44}. The administration of furosemide was found associated with increased excretion of 20-HETE, correlated with changes in urinary sodium, in salt resistant and sensitive hypertensive subjects. Both sodium and 20-HETE excretion were altered in salt sensitive subjects, thus suggesting that 20-HETE modulates the natriuretic response to furosemide and that impaired natriuretic response in salt sensitive subjects implicates a mechanism controlling 20-HETE release \cite{45}. In all these study correlation was found between urinary 20-HETE and BMI of hypertensive subject\cite{45}. When obese hypertensive subjects were studied, not only correlation with BMI was confirmed, but also correlation between increased circulating insulin, not insulin resistance per se, and reduced urinary excretion of 20-HETE was found. The mechanism may implicate the inhibition of renal CYP4A by insulin, that may link obesity, high insulin levels and hypertension\cite{46}. However, in a study including lean to overweight untreated hypertensive and normotensive subjects, despite positive correlation between urinary 20-HETE and BMI, correlation of urinary 20-HETE with serum insulin or insulin resistance was not confirmed\cite{47}. Correlation between 20-HETE and markers of oxidative stress (F2-isoprostanes and γ-GT) as well as with 24-h diastolic blood pressure was observed in treated hypertensive subjects\cite{48}. Interestingly, in hypertensive women, but not in men, the urinary excretion of 20-HETE was higher compared to normotensive subjects and correlated with diastolic and systolic blood pressure\cite{49}.

A limited number of studies explored the relation between CYP-derived eicosanoids and endothelial function in humans. The urinary excretion of 20-HETE was similar in non-treated hypertensive and normotensive subjects, although a negative correlation between urinary 20-HETE and endothelium-dependent vasodilation, as assessed using the method of flow-mediated dilation (FMD) of the brachial artery, has been observed\cite{49}. The role of EETs as endothelium-derived hyperpolarizing factor (EDHF) was explored by using the CYP2C9 inhibitor sulfaphenazole. sulfaphenazole blunted the dilatory response to acetylcholine and bradykinin in the forearm microcirculation of essential
hypertensive patients, while in normotensive control subjects only vasodilation residual to the
effects of cyclooxygenase and nitric oxide synthase inhibitors was reduced by sulfaphenazole [50].
When urinary 20-HETE, EETs and DHETs levels were measured in plasma and urine of patients
with reno-vascular disease in comparison with matched essential hypertensive and normotensive
controls[51], plasma 20-HETE was found to be elevated and to correlate with plasma renin activity.
This finding suggests that vasoconstrictor 20-HETE might mediate the increase in blood pressure
driven by the activation of the renin-angiotensin system, typical of reno-vascular disease[51].

Genetic variations, cardiovascular diseases and sex specificity
A detailed discussion of the strength and weakness of all the studies performed to test the
association between cardiovascular outcome and genetic variants in genes implicated in 20-HETE,
EETs metabolism will not be discussed in details in the present review. Interest readers could refer
to a recent review[52] and for the effect on coronary artery disease to a recent meta-analysis[53].
The present review will be focused on the results of a number of association studies showing an
uneven sex prevalence in cerebro- and cardiovascular events, supporting the hypothesis of a sex-
specific effect of altered CYPs/sEH bioactivity in hypertension. The focus has been put especially
on functional SNPs. Table 1 to 4 show a summary of the results from the most studied functional
SNPs, with indication of the studies identifying a sex-specific association with hypertension and
cerebro- and cardiovascular diseases.

CYP2C8, CYP2C9, hypertension and cardiovascular diseases
CYP2C8*3 (Arg139Lys and Lys399Arg, which are in 100% linkage disequilibrium; dbSNP
accession number rs11572080 and rs10509681) has been shown to be associated with reduced
arachidonic acid epoxygenase activity and reduced EETs production[54]. Similar observations are
reported for the CYP2C9*2 (Arg144Cys, rs1799853) and CYP2C9*3 (Ile359Leu; rs1057910) polymorphisms.[55]
Conflicting results have been obtained from studies on CYP2C8 polymorphisms and hypertension.
Despite the evidence of decreased epoxygenases activity with consequent reduced formation of the
anti-hypertensive EETs[54], no association between the CYP2C8*3 genotype and hypertension was found in small sized studies (table 1)[56;56;57] and from a large case-control study concerning the risk of myocardial infarction in Caucasians[58]. In the latter study also the CYP2C9*2 and CYP2C9*3 polymorphisms were explored for a possible association with hypertension with negative results, independently of sex. A trend towards increased risk for acute myocardial infarction was found in a previous study analyzing the polymorphisms CYP2C8*3, CYP2C9*2 and CYP2C9*3 (table 3)[58]. In subjects enrolled in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, no significant differences in coronary artery disease between individuals carrying different CYP2C8 and 2C9 genotypes. When the population carrying the CYP2C9*3 allele was split according to sex, increased risk of MI was found in men and decreased risk in women[59]. The use of CYP2C9 substrates and inhibitors was associated with a significant increase in the risk of myocardial infarction especially in female harbouring a variant allele (CYP2C8*2, CYP2C9*3) [60]. No association between CYP2C9*2 and CYP2C9*3 polymorphisms and risk of subclinical atherosclerosis, ischemic vascular disease (ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease and ischemic stroke) or cardiovascular death was found in a very large Danish study[61]. Similarly, an association with coronary atherosclerosis, detected by angiography, was found in a small study performed in Turkey[62]. In a mixed population from the USA, mostly of Caucasian ethnicity, no association with myocardial infarction and stroke was observed in a haplotype analysis using 8 and 7 tag SNPs for the CYP2C8 and CYP2C9, respectively[63].

In conclusion, as for CYP2C8 and CYP2C, limited evidence exists of an association with increased cardiovascular risk and the presence of a sex-specific effect.

**CYP2J2, hypertension and cardiovascular diseases**

The CYP2J2 -50G>T (rs890293; sometimes referred as CYP2J2*7) interferes with a binding site for the transcription factor Sp1 with consequent reduction in the plasma levels of EETs in vivo[64;65]. Carriers of the common polymorphism CYP2J2*7 within the promoter region display reduced expression of CYP2J2 mRNA in the heart[66]. In a Chinese population, plasma levels of 14,15-
dihydroxyeicosatrienoic acid were found significantly lower in CYP2J2*7 T allele carriers than in GG subjects (table 1) [67]. In a case-control study in African American, several SNPs in CYP genes, including the CYP2J2*7, were studied and no association was found with hypertension[57]. In a study involving 168 African American and 251 Caucasians the prevalence of the CYP2J2*7 variant allele was found significantly different among hypertensive and normotensive Caucasians but not African-Americans. Interestingly, the variant allele was found to be protective in Caucasian men, but not in women[56]. In a Han Chinese population, despite no apparent effect for the functional variant CYP2J2*7 in the entire cohort, an association with hypertension and high systolic blood pressure was found in women homozygote for the T-allele of the intronic rs1155002 variant[68]. In more than 5,000 Swedish participants to an urban-based survey, the Malmo Diet and Cancer cardiovascular arm (MDC-CVA), no difference in the prevalence of hypertension was detected between carriers of the CYP2J2*7 variant allele either when the entire cohort was considered or when subjects were stratified according to sex[69].

In previous case-control studies statistically significant association of the G-50T SNP was found with coronary artery disease in Germans and with premature myocardial infarction in a Taiwanese, but not in a Chinese population (table 3)[53,65,67]. Similarly, no association with cardiovascular disease was found in the aforementioned urban-based study in Swedes, in two studies including subjects at high risk for myocardial infarction and in a case-control study in Germans[69-71]. No association between the CYP2J2*7 and coronary heart disease was observed in White Americans whereas an inverse association was evident in African Americans recruited in the ARIC study[72].

In a mixed population in the USA including mostly (90%) Caucasians, an association with myocardial infarction, but not with stroke, was found for two intronic SNPs in CYP2J2[63]. Also in a meta-analysis of previous studies, no association for the common CYP2J2*7 and cardiovascular disease was found[53]. The frequency of CYP2J2-50T allele was not significantly different in cases with ischemic stroke compared with controls in a cross-sectional study performed in a Chinese population and in a longitudinal study in Swedes[69,73]. Thus, in all the studies focusing on
cerebro- and cardiovascular events, no sex-specific effects were reported or, in most cases, not even explored.

**CYP4A11, hypertension and cardiovascular diseases**

The c.8590 T>C polymorphism (rs1126742) of the CYP4A11 gene leads to the missense variant Phe434Ser, associated with a reduced metabolizing capacity of CYP4A11 in vitro[74] with consequent reduction in the urinary excretion of 20-HETE.[75] Carriers of the same polymorphism have higher aldosterone/renin and waist/hip ratios and lower furosemide-induced fractional excretions of sodium and potassium than 8590TT homozygotes[76]. All these data are coherent with the selective localization of CYP4A11 in the S2 and S3 segments of proximal tubule epithelia in the cortex and outer medulla[77], so that a decreased catalytic activity should result in the reduction of 20-HETE formation and a consequent decrease in natriuresis. Therefore, the net result of having the variant allele should result in a pro-hypertensive phenotype.

Indeed, CYP4A11 c.8590 CC-homozygotes compared to carriers of at least one “wild-type” T allele show endothelial dysfunction as demonstrated by coronary vasoconstriction induced by infusion with acetylcholine[78].

The 8590C allele of CYP4A11, was found associated with hypertension in the general population in Caucasian subjects from Tennessee, later replicated in the Framingham population[74], in Germans participating to the MONICA study[79] and in Swedes enrolled in the MDC-CVA study [80] (table 2). No association with hypertension was evident between the same 8590C allele and hypertension in a case control study in Australian population[75]. The 8590C allele of CYP4A11 was associated with increased systolic blood pressure in Caucasian survivors of myocardial infarction recruited from the MONICA Augsburg myocardial infarction registry and followed up for up to 6 years[81].

In a study involving a Japanese population, the 8590C allele was associated with low risk of hypertension in the whole sample and in men[82]. In the same ethnic group, a common haplotype, containing the 8590C allele, conferred protection towards cerebral infarction (table 4) [83]. Not the functional CYP4A11 8590T>C but other SNPs, such as the rs9333025 and the C-296T, were found
associated with cerebrovascular events in Japanese and Chinese populations, respectively[83,84]. Interestingly, the association of the rs9333025 polymorphism with cerebro-vascular events was especially evident in men[83]. In the Swedish cohort no effect of the 8590C allele of CYP4A11 was detectable for coronary events and ischemic strokes in the whole population as well as in men and women separately[80]. In black Americans with hypertensive renal disease participating in the African American Study of Kidney Disease (AASK), men with the 8590CC genotype had significantly higher systolic blood pressure at baseline, while no association was observed in women. The same genotype was also associated with increased cumulative incidence of end stage renal disease and death[85].

**CYP4F2, hypertension and cardiovascular diseases**

The rs2108622 CYP4F2 c.1347G>A (Val433Met) polymorphism causes in vitro a reduced arachidonic acid metabolizing capacity[86]. Consistent with this finding is the observation that carriers of the 433Met allele have decreased capacity to metabolize vitamin K[87], and tocopherol[88]. At variance with the results of these studies, the CYP4F2 433Met, but not the 433Val allele, increases the urinary excretion of 20-HETE in a mixed sample of hypertensive and normotensive subjects, suggesting an increased, rather than a decreased, metabolizing capacity [75]. Moreover, the increase in 20-HETE urine excretion in 433Met carriers suggests that the prohypertensive effect of the variant allele may be due to excess renal vascular production of the vasoconstrictor 20-HETE by CYP4F2. This apparently conflicts with the evidence that CYP4F2 is expressed only in the S2 and S3 segments of renal proximal tubule epithelia in the cortex and outer medulla[77].

The CYP4F2 Val433Met genotype was not associated with increased prevalence of hypertension in the MDC-CVA, when the entire cohort was analyzed. However, an association was found in male carriers of the 433Met allele when stratifying the sample by sex (table 2) [80]. In a case-control study in Indians with stroke, an association with hypertension was found with the CYP4F2 433Met allele without differences between sexes[89]. In Australians the CYP4F2 GA/AA genotype (433Met
allele) was significantly associated with an increase in systolic blood pressure[75] whereas in Japanesees the prevalence of the same 433Met allele was not significantly different between hypertensive cases and normotensive controls[90]. In the latter study a common haplotype and the CC genotype of the rs1558139 were associated with hypertension only in men[90]. A CYP4F2 construct haplotype, was associated with increased excretion of 20-HETE and increased prevalence of hypertension in a case-control population study performed in China[91]. This finding was subsequently confirmed by the results of a family-based sub-analysis[91]. Notably, the CYP4F2 433Met allele was associated with ischemic stroke in Swedish men participating to the MDC-CVA study (table 4 and figure 1) and in a case control study in Indians[80,89]. In the latter study the association with cardioembolic stroke was strong, but no data concerning the prevalence of events in relation to sex were available. Two case-control studies were performed in Han Chinese: one showing a positive association of the 433Met allele with stroke in men [84], the second showing increased risk of ischemic stroke in male carriers of the 433Val allele. Also in a case-control study in Japanese, an association with cerebral infarction was observed in male carriers of the 433Val allele[92]. Anyhow, the sample size of the latest studies was quite limited. In the MDC-CVA no association with coronary events was found in both sexes whereas in a case-control study performed in Han Chinese the 433Val allele was more frequently observed in cases of myocardial infarction than in controls[93].

Thus, even if a discrepancy exists in the direction of the association in people of Asian ethnicity with respect to Caucasians, a sex-specific effect is almost always present for this SNP, when explored.

Interestingly, another functional variant, c.-91T>C, included in a common haplotype (Hap I) containing SNPs in the CP4F2 regulatory region, when transfected into HEK293 cells, exhibited significantly greater LPS-stimulated activity than Hap II, that may be driven by a different NF-kappaB binding affinity between the two constructs. In vivo, a case-control study demonstrated that homozygosity for Hap I doubles the risk for hypertension in a Chinese population and is associated
with higher 20-HETE excretion[91].

**EPHX2 polymorphisms and cardiovascular diseases**

*In-vitro* studies testing the functional capacity of 164A>G Lys55Arg (rs41507953) and the c.860G>A Arg287Gln (rs751141) polymorphisms in *EPHX2* have shown increased and diminished sEH activity, respectively[94-96], suggesting an association with lower EETs and a pro-hypertensive phenotype and higher EETs and a vasodilatory effect in the two genetic variants. In fact, a decrease in the ratio of 12,13-epoxyoctadecenoic acid to dihydroxyoctadecenoic acid was found in carriers of the *EPHX2* Lys55Arg genotype, together with higher sEH activity *ex vivo*[97]. Moreover, the *EPHX2* 287Gln variant is associated with increased neuronal survival after ischemic injury in a cell culture model[98]. Finally, the functionality of these SNPs was tested on endothelial dependent and independent vasodilatory capacity, using intra-arterial infusion of vasoactive compounds and strain-gauge venous occlusion plethysmography. A decrease in forearm blood flow, index of impaired endothelial function, was detected in white Americans for the 55Arg variant in response to the intra-arterial infusion of bradykinin, methacholine and sodium nitroprussiate[99]. Decreased vascular resistance, suggesting a potential protective effect towards the development of hypertension, was found in Black American carriers of the *EPHX2* 287Gln variant with respect to “wild type” subjects[99].

In the MDC-CV A study a positive association of the *EPHX2* Lys55Arg variant with blood pressure/hypertension was found in men with unexpectedly high systolic blood pressure values in homozygotes with respect to carriers of at least one wild type allele[100]. In the same cohort of Swedish and in a cohort of African Americans no evidence for the *EPHX2* Arg287Gln variant influencing the development of hypertension was found[57,100].

In the Atherosclerosis Risk in Communities (ARIC) study, the *EPHX2* Lys55Arg and the Arg287Gln did not show any association with ischemic stroke, whereas two common *EPHX2* haplotypes show significant association in African-Americans and Whites respectively[101]. Different *EPHX2* haplotypes were associated with stroke also in Caucasians[102]. In the same
sample, the 287Gln was associated with increased risk of cerebral ischemia, whereas no association was detectable for the Lys55Arg polymorphism[102]. Among Swedish men, those who were homozygotes for the 55Lys variant were at increased risk of ischemic stroke, while no association was evident for those carrying the Arg287Gln genotype [100]. Carriers of the EPHX2 287Gln allele had 50% lower risk of ischemic stroke in a Chinese population compared to 287Arg homozygotes with a positive interaction with smoke[73].

In the ARIC study, Caucasian but not African-Americans individuals harbouring the Lys55Arg polymorphism were at increased risk of coronary heart disease, with a positive association also in the analysis testing common haplotypes[97]. In the CARDIA study the Arg287Gln polymorphism, tagging a common haplotype, was associated with the presence of coronary artery calcified plaque in African Americans, while a common haplotype uniquely tagged by a polymorphism in Intron 11 was associated with significantly greater risk for coronary artery calcified plaques, in Whites[103]. In the MDC-CVA study, despite the already cited association with stroke, no association with coronary events was detected both in men and women[100]. No association was also observed in a recent study in a Chinese population and in a meta-analysis including previous studies[53].

Conclusions

Experimental models and pathophysiological studies in humans suggest that enzymes involved in 20-HETE and EETs biosynthesis and metabolism have a role in the control of blood pressure with a sex-specific effect. At least in animal models, clear evidence exists that sex hormones, especially testosterone, modulate CYPs expression and activity favouring higher blood pressure levels. Also in humans the interaction of sex hormones with CYPs expression could have a role in the well-established sexual dimorphism in blood pressure levels and cardiovascular risk described by epidemiologic studies[11,104]. In fact, SNPs in genes that code for some of these enzymes are associated with hypertension and vascular disease, especially stroke, with notable sex-specific effect, as is the case of CYP4F2 and EPHX2. Figure 2 shows the putative mechanisms by which androgens interacting with the wild type or mutant isoform of CYP4F2 could respectively
increment or diminish tubular 20-HETE with subsequent modification of the individual susceptibility to hypertension and/or stroke. This model is coherent with the diminished activity of mutant CYP4F2 in vitro, the localization of the enzyme in the renal tubuli and his absence in the vasculature[77,105].

At the present time no specific pharmacological tools are available for studies in humans, necessary to confirm the role of CYP-derived eicosanoids in the control of blood pressure and to further investigate the proposed sex-specificity of their cardiovascular effects. Unexplored are also the potential interactions of genetic polymorphisms with environmental conditions, particularly food and dietary elements. An intriguing hypothesis to be tested is that n-3 fatty acids, acting as competitive substrates of CYPs may modulate the biosynthesis of 20-HETE and EETs and generate compounds with different vasoactive properties[106]. Future studies, in which genetic and “omic” approach are integrated with clinical and epidemiologic data, may aid to unravel the described complex tangle.

Figure legend.

Figure 1: Cumulative incidence (percentage) of ischemic strokes (left panel) and coronary events (right panel) in the Malmö Diet and cancer-cardiovascular arm according to the CYP4F2 M433V genotype in the entire cohort (a and d), females (b and e) and males (c and f). From Fava et al Hypertension 2008;52(2):373-80.

Figure 2: Putative mechanisms by which androgens, interacting with the wild type or mutant isoforms of CYP4F2, may increment or diminish tubular 20-HETE biosynthesis with subsequent modification of the individual susceptibility to hypertension and/or stroke.
<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP/SNPs (possible functionality in vitro or ex vivo)</th>
<th>Ethnicity</th>
<th>MAF %</th>
<th>HT / CT</th>
<th>Effect of the variant allele in the entire cohort</th>
<th>Sex-specific findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP2C8</strong></td>
<td>rs11572080→CYP2C8*3→Arg139Lys (n.b. associated with reduced arachidonic acid epoxygenase activity with subsequent reduced EETs production [54])</td>
<td>Caucasian</td>
<td>9.5</td>
<td>No</td>
<td>No difference</td>
<td>No difference</td>
<td>Yasar [58]</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>3.3</td>
<td>No</td>
<td>N.E.</td>
<td></td>
<td>King [56]</td>
</tr>
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<td>Caucasian</td>
<td>11.9</td>
<td>No</td>
<td>N.E.</td>
<td></td>
<td>King [56]</td>
</tr>
<tr>
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<td></td>
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<td>3.3</td>
<td>No</td>
<td>N.E.</td>
<td></td>
<td>Dreisbach [57]</td>
</tr>
<tr>
<td><strong>CYP2C9</strong></td>
<td>rs1799853→CYP2C9<em>2→Arg144Cys rs1057910→CYP2C9</em>3→Ile359Leu (n.b. both associated with reduced arachidonic acid epoxygenase activity with subsequent reduced EETs production[55])</td>
<td>Caucasian</td>
<td>11.3</td>
<td>No</td>
<td>No difference</td>
<td>No difference</td>
<td>Yasar [58]</td>
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<td>Caucasian</td>
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<td>No difference</td>
<td>No difference</td>
<td>Yasar [58]</td>
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<td>African American</td>
<td>3.3</td>
<td>No</td>
<td>N.E.</td>
<td></td>
<td>Dreisbach [57]</td>
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<td></td>
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<td>2.6</td>
<td>No</td>
<td>N.E.</td>
<td></td>
<td>Dreisbach [57]</td>
</tr>
<tr>
<td><strong>CYP2J2</strong></td>
<td>rs890293→CYP2J2*7→-50 G&gt;T (n.b. associated with the interference with a binding site for the transcription factor Sp1 with consequent reduction in the plasma levels of EETs in vivo[64,65].)</td>
<td>African American</td>
<td>10.2</td>
<td>No</td>
<td>N.E.</td>
<td></td>
<td>Dreisbach [57]</td>
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<td>7.7</td>
<td>Protective</td>
<td>Protective only in males</td>
<td></td>
<td>King [56]</td>
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<tr>
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<td></td>
<td>Han Chinese</td>
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<td>Russian</td>
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<td>295/281</td>
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<td>Caucasian</td>
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<td>36/48/1658</td>
<td>No effect in both sexes</td>
<td></td>
<td>Fava [69]</td>
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<td><strong>EPHX2</strong></td>
<td>rs751141→c.860G&gt;A→Arg287Gln (n.b. associated with diminished sEH activity [94-96])</td>
<td>African American</td>
<td>3.6</td>
<td>108/106</td>
<td>N.E.</td>
<td></td>
<td>Dreisbach [57]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>10.6</td>
<td>3719/2108</td>
<td>N.E.</td>
<td></td>
<td>Fava [100]</td>
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<tr>
<td><strong>EPHX2</strong></td>
<td>rs41507953→c.164A&gt;G→Lys55Arg; (n.b. associated with diminished sEH activity [94-96])</td>
<td>Caucasian</td>
<td>9.2</td>
<td>3746/2129</td>
<td>No</td>
<td>Evident in males but not females</td>
<td>Fava [100]</td>
</tr>
</tbody>
</table>

HT: hypertension; CT: either control or normotensive subjects; N.E. non explored; BP, blood pressure
<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP/SNPs</th>
<th>Ethnicity</th>
<th>MAF</th>
<th>HT/CT</th>
<th>Effect of the variant allele in the entire cohort</th>
<th>Sex-specific findings</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>CYP4A11</td>
<td>rs1126742&gt;c.8590 T&gt;C &gt; Phe434Ser (n.b. associated with reduced metabolizing capacity of CYP4A11 \textit{in vitro}[74] with consequent reduction in the urinary excretion of 20-HETE.[75])</td>
<td>African-American</td>
<td>30.0</td>
<td>60/60</td>
<td>No effect</td>
<td>No difference</td>
<td>Gainer[74]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White American</td>
<td>15.7</td>
<td>197/195</td>
<td>Deleterious</td>
<td>No difference</td>
<td>Gainer[74]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White American</td>
<td>12.5</td>
<td>868/670</td>
<td>Deleterious</td>
<td>No difference</td>
<td>Gainer[74]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>13.3</td>
<td>649/748</td>
<td>Higher prevalence of HT</td>
<td>No difference</td>
<td>Meyer[79]</td>
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<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>15.1</td>
<td>228/332</td>
<td>Higher SBP in survivors of MI</td>
<td>N.E.</td>
<td>Meyer[81]</td>
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<tr>
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<td>Australian</td>
<td>15.3</td>
<td>161/74</td>
<td>None for HT and BP</td>
<td>No difference</td>
<td>Ward[75]</td>
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<td></td>
<td></td>
<td>Caucasian</td>
<td>12.5</td>
<td>3805/2170</td>
<td>Higher BP and HT prevalence in CC-HZ</td>
<td>No differences</td>
<td>Fava[80]</td>
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<tr>
<td></td>
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<td>Japanese</td>
<td>20.6</td>
<td>304/207</td>
<td>Protective</td>
<td>Evident in males but not females</td>
<td>Fu[82]</td>
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<tr>
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<td></td>
<td>African American</td>
<td>30.9</td>
<td>732</td>
<td>Higher SBP in CC-HZ</td>
<td>Evaluated only in men</td>
<td>Gainer[85]</td>
</tr>
<tr>
<td>CYP4F2</td>
<td>rs2108622&gt;c.1347G&gt;A &gt; Val433Met (n.b. associated with a reduced arachidonic acid metabolizing capacity[86] but contrarily to what could be expected with increased urinary excretion of 20-HETE[75])</td>
<td>Australian</td>
<td>28.7</td>
<td>161/74</td>
<td>None on HT and BP</td>
<td>Higher SBP in female A-carriers</td>
<td>Ward[75]</td>
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<tr>
<td></td>
<td></td>
<td>Caucasian</td>
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<td>3700/2092</td>
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<td>Higher BP and HT only in males</td>
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<tr>
<td></td>
<td></td>
<td>Japanese</td>
<td>27.4</td>
<td>249/238</td>
<td>No effect</td>
<td>No differences</td>
<td>Fu[90]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indians</td>
<td>50</td>
<td>427/567</td>
<td>Higher prevalence of HT</td>
<td>N.E.</td>
<td>Munshi[89]</td>
</tr>
</tbody>
</table>

HT: hypertension, CT: either control or normotensive subjects, N.E. non explored, BP, blood pressure; HZ, Homozygotes
Table 3: Association between the most extensively studied SNPs in genes responsible for EETs formation/metabolism and cardiovascular diseases in humans

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP/SNPs</th>
<th>Ethnicity</th>
<th>MAF %</th>
<th>Cases /CT</th>
<th>Effect of the variant allele in the entire cohort</th>
<th>Sex-specific findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C8</td>
<td>rs11572080→CYP2C8*3→Arg139Lys</td>
<td>African American</td>
<td>1.9</td>
<td>224/309</td>
<td>No for CHD</td>
<td>N.E.</td>
<td>Lee[72]</td>
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<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>10.9</td>
<td>773/577</td>
<td>No for CHD</td>
<td>N.E.</td>
<td>Lee[72]</td>
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<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>9.5</td>
<td>1172/1503</td>
<td>Trend vs. higher prevalence in MI cases</td>
<td>No difference</td>
<td>Yasar[58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>10.1</td>
<td>1053/615</td>
<td>No difference</td>
<td>No difference</td>
<td>Haschke-Becher[59]</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>rs1799853→CYP2C9<em>2→Arg144Cys rs1057910→CYP2C9</em>3→Ile359Leu</td>
<td>Caucasian</td>
<td>11.3</td>
<td>1172/1503</td>
<td>Trend vs. higher prevalence in MI cases</td>
<td>No difference</td>
<td>Yasar[58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>11.4</td>
<td>1053/615</td>
<td>No difference in MI and CAD</td>
<td>Men and women carrying the CYP2C9*3 allele had respectively an increased and decreased risk of IHD</td>
<td>Haschke-Becher[59]</td>
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<tr>
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<td></td>
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<td>12.8</td>
<td>223/5753</td>
<td>No difference</td>
<td>Higher risk in women with a variant allele</td>
<td>Visser[60]</td>
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<td></td>
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<td>9469/51898</td>
<td>No difference for IHD, MI</td>
<td>N.E.</td>
<td>Kaur-Knudsen[61]</td>
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<td>Turkish</td>
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<td>10890</td>
<td>No difference in CAD</td>
<td>N.E.</td>
<td>Ercan[62]</td>
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<td>CYP2J2</td>
<td>rs890293→CYP2J2*7→-50 G&gt;T</td>
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<td>289/255</td>
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<td>Specker[65]</td>
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<td>N.E.</td>
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<td>2547/697</td>
<td>No association with CAD, MI and death</td>
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<td>Hoffmann[71]</td>
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<td>Han Chinese</td>
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<td>1344/1267</td>
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<td>Xu[53]</td>
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<td>15.5</td>
<td>224/309</td>
<td>lower risk of incident CHD events</td>
<td>N.E.</td>
<td>Lee[72]</td>
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<td>773/577</td>
<td>No for CHD</td>
<td>N.E.</td>
<td>Lee[72]</td>
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<td>2617/5478</td>
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<td>No effect in both sexes</td>
<td>Fava[69]</td>
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<tr>
<td>EPHX2</td>
<td>c.860G&gt;A Arg287Gln</td>
<td>c.164A&gt;G Lys55Arg</td>
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<tr>
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</tr>
</tbody>
</table>

N.E., non explored; CAC, coronary artery calcified plaques; CAD, coronary artery disease; CAD, coronary heart disease; IHD, ischemic heart disease; MI, myocardial infarction; IS, ischemic stroke
Table 4: Association between the most extensively studied SNPs in genes responsible for 20-HETE formation and cardiovascular diseases in humans

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP/SNPs</th>
<th>Ethnicity</th>
<th>MAF %</th>
<th>Cases /CT</th>
<th>Effect of the variant allele in the entire cohort</th>
<th>Sex-specific findings</th>
<th>Reference</th>
</tr>
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<tbody>
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<td></td>
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<td></td>
</tr>
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<td>CYP4A11</td>
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<td>Caucasian</td>
<td>12.5</td>
<td>276/5554</td>
<td>No difference in coronary events</td>
<td>No differences</td>
<td>Fava[80]</td>
</tr>
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<td></td>
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<td>Caucasian</td>
<td>12.5</td>
<td>199/5554</td>
<td>No difference in stroke</td>
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<td>Fava[80]</td>
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<tr>
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<td>Japanese</td>
<td>20.5</td>
<td>174/293</td>
<td>No effect for cerebral infarction</td>
<td>No differences</td>
<td>Fu[83]</td>
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<td>Chinese</td>
<td>20.0</td>
<td>779/557</td>
<td>No effect for ischemic stroke</td>
<td>No differences</td>
<td>Ding[84]</td>
</tr>
<tr>
<td>CYP4F2</td>
<td>rs2108622</td>
<td>Japanese</td>
<td>28.7</td>
<td>175/246</td>
<td>No effect for cerebral infarction</td>
<td>The G allele is associated with cerebral infarction in men</td>
<td>Fu[92]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Indians</td>
<td>50</td>
<td>507/487</td>
<td>Higher prevalence in stroke</td>
<td>N.E.</td>
<td>Munshi[89]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>26.1</td>
<td>276/5554</td>
<td>No effect for coronary events</td>
<td>No differences</td>
<td>Fava[80]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caucasian</td>
<td>26.1</td>
<td>199/5554</td>
<td>No effect for strokes</td>
<td>Higher prevalence in men with stroke</td>
<td>Fava[80]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chinese</td>
<td>22.9</td>
<td>779/557</td>
<td>Associated with ischemic stroke</td>
<td>Evident only in men</td>
<td>Ding[84]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Han Chinese</td>
<td>29.1</td>
<td>302/350</td>
<td>No effect for stroke</td>
<td>The wild type was associated with stroke only in men</td>
<td>Deng[108]</td>
</tr>
</tbody>
</table>

N.E., non explored; CAC, coronary artery calcified plaques; CAD, coronary artery disease; CAD, coronary heart disease; IHD, ischemic heart disease; MI, myocardial infarction; IS, ischemic stroke
Figure 1

a. p=0.11
   by log-rank test
   H.R. 1.76
   95%CI 0.95-1.64
   M-carriers
   V436V homozygotes

b. p=0.58
   by log-rank test
   H.R. 0.99
   95%CI 0.95-1.32
   M-carriers
   V436V homozygotes

c. p=0.006
   by log-rank test
   H.R. 1.71
   95%CI 1.16-2.49
   M-carriers
   V436V homozygotes

d. p=0.88
   by log-rank test
   H.R. 1.24
   95%CI 0.93-1.50
   M-carriers
   V436V homozygotes

e. p=0.89
   by log-rank test
   H.R. 0.87
   95%CI 0.65-1.15
   M-carriers
   V436V homozygotes

f. p=0.89
   by log-rank test
   H.R. 1.28
   95%CI 0.95-1.66
   M-carriers
   V436V homozygotes
Interaction with androgens

CYP4F2

V433M/M433M

↓

↑

tubular 20-HETE

susceptibility to hypertension and stroke

CYP4F2

V433V

↑

↓

tubular 20-HETE

susceptibility to hypertension and stroke

Arachidonic Acid

Other factors (insulin, obesity, oxidative stress…)

Figure 2


99. Lee CR, Pretorius M, Schuck RN, Burch LH, Bartlett J, Williams SM et al. Genetic variation in soluble epoxide hydrolase (EPHX2) is associated with forearm vasodilator...


