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Original Contribution

Cytochrome P450 and matrix metalloproteinase genetic modifiers of disease severity in Cerebral Cavernous Malformation type 1

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ABSTRACT

Background: Familial Cerebral Cavernous Malformation type 1 (CCM1) is an autosomal dominant disease caused by mutations in the Krev Interaction Trapped 1 *(KRIT1/CCM1)* gene, and characterized by multiple brain lesions. CCM lesions manifest across a range of different phenotypes, including wide differences in lesion number, size and susceptibility to intracerebral hemorrhage (ICH). Oxidative stress plays an important role in cerebrovascular disease pathogenesis, raising the possibility that inter-individual variability in genes related to oxidative stress may contribute to the phenotypic differences observed in CCM1 disease. Here, we investigated whether candidate oxidative stress-related cytochrome P450 (CYP) and matrix metalloproteinase (MMP) genetic markers grouped by superfamilies, families or genes, or analyzed individually influence the severity of CCM1 disease.
Methods: Clinical assessment and cerebral susceptibility-weighted magnetic resonance imaging (SWI) were performed to determine total and large (≥ 5 mm in diameter) lesion counts as well as ICH in 188 Hispanic CCM1 patients harboring the founder *KRIT1/CCM1* 'common Hispanic mutation' (CCM1–CHM). Samples were genotyped on the Affymetrix Axiom Genome-Wide LAT1 Human Array. We analyzed 1,122 genetic markers (both single nucleotide polymorphisms (SNPs) and insertion/deletions) grouped by CYP and MMP superfamily, family or gene for association with total or large lesion count and ICH adjusted for age at enrollment and gender. Genetic markers bearing the associations were then analyzed individually.
Results: The CYP superfamily showed a trend toward association with total lesion count ($P=0.057$) and large lesion count ($P=0.088$) in contrast to the MMP superfamily. The CYP4 and CYP8 families were associated with either large lesion count or total lesion count ($P=0.014$), and two other families (CYP46 and the MMP Stromelysins) were associated with ICH ($P=0.011$ and 0.007, respectively). *CYP4F12* rs11085971, *CYP8A1* rs5628, *CYP46A1* rs10151332, and *MMP3* rs117153070 single SNPs, mainly bearing the above-mentioned associations, were also individually associated with CCM1 disease severity.
Conclusions: Overall, our candidate oxidative stress-related genetic markers set approach outlined CYP and MMP families and identified suggestive SNPs that may impact the severity of CCM1 disease, including the development of numerous and large CCM lesions and ICH. These novel genetic risk factors of

Abbreviations: CCM, Cerebral Cavernous Malformation; CHM, common hispanic mutation; ICH, intracerebral hemorrhage; CYP, cytochrome P450; MMP, matrix metalloproteinase; ROS, reactive oxygen species; ECM, extracellular matrix; NVU, neurovascular unit; BBB, blood–brain barrier; SNP, single nucleotide polymorphism; AA, arachidonic acid; PG, prostaglandins; LT, leukotrienes; EET, epoxyeicosatrienoic acids; 24-OHC, 24(S)-hydroxycholesterol; 27-OHC, 27-hydroxycholesterol; 25-OH-D_3, 25-hydroxyvitamin D3; 1,25-OH_2-D_3, 1,25-dihydroxyvitamin D3

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prognostic value could serve as early objective predictors of disease outcome and might ultimately provide better options for disease prevention and treatment.

1. Background

Cerebral Cavernous Malformation (CCM) is a major cerebrovascular disease consisting of closely clustered, abnormally dilated and leaky capillary malformations, which can be single or multiple (even hundreds), ranging in size from a few millimeters to a few centimeters [1–4]. Despite their high prevalence in the general population (0.3–0.5%), only approximately 30% of people with CCM lesions will eventually develop clinical symptoms, including headaches, focal neurological deficits, seizure, stroke, and fatal intracerebral hemorrhage (ICH) [5]. Symptomatic disease usually occurs between the 2nd and 5th decades of life, although it has been described in all age groups, including young children [3].

Although advances have been made toward understanding the natural history and pathogenic mechanisms of CCM disease, the clinical behavior in individual patients, including the development of numerous and large symptomatic lesions and the risk of serious complications such as ICH, is highly unpredictable [6]. Identification of modifiable risk factors of prognostic value associated with clinical severity of CCM disease is therefore needed to ultimately provide better options for disease prevention and treatment.

CCM disease may arise sporadically or can be inherited as an autosomal dominant condition with incomplete penetrance and variable expressivity. The familial form has been linked to loss-of-function mutations in any of three known CCM genes (*KRIT1*/*CCM1*, *MGC4607*/*CCM2* and *PDCD10*/*CCM3*), and is characterized by the presence of multiple CCM lesions whose number, size and susceptibility to ICH vary widely even among carriers of the same gene mutation [7,8]. The origin of this variability is still largely unknown, although it is likely that multiple factors contribute to CCM disease severity, including genetic, environmental and lifestyle risk factors. Consistently, we have recently reported the influence of cardiovascular risk factors [7], and inflammatory and immune response genetic factors [8] on the severity of familial CCM1 disease in a cohort of Hispanic CCM patients, all carrying the founder *KRIT1*/*CCM1* Q455X 'Common Hispanic Mutation' (CCM1–CHM).

Comprehensive studies in CCM animal models have revealed a causal link between loss-of-function of CCM genes and the development of vascular malformations mimicking human CCM lesions [9–13]. However, experiments in inducible, endothelial-specific knockout mouse models have clearly demonstrated that lesion development is highly restricted, both spatially and temporally, despite the pan-endothelial deletion of CCM genes, suggesting that the homozygous loss of CCM genes is not fully sufficient to induce CCM lesions, and raising the possibility that additional events occurring locally within the neurovascular microenvironment, including stress events, are necessary to trigger CCM lesion formation [14–16]. Among the microenvironmental stress events that might account for a sort of environmental second hit, triggering CCM lesion formation, there is oxidative stress. This may occur as a consequence of an endogenous imbalance between molecular mechanisms of production and scavenging of reactive oxygen species (ROS), as well as by exogenous oxidative insults, including cell exposure to xenobiotics, ionizing radiations, or pro-oxidant factors that may be released locally following inflammatory responses, impaired neurovascular coupling, and ischemia-reperfusion events [17,18]. Remarkably, there is now a wealth of evidence indicating that oxidative stress is a major cause of vascular remodeling and neurovascular unit (NVU) dysfunction associated with cerebrovascular diseases [17,19,20]. Indeed, oxidative stress has been clearly implicated in major molecular and cellular dysfunctions related to CCM disease, including destabilization of VE-cadherin-mediated endothelial cell-cell junctions and blood–brain barrier (BBB) integrity, increased β1 integrin activation, reduced cells' ability to maintain a quiescent state, and increased vascular permeability and angiogenic activity [18,19,21–23], suggesting that it might significantly contribute in driving the onset and progression of CCM disease. Consistently, considerable evidence from both *in vitro* and *in vivo* studies demonstrates that CCM proteins, including KRIT1/CCM1, CCM2 and PDCD10/CCM3, are implicated in the modulation of various redox-sensitive signaling pathways and cellular responses to oxidative stress [16,22,24–29]. In particular, original findings indicated that KRIT1 is involved in the maintenance of intracellular ROS homeostasis through the modulation of master regulators of cellular responses to oxidative stress, including FoxO1 and SOD2, which prevent the accumulation of superoxide anions produced at the mitochondrial electron transport chain and consequent mitochondrial and cellular dysfunctions [24]. Since then, accumulating evidence has provided strong support for a role of oxidative stress in CCM pathogenesis, demonstrating that CCM proteins play an important role in limiting pro-oxidant and pro-inflammatory pathways and mechanisms, including c-Jun-dependent pathways [22] and defective autophagic removal of dysfunctional mitochondria [28]. Furthermore, there is also evidence that compounds endowed with antioxidant properties are effective in rescuing major disease phenotypes associated with loss-of-function of CCM genes [16,22,24,26,27], including decreased endothelial barrier function and increased lesion burden in a mouse model of CCM disease [16].

While pointing to a novel pathogenic mechanism whereby the loss-of-function of CCM proteins sensitize vascular cells to local oxidative stress events, these findings raised also the possibility that inter-individual variability in susceptibility to oxidative stress may contribute to CCM disease pathogenesis [29].

Notably, several genes encoding proteins involved in ROS metabolism and vascular responses to oxidative stress are characterized by single nucleotide polymorphisms (SNPs) that confer substantial inter-individual variability in susceptibility to various oxidative stress-related pathologies, including vascular diseases [30–33], suggesting that inter-individual variation in polymorphisms of genes related to oxidative stress responses might contribute to the phenotypic differences characterizing the familial form of CCM disease.

In particular, among several polymorphic candidate genes related to oxidative stress that might be associated with CCM disease development and severity, we focused our analysis on the highly polymorphic cytochrome P450 (CYP) and matrix metalloproteinase (MMP) genes. Indeed, genetic variants in these genes have been shown to be significant indicators for susceptibility to the most severe forms of well-established oxidative stress-related diseases [34,35].

The human CYP superfamily comprises 57 genes encoding for monooxygenases performing a variety of oxidation reactions implicated in important physiopathological processes [36]. High levels of CYP activity are associated with increased oxygen radical formation and cellular oxidative stress, which can damage cellular macromolecules, including proteins, lipids and DNA [37,38]. Importantly, whereas most CYP enzymes exhibit a large variability in

expression and activity among tissues, among individuals of a population and in a given individual under various conditions [39], compelling evidence supports the expression of these enzymes by BBB endothelial cells and astrocytes associated with cerebral microvessels [40–43]. Indeed, it has been demonstrated that variations in the expression and activity of CYPs can influence the local regulation of cerebral blood flow and vascular homeostasis, and contribute to cerebrovascular diseases [44,45]. Consistently, genetic polymorphisms in several *CYPs* have been associated with inter-individual differences in susceptibility to various vascular and neurological disorders [39,46–48].

The human matrix metalloproteinase (MMP) superfamily comprises 24 genes encoding proteolytic enzymes that degrade various components of the extracellular matrix (ECM) in both physiological and pathological processes, mediating ECM remodeling and supplying bioactive molecules that impact cellular function. In vascular tissues, including cerebral microvessels, the expression and activity of MMPs are regulated by major drivers of vascular remodeling, such as ROS [49] and oxidative stress [50,51], and may contribute to oxidative stress-mediated vascular dysfunctions and BBB breakdown in cerebrovascular diseases [52,53]. Indeed, once activated, MMP enzymes mediate the proteolytic degradation of endothelial basal lamina components and the release of ECM-bound latent bioactive molecules, including pro-angiogenic growth factors and cytokines such as VEGF and TGF-β1, thus playing a pivotal role in vascular remodeling and BBB rupture induced by various physiological stimuli and pathological stresses, and contributing to oxidative stress-related signaling events [49,54–56]. Consistently, polymorphisms in some *MMP* genes are important genetic risk factors influencing inter-individual differences in susceptibility to the onset and/or severity of various vascular diseases, including cerebrovascular diseases [57–62].

Overall, our analysis of polymorphic candidate genes related to oxidative stress identified suggestive SNPs in *CYP* and *MMP* genes that showed a statistically significant association with phenotypic markers of CCM1 disease severity, indicating that variation in these genes may contribute to inter-individual differences in susceptibility to CCM disease.

2. Methods

2.1. Study population

The study sample comprised 188 CCM1 subjects, all confirmed carriers of the *KRIT1/CCM1*–CHM mutation by genetic testing as previously described [7], and with both genotype and phenotype data available. Subjects were recruited through the Brain Vascular Malformation Consortium (BVMC) study at the University of New Mexico (UNM) and the Angioma Alliance patient advocacy group's DNA & Tissue Bank study as previously described [8]. The study was approved by the local institutional review boards at UNM, University of California, San Francisco (UCSF), and Quorum IRB (Angioma Alliance), and by the National Institutes of Neurological Disorders and Stroke (NINDS). Written informed consent was obtained from all participants.

2.2. Clinical characteristics

Clinical assessment included information on characteristics of presenting symptoms leading to CCM diagnosis and classified as acute cerebral hemorrhages, seizures, focal neurological symptoms, and headaches using standardized guidelines [63]. Cerebral magnetic resonance imaging (MRI) was performed for all patients at study enrollment using a volume T1-weighted magnetization prepared rapid gradient echo acquisition (MPRAGE, 1-mm slice reconstruction) and axial T2-weighted turbo spin echo (TSE T2), T2 gradient-recalled echo (GRE), susceptibility-weighted imaging (SWI), and FLAIR sequences. Lesion counting was based on concurrent evaluation of axial SWI as previously described, and lesion size was measured on TSE T2 images [7]. Three phenotypes were used as markers of CCM1 disease severity: (1) total lesion count, (2) large lesion count (≥ 5 mm), and (3) history of ICH at baseline. In our sample, 30.3% of CCM1–CHM subjects had a history of ICH and 90% had multiple lesions on MRI as previously reported [8], with total lesions mean $\pm$ SD of 60.1 $\pm$ 115.1 (range from 0 to 713) and large lesions mean $\pm$ SD of 4.9 $\pm$ 8.7 (range from 0 to 104).

2.3. Genotyping and quality control

Blood or saliva samples were collected and genomic DNA was extracted using standard protocols. DNA samples were genotyped at the UCSF Genomics Core Facility using the Affymetrix Axiom® Genome-Wide LAT 1 (Axiom GW LAT) Human Array [64], as previously described [8]. This array is designed to provide optimized genome-wide coverage of both common and rare variants in populations with West African, European, and Native American ancestries and consists in a total of 817,810 genetic markers, including 813,551 SNPs and 4259 insertion/deletions (Indels). All samples had a genotyping call rate of 97% or greater, and the two Affymetrix Reference DNA controls were concordant, as previously reported [8]. Further quality control steps, including sex check, Mendelian errors and cryptic relatedness, were applied to genotype data using PLINK software (v1.07) before data analysis. Neither sex discordance nor Mendelian errors were identified [8].

2.4. Candidate genes and genetic markers selection

Candidate genes were selected for association studies on the basis of their involvement in vascular responses to oxidative stress and potential influence on major phenotypic markers of familial CCM1 disease severity, including total lesion burden, total large lesion burden, and history of ICH at baseline, as suggested by their physiopathological functions and impact on cerebrovascular diseases [31,43,44,53,65].

As a preliminary approach, candidate SNPs in multiple biologically relevant oxidative stress-related genes, including those implicated in modulating vascular homeostasis and remodeling in response to oxidative stress, were screened as putative genetic modifiers of CCM disease severity in our homogeneous cohort of Hispanic CCM1–CHM subjects, using the Axiom GW LAT Array as previously described [8].

Based on the outcomes of our preliminary screening (data not shown), we focused on the in-depth investigation of the two following gene superfamilies:

1) CYP superfamily consisting of 57 genes, divided among 18 families, encoding monooxygenase enzymes involved in the metabolism of xenobiotics and endobiotics, and the production of bioactive oxidation metabolites and reactive oxygen species that may affect the vasculature.
2) MMP superfamily consisting of 24 genes, divided among 6 families, encoding proteolytic enzymes that degrade the ECM scaffold, including the vascular basal lamina, enabling vascular remodeling in response to various physiological stimuli and pathological stresses.

The complete list of the members of CYP and MMP superfamilies is given in Supplementary Table 1. CYP and MMP proteins were classified into 18 and 6 families, respectively, according to their structures, substrate specificity and cellular localization [39,66]. For example, MMP families include: Collagenases (MMP-1,

-8, -13), Gelatinases (MMP-2, -9), Stromelysins (MMP-3, -10, -11, -12), Matrilysins (MMP-7, -26), membrane type MMPs (MMP-14, -15, -16, -17, -24, -25) and other MMPs (MMP-19, -20, -21, -27, -28) (Supplementary Table 1).

Gene loci were defined as ± 5 kb upstream and downstream of the sequence using UCSC Genome Browser Assembly Feb. 2009 (GRCh37/hg19). The following cut-off points were used: genotype call rate < 98%, a minor allele frequency (MAF) < 1%, or deviation from Hardy-Weinberg equilibrium ($P < 0.001$). We included 1122 genetic markers (1116 SNPs and 6 Indels) that fulfilled the above-mentioned criteria and extracted the genotypes from the Axiom GW LAT Human Array.

2.5. Statistical analysis

Residuals of log-transformed total or large lesion count were obtained after adjustment for age at enrollment and gender (R v2.10.1 software). To analyze the sets of genetic markers grouped by superfamily, family or gene, we used the genetic markers "set-based test" in PLINK v1.07 which takes account of the linkage disequilibrium (LD) between the genetic markers and corrects *P*-values for the multiple markers tested within a set using 1,000 permutations. In addition to this correction for multiple genetic markers tested, we considered statistically significant associations with *P*-values ≤ 0.017 (0.050/3 phenotypes tested). The best SNP bearing each association between CYP or MMP families or genes and CCM1 disease severity was selected for further association analyses: (1) linear regression to assess association between residuals of log-total or large lesion count and single SNP (assuming an additive genetic model, i.e., 0, 1, or 2 copies of the minor allele) using STATA 12.1 statistical software (StataCorp, College Station, Tex., USA), and (2) logistic regression to assess association between ICH and single SNP, adjusting for age at enrollment and gender. All analyses accounted for clustering within families by using robust standard errors, except for *MMP3* rs117153070. Since ICH analysis produced an infinite estimated odds ratio (OR) for *MMP3* rs117153070, we calculated a one-sided 95% confidence interval (CI) based on the profile likelihood, and derived a *p*-value from the likelihood ratio test comparing models with and without the polymorphism. To ensure familial clustering could be disregarded for this specific analysis, we tested the effect of family with a mixed-effects logistic regression model.

3. Results

3.1. Association of CYP or MMP superfamilies with CCM1 disease severity

Growing evidence suggests that oxidative stress plays an important role in CCM disease pathogenesis [16,22,24]. Moreover, it is known that polymorphic genes related to oxidative stress and implicated in modulating vascular homeostasis and remodeling, including *CYP* and *MMP* genes encoding major enzymatic players of redox metabolism and ECM remodeling, are characterized by SNPs that confer greater susceptibility to vascular diseases [39,47,48,61,67–69], thus representing potential genetic modifiers of CCM disease severity.

In this light, we tested the possibility that genetic inter-individual variability in *CYP* and *MMP* genes influences markers of familial CCM1 disease severity, including total lesion burden, total large lesion burden, and history of hemorrhage at baseline. To this end, we first assessed the impact of the whole CYP and MMP superfamilies on CCM1 disease severity by performing a genetic marker set association analysis. The CYP superfamily, including 765 genetic markers, showed a trend toward association with total lesion count ($P=0.057$) and large lesion count ($P=0.088$) after correction for multiple markers tested (Table 1). In contrast, the MMP superfamily, including 357 genetic markers, was not associated with phenotypic markers of disease severity.

Table 1
Association of CYP or MMP superfamilies and families with phenotypic markers of CCM1 disease severity.

	Genetic markers set	N genetic markers	*P*-value		
			Total lesions	Large lesions	ICH
Superfamily	**CYP**	765	0.057	0.088	0.33
Families	CYP1	16	1	0.069	1
	CYP2	176	0.079	0.24	0.84
	CYP3	39	0.15	0.61	0.32
	CYP4	169	0.10	**0.014**	0.21
	CYP5	80	0.60	0.058	0.56
	CYP7	21	1	0.24	0.078
	CYP8	47	**0.014**	0.10	0.61
	CYP11	29	0.14	1	1
	CYP17	5	0.082	1	1
	CYP19	51	0.23	0.14	0.035
	CYP20	11	0.22	1	1
	CYP21	1	1	1	1
	CYP24	27	0.045	0.32	0.24
	CYP26	25	1	0.062	1
	CYP27	24	0.052	0.16	0.044
	CYP39	21	0.40	0.56	1
	CYP46	19	0.099	0.17	**0.011**
	CYP51	4	0.041	1	1
Superfamily	**MMP**	357	0.33	0.54	0.37
Families	Collagenases	45	0.74	0.61	0.47
	Gelatinases	47	0.25	0.14	1
	Stromelysins	41	0.63	1	**0.007**
	Matrilysins	24	0.52	0.56	0.34
	Membrane-type MMPs	141	0.17	0.18	0.52
	Other MMPs	59	0.17	0.73	1

P-values are corrected for the multiple markers within a set (taking account of the LD between these genetic markers).
P-values in bold are considered statistically significant ($P \leq 0.017$).

3.2. Association of CYP or MMP families or genes with CCM1 disease severity

We then evaluated whether the CYP and MMP families were associated with phenotypic markers of disease severity by grouping genetic markers by families (Supplementary Table 1). For CYP or MMP families showing a significant or borderline association with CCM1 disease severity, we performed additional association analyses by grouping genetic markers by genes. Results are presented in Table 2.

The CYP4 family was associated with large lesion count ($P=0.014$) (Table 1), and, among the 12 genes belonging to the CYP4 family, the strongest association was exhibited by the *CYP4F2* followed by *CYP4F11* and *CYP4F12* genes (Table 2). *CYP4F2*, *CYP4F8* and *CYP4Z1* genes were also modestly associated with total lesion count (Table 2). The CYP8 family was associated with total lesion count ($P=0.014$) (Table 1), and, of the two *CYP8* genes analyzed, *CYP8A1* encoding the prostaglandin I2 (prostacyclin) synthase (PTGIS) was significantly associated with total lesion count ($P=0.013$) (Table 2). In addition, two families (CYP46 and MMP Stromelysins) were significantly associated with ICH ($P=0.011$ and 0.007, respectively), with *CYP46A1* and *MMP3* genes bearing these associations (Tables 1–2). Moreover, CYP19, CYP24, CYP27 and CYP51 families were modestly associated

Table 2
CYP or MMP families associated with phenotypic markers of CCM1 disease severity and association of related genes.

	Genetic markers set	N genetic markers	*P*-value		
			Total lesions	Large lesions	ICH
Family	**CYP4**	169	0.10	**0.014**	0.21
Genes	*CYP4A11*	6	1	1	1
	CYP4A22	5	1	1	1
	CYP4B1	12	1	0.26	1
	CYP4F2	13	0.027	**0.007**	0.16
	CYP4F3	18	1	0.21	1
	CYP4F8	11	0.021	1	1
	CYP4F11	19	0.098	0.041	0.044
	CYP4F12	28	0.086	0.030	0.16
	CYP4F22	17	0.11	1	1
	CYP4V2	25	0.34	1	0.35
	CYP4X1	10	0.19	1	1
	CYP4Z1	5	0.039	1	1
Family	**CYP8**	47	**0.014**	0.10	0.61
Genes	*CYP8A1 (PTGIS)*	39	**0.013**	0.12	0.56
	CYP8B1	8	1	0.079	1
Family/ Gene	**CYP19**/*CYP19A1*	51	0.23	0.14	0.035
Family/ Gene	**CYP24**/*CYP24A1*	27	0.045	0.32	0.24
Family	**CYP27**	24	0.052	0.16	0.044
Genes	*CYP27A1*	11	1	0.036	0.041
	CYP27B1	4	0.032	0.099	1
	CYP27C1	9	1	1	1
Family/ Gene	**CYP46**/*CYP46A1*	19	0.099	0.17	**0.011**
Family/ Gene	**CYP51**/*CYP51A1*	4	0.041	1	1
Family	**Stromelysins**	41	0.63	1	**0.007**
Genes	*MMP3*	7	1	1	**0.002**
	MMP10	19	0.39	1	0.062
	MMP11	7	0.17	1	1
	MMP12	8	1	1	1

P-values are corrected for the multiple markers within a set (taking account of the LD between these genetic markers).
P-values in bold are considered statistically significant ($P \leq 0.017$).

with one of the three markers of disease severity tested, with nominal associations of *CYP19A1*, *CYP24A1*, *CYP27A1*, *CYP27B1* and *CYP51A1* genes (Tables 1–2).

3.3. Genetic markers bearing associations between CYP or MMP families or genes and CCM1 disease severity

We then focused on the genetic markers bearing associations of CYP or MMP families and genes with CCM1 disease severity. As a note, none of the six Indels tested explained the observed associations or were individually associated with lesion counts or ICH (Supplementary Table 2).

However, we found that an intronic SNP (rs11085971) of *CYP4F12* was mainly driving the association of CYP4 family and *CYP4F12* gene with large lesion count, and was consistently individually associated with this marker of disease severity ($P < 0.001$) (Table 3). Further, *CYP4F2* rs3093088, *CYP4F8* rs28669833 and *CYP4F11* rs12610962, bearing associations of *CYPF2*, *CYP4F8* and *CYP4F11* genes, respectively, were individually associated with at least one disease severity phenotype (Table 3).

A synonymous SNP (rs5628, Leu256Leu) of *CYP8A1* was mainly bearing the association of CYP8 family and *CYP8A1* gene with total lesion count. Analyzed individually, this SNP was associated with both total lesion and large lesion counts, as the minor allele carriers of rs5628 had 79% more total lesions ($P=0.013$) and 40% more large lesions ($P=0.006$) at baseline, in comparison to non-carriers (Table 3).

Additional SNPs were found to be individually associated with at least one disease severity phenotype, consistently with associations reported for CYP or MMP families and genes (Table 3). The *CYP27B1* rs1048691 and *CYP51A1* rs2049900 polymorphisms were associated with smaller total lesion counts, while the *CYP27A1* rs645163 and *CYP46A1* rs10151332 were associated with greater large lesion counts and ICH (Table 3). Further, *CYP19A1* rs72727199 was significantly associated with ICH ($P=0.002$), while the CYP19 family or *CYP19A1* gene was modestly associated with this phenotype ($P=0.035$) (Tables 2 and 3).

Finally, an intronic SNP (rs117153070) of *MMP3,* which was mainly driving the association of Stromelysin family and *MMP3* gene with ICH, was strongly associated with ICH, as all the minor allele carriers of rs117153070 had a history of ICH (Table 3). Thus, the results of the individual SNP analyses supported the findings obtained with the family and gene sets.

Table 3
SNPs bearing associations of CYP or MMP families and genes with CCM1 disease severity.

Family	Gene	SNP	MAF	Variant function class	Total lesion count				Large lesion count				ICH			
					PI	95% C.I.		*P*	PI	95% C.I.		*P*	OR	95% C.I.		*P*
CYP4	***CYP4F2***	rs3093088	0.13	Upstream	0.62	0.39	0.99	0.046	0.71	0.54	0.94	0.019	0.82	0.39	1.70	0.59
	CYP4F8	rs28669833	0.14	Intron	1.50	1.11	2.02	**0.009**	1.08	0.85	1.36	0.52	0.80	0.43	1.48	0.47
	CYP4F11	rs12610962	0.46	3'UTR	1.18	0.93	1.50	0.16	1.21	1.04	1.40	**0.014**	1.94	1.19	3.16	**0.007**
	CYP4F12	rs11085971	0.03	Intron	0.60	0.29	1.26	0.18	0.49	0.40	0.59	**< 0.001**	0.61	0.19	1.90	0.39
	CYP4Z1	rs1502924	0.11	Intron	1.61	0.98	2.65	0.059	1.12	0.90	1.40	0.29	1.01	0.43	2.35	0.98
CYP8	***CYP8A1***	rs5628	0.13	Synonymous (L256L)	1.79	1.13	2.84	**0.013**	1.40	1.10	1.79	**0.006**	1.33	0.77	2.31	0.31
CYP19	***CYP19A1***	rs72727199	0.12	Intron	0.93	0.67	1.29	0.65	0.87	0.71	1.06	0.17	0.25	0.10	0.60	**0.002**
CYP24	***CYP24A1***	rs11907350	0.03	3'UTR	0.33	0.11	0.99	0.048	0.71	0.34	1.47	0.35	1.23	0.27	5.69	0.79
CYP27	***CYP27A1***	rs645163	0.15	Downstream	1.02	0.69	1.50	0.92	0.75	0.62	0.92	**0.006**	0.35	0.16	0.76	**0.008**
	CYP27B1	rs1048691	0.33	Downstream	0.70	0.55	0.89	**0.004**	0.94	0.77	1.13	0.50	1.00	0.61	1.64	0.99
CYP46	***CYP46A1***	rs10151332	0.46	Intron	0.96	0.73	1.25	0.76	0.80	0.67	0.94	**0.008**	0.45	0.26	0.79	**0.005**
CYP51	***CYP51A1***	rs2049900	0.07	Intron	0.57	0.39	0.84	**0.004**	0.85	0.64	1.12	0.24	0.80	0.33	1.91	0.62
Stromelysin	***MMP3***	rs117153070	0.02	Intron	0.87	0.43	1.75	0.69	1.27	0.88	1.84	0.19	n/a*	8.50*	∞*	**< 0.001***

Table gives odds ratio (OR) or proportional increase (PI, or decrease if less than 1) in either total or large lesion count, along with 95% confidence intervals as well as *P*-values. *P*-values in bold are considered statistically significant ($P \leq 0.017$).
*The OR cannot be determined, because 100% (6/6) of heterozygous carriers have a history of ICH, compared to 70% of patients all without ICH. We show a one-sided 95% confidence interval (CI) based on the profile likelihood and a p-value from the likelihood ratio test comparing models with and without the polymorphism. A mixed-effects logistic regression model found no estimated family effect; hence, the model was not adjusted for familial clustering.

The allele distribution of these SNPs individually associated with CCM1 severity in Hispanic CCM1–CHM subjects were then compared with those available in general and specific Latin populations, using the public databases from dbSNP (SNP database) (http://www.ncbi.nlm.nih.gov/), 1000 Genomes Project global population and HapMap MEX (Mexican Ancestry in Los Angeles) Hispanic population. No significant differences were found in allele frequencies of SNPs reported in Table 3 in comparison to general populations (data not shown).

3.4. Function of significantly associated CYPs and MMPs

To gain insight into potential regulatory mechanisms through which these *CYP* or *MMP* genes and variants might impact CCM1 disease severity, we applied complementary methods, including *in silico* analyses and extensive manual review of the literature. Results are summarized in Supplementary Table 3.

We first asked whether the encoded proteins might be grouped into specific categories according to their functions and substrates using the DAVID Gene Functional Classification Tool [70]. We found that the most significantly associated CYPs can be grouped into specific categories by both their endogenous substrates and bioactive products. Indeed, they are all monooxygenases that play key roles in the metabolism of important endogenous compounds in the brain, including arachidonic acid (AA), AA derivatives, cholesterol, steroid hormones and vitamin D3, contributing to the production of important bioactive metabolites, such as eicosanoids, oxysterols, estrogens and active vitamin D3, which act as signaling molecules in redox-related pathways and may regulate cerebrovascular functions (Supplementary Table 3). Consistently, we found compelling evidence in the literature that supports the expression of CYP enzymes at the blood–brain interface, including in cellular components of the NVUs, where they participate in the local regulation of BBB function, vascular homeostasis and cerebral blood flow through biologically active molecules produced by the metabolism of xenobiotics and endobiotics [40–43,71].

Specifically, members of the CYP4F subfamily have been involved in the metabolism of vitamins (K1 and E), xenobiotics, including drugs, and endobiotics, including polyunsaturated fatty acids (PUFAs) and their derivatives [72]. In particular, there is clear evidence that CYP4F enzymes catalyze the ω-hydroxylation of arachidonic acid and eicosanoids, such as prostaglandins (PG), leukotrienes (LT) and epoxyeicosatrienoic acids (EET), which act as autocrine and paracrine factors in various biological processes affecting the vasculature, including the modulation of ROS formation, inflammatory responses, and vascular tone, permeability and remodeling [43,72–74].

CYP8A1 encodes the prostaglandin I2 (PGI2) synthase, which catalyzes the conversion of prostaglandin H2 (PGH2) to PGI2 (prostacyclin), a potent vasodilator involved in local regulation of cerebral blood flow and vascular homeostasis [75–77]. Remarkably, recent evidence demonstrates a role of prostacyclin in KRIT1-dependent vascular endothelial cell barrier regulation [78].

CYP19A1 (also called aromatase or estrogen synthase) is expressed in major cellular components of the NVU, such as astrocytes and endothelial cells, and catalyzes the key aromatization step that converts androgens into estrogens, thus playing a role in local modulation of BBB permeability and cerebrovascular homeostasis mediated by sex steroid hormones [79,80]. In particular, estrogens are known to favor vasculoprotection from oxidative stress by inducing the upregulation of distinct proteins that exert anti-oxidant and anti-inflammatory functions and contribute to cerebrovascular homeostasis, including Mn superoxide dismutase (SOD2) and prostacyclin synthase (CYP8A1) [79,81].

CYP51A1 (lanosterol 14α-demethylase), CYP46A1 (cholesterol 24-hydroxylase) and CYP27A1 (sterol 27-hydroxylase) are the major CYP enzymes involved in cholesterol metabolism in the brain. In particular, CYP51A1 is involved in cholesterol synthesis, whereas CYP46A1 and CYP27A1 catalyze the conversion of excess cholesterol into the oxysterols 24(S)-hydroxycholesterol (24-OHC) and 27-hydroxycholesterol (27-OHC), respectively [82,83]. Notably, local excess levels of biologically active oxysterols, such as 24-OHC and 27-OHC, may trigger and sustain local oxidative stress reactions leading to vascular dysfunctions and cerebrovascular diseases [84–86].

CYP27A1 is also involved in the metabolism of vitamin D3, along with CYP27B1. Indeed, these two CYP enzymes catalyze the two key hydroxylation steps that convert the inactive form of vitamin D3 (cholecalciferol) to calcifediol (25-hydroxyvitamin D3) and calcitriol (1,25-dihydroxyvitamin D3 or active vitamin D3), respectively [87,88]. Notably, vitamin D3 is endowed with antioxidant properties in the endothelium, which may prevent peripheral vascular diseases [89,90]. Consistently and importantly, vitamin D3 supplement has been recently demonstrated to decrease vascular oxidative stress and CCM lesion burden in a mouse model of CCM disease [16].

Matrix metalloproteinase-3 (MMP-3), also known as stromelysin-1, is a broad-spectrum extracellular matrix degrading enzyme that acts on collagen types II, III, IV, IX, and X, proteoglycans, fibronectin, laminin, and elastin [91]. In addition, MMP-3 can also activate other MMPs such as MMP-1, MMP-7, and MMP-9, rendering MMP-3 crucial in extracellular matrix remodeling both in physiological and pathological processes (Supplementary Table 3). Notably, MMP activity has been clearly implicated in focal degradation of the vascular extracellular matrix underlying the onset and progression of cerebrovascular diseases [53,92,93]. Furthermore, increased endothelial expression of MMPs has been previously suggested to contribute to the formation, enlargement and rupture of human CCM lesions by affecting the stability of the vascular extracellular matrix scaffold [94].

We then asked whether specific redox-related pathways were affected by the CCM disease-associated CYP or MMP genes and SNPs using the NCBI BioSystems database [95] and Reactome, a curated pathway database (http://www.reactome.org/), as well as a comprehensive review of the literature.

All CYPs are involved in biological oxidations, including oxidative metabolism of endogenous and exogenous compounds by monooxygenase activity. Moreover, CYP enzymes are among the oxidoreductases that are potential sources of superoxide anions in mammalian cells [38]. Indeed, compelling evidence indicates that poor coupling of the CYP catalytic cycle may result in continuous ROS production and consequent formation of lipid peroxidation products, such as 4-hydroxynonenal (HNE), which in turn may activate the redox-sensitive JNK/c-Jun signaling pathway, leading to cellular oxidative stress [38,96]. Furthermore, CYP enzymes may also influence redox-related pathways modulated by some of their bioactive metabolic products, including eicosanoids, oxysterols, steroid hormones and vitamin D3 [37,39,48,97]. Intriguingly, whereas there is clear evidence that some CYP enzymes may act as either regulators or targets of redox-sensitive pathways, including the JNK/c-Jun pathway [98–100] (Supplementary Table 3), we have previously demonstrated that the JNK/c-Jun pathway is influenced by KRIT1 loss-of-function [22], suggesting a potential functional connection between CYP and KRIT1 proteins.

On the other hand, there is consistent evidence that also MMP enzymes may act as either targets or regulators of oxidative stress-related signaling events [49,54,55]. In particular, MMP3 has been involved in distinct redox-sensitive pathways, including PKC, PI3K/AKT and ERK1/2 pathways [56] (Supplementary Table 3).

Importantly, none of the CCM disease-associated SNPs was previously reported to affect specific redox related pathways. Most of these SNPs were in non-coding regions of the candidate genes

(Table 3) and therefore do not directly alter protein sequence. However, we cannot exclude a role of these non-coding SNPs in gene regulation, as there is clear evidence that not only polymorphisms in the open reading frame are capable of modifying the function of a given gene. Indeed, non-coding SNP may be functionally relevant by affecting the promoter region [101], splicing sites [102], or intronic microRNAs [103]. Accordingly, the expression of CYP enzymes is regulated through an extremely complex network of nuclear receptors, microRNAs and genetic/epigenetic factors [39].

To evaluate the potential importance of these non-coding variants in gene regulation, we searched for phylogenetic conservation using *in silico* tools, such as Ensembl Phylogenetic Context [104]. We found that a high evolutionary conservation among species was observed for the rs5628, rs72727199, rs645163, rs1048691, rs2049900 and rs117153070 variants in up to 23 vertebrates, including mammalian, reptile, and bird classes. Further functional investigations using relevant human tissues and cells are required to robustly assess the regulatory role of these non-coding variants.

4. Discussion

The results of this study indicate that oxidative stress-related CYP and MMP genetic markers grouped by superfamilies, families, genes or analyzed individually influence the severity of familial CCM1 disease, as manifested by ICH and greater total or large lesion counts. These phenotypic markers vary widely for unknown reasons, even among family members of similar ages harboring the same *CCM1* germline mutations, suggesting the influence of environmental and/or other genetic factors [7,8]. Consistently, in contrast to the wide intrafamilial variability usually observed between siblings, a great similarity of disease onset and phenotypic markers has been found in monozygotic twins with a *CCM1* germline mutation, suggesting that the expression of CCM disease in non-twin siblings is significantly influenced by additional genetic modifiers [105].

In our homogeneous CCM1–CHM cohort, we reported that seven CYP (CYP4, CYP8, CYP19, CYP24, CYP27, CYP46 and CYP51) and one MMP (Stromelysins) families contribute to the wide differences observed between patients in number of total/large lesions, or susceptibility to ICH. In particular, ten polymorphisms, including *CYP4F8* rs28669833, *CYP4F11* rs12610962, *CYP4F12* rs11085971, *CYP8A1* rs5628, *CYP19A1* rs72727199, *CYP27A1* rs645163, *CYP27B1* rs1048691, *CYP46A1* rs10151332, *CYP51A1* rs2049900 and *MMP3* rs117153070, mainly bearing the above-mentioned associations, were individually significantly associated with at least one marker of CCM1 disease severity. Thus, our study supports a role of oxidative stress-related genetic risk factors in predicting CCM1 disease outcomes.

Our findings are consistent with the expression of the identified CYP genes at the blood–brain interface, where they contribute to the production of many bioactive metabolites that affect vascular tone and homeostasis and BBB stability, including oxidative derivatives of arachidonic acid and cholesterol, such as eicosanoids and oxysterols, steroid hormones and vitamin D3 [36,40–43,71,106]. Further support is provided by compelling evidence that CYP activity is associated with enhanced local formation of ROS and lipid peroxidation products, which may alter redox signaling pathways and cause oxidative stress-mediated vascular dysfunctions [37,38,71,96,97,107]. Importantly, they are also consistent with previous studies reporting the association of *CYP* genetic polymorphisms with different degrees of susceptibility to various cerebrovascular diseases [44,46]. In turn, this may reflect the highly polymorphic nature and significant inter-individual and inter-tissue variability in the expression and activity of CYP enzymes, and the consequent variability in the occurrence of local oxidative stress events [39,48,108].

Specifically, our findings that SNPs in CYP8A1, CYP4F8, CYP4F11 and CYP4F12 are associated with at least one marker of CCM1 disease severity are consistent with the established role of these enzymes in the production of eicosanoids, such as prostaglandins (PGs), leukotrienes (LTs) and epoxyeicosatrienoic acids (EETs), which are long recognized as key regulators of local cerebrovascular functions, as well as therapeutic targets for cerebrovascular diseases [43,72–74]. Accordingly, genetic variations in CYP enzymes involved in eicosanoid pathways are generally considered one of the determinants of individual susceptibility to vascular diseases, including cerebrovascular diseases [39,48], supporting the possibility that inter-individual variations in local eicosanoid metabolism and signaling influence CCM1 disease severity. Indeed, consistent with its potential modulating role in CCM1 disease, the rs5628 polymorphism of the *CYP8A1* gene, which encodes the prostacyclin (PGI2) synthase, has been previously associated with genetic predisposition to hemorrhagic stroke in Korean population [109]. Moreover, further support is provided by recent evidence demonstrating a role of prostacyclin in KRIT1-dependent vascular endothelial cell barrier regulation [78].

The association between CYP19A1 rs72727199 and ICH is consistent with the important role of CYP19A1 (estrogen synthase) in the local modulation of BBB permeability and cerebrovascular homeostasis mediated by sex steroid hormones [79,80]. Indeed, CYP19A1 is expressed at the NVU and catalyzes a key step in the biosynthesis of estrogens, which are known to favor vasculoprotection from oxidative stress by increasing cerebrovascular levels of proteins endowed with antioxidant and anti-inflammatory properties, including SOD2 and prostacyclin synthase (CYP8A1) [79,81]. Intriguingly, whereas there is evidence that deficiencies in estrogen-mediated regulation of cerebrovascular homeostasis may contribute to an increased risk of cerebral aneurysm pathogenesis and rupture [110,111], it has been reported that risk of recurrent ICH due to CCM is greater for women than men [63], suggesting that cerebrovascular levels of sex hormones may have a role in the variable expression of CCM disease.

The association of CYP46A1 rs10151332 and CYP27A1 rs645163 polymorphisms with both large CCM lesion count and susceptibility to ICH is consistent with a substantial body of evidence indicating that variations in CYPs involved in cholesterol metabolism in the brain, such as the cholesterol 24-hydroxylase CYP46A1 and the sterol 27-hydroxylase CYP27A1, can lead to local excess levels of biologically active 24-OHC and 27-OHC oxysterols [84,85,112–115]. Indeed, local accumulation of these cholesterol oxidation derivatives can trigger and sustain local oxidative stress and inflammatory reactions that contribute to distinct human disorders, including cerebrovascular diseases [83,86,116]. Accordingly and importantly, markers of cholesterol metabolism in the brain, including the 24-OHC and 27-OHC oxysterols, showed strong associations with cerebrovascular diseases [86].

CYP27A1 is also involved in the metabolism of vitamin D3 along with CYP27B1, acting as vitamin D3 25-hydroxylase and 25-hydroxyvitamin D3 1α-hydroxylase, respectively [87,88,117]. Consistent with a potential impact on CCM disease severity of genetic variations in CYP enzymes involved in vitamin D3 metabolism, including the identified CYP27A1 rs645163 and CYP27B1 rs1048691 SNPs, recent *in vitro* and *in vivo* studies have demonstrated the effectiveness of vitamin D3 in decreasing vascular oxidative stress and CCM lesion burden in a mouse model of CCM disease [16]. Accordingly, vitamin D3 is endowed with antioxidant properties in the endothelium, and may prevent peripheral vascular diseases [89,90].

In this study, we also identified an association between the *MMP3* rs117153070 polymorphism and ICH, which is in line with previous studies reporting associations between MMPs and ICH in human [65]. Accordingly, MMP-related genetic variations, including SNPs, may contribute to heterogeneity in vascular remodeling and consequent presentation and natural history of cerebrovascular diseases [53,93]. Indeed, there is compelling evidence that polymorphisms in MMP genes, including *MMP3*, may influence inter-individual differences in susceptibility to the onset and severity of cerebral aneurysms [57], brain arteriovenous malformations [58–60], ischemic white matter injury [118], and Moyamoya disease [62]. Furthermore and consistent with a potential impact of MMP genetic variations on CCM disease, increased endothelial expression of MMPs has been previously observed in human CCM lesions and suggested to contribute to their formation, enlargement and rupture by affecting the stability of the vascular extracellular matrix scaffold [94]. In addition, whereas there is evidence that polymorphisms in MMP genes may influence cerebral small vessel disease associated with white matter lesions [68], an increased frequency of white matter lesions among familial cases of CCM disease has been recently reported [119].

Some of the identified SNPs were concomitantly associated with two phenotypic makers of CCM disease severity, including *CYP8A1* rs5628, which was associated with both total and large number of CCM lesions, and *CYP4F11* rs12610962, *CYP27A1* rs645163 and *CYP46A1* rs10151332, which were associated with large lesion count and susceptibility to ICH phenotypes. However, no significant concomitant association with all three phenotypic markers of CCM disease severity was observed, suggesting that multiple genetic risk factors contribute to the distinct most severe phenotypes of the disease.

The study was centered on an ethnically homogeneous cohort of 188 Hispanic CCM patients carrying the founder *KRIT1/CCM1*–CHM mutation, which avoided confounding factors due to differences in population genetic structure and disease-causing mutation. Furthermore, it was based on a genetic marker set association analysis, which is more powerful than testing each genetic marker individually [120,121]. Despite some limitations, including the limited ethnic group and sample size of our CCM1 cohort and the fact that our selection of common genetic markers cannot exclude the possibility for rare functional variants in these families to influence the severity of CCM1 disease, our study has the important value of opening a novel avenue for future basic and translational research in the field of CCM disease. Indeed, our experimental outcomes prompt future replication studies with independent and random cohorts of CCM subjects to verify whether the genetic modifiers of CCM1 disease severity identified in our *KRIT1/CCM1*–CHM cohort are replicated in subjects affected by other forms of CCM disease, including sporadic forms and familial forms linked to different mutations in any CCM gene, as well as across different ethnic groups. The confirmation and extension of our results might lead to the recognition of subsets of CCM patients who are predisposed to develop the most severe disease phenotypes, including ICH, offering the opportunity to prevent these events and related complications, and improve outcomes. Furthermore, our findings pave the way for future basic research investigations aimed at addressing the role of specific CYP enzymes and metabolites in CCM disease pathogenesis and severity, providing a novel useful framework for a better understanding of pathogenic mechanisms, the identification of new diagnostic, prognostic and predictive biomarkers, and the development of specific and effective preventive and therapeutic strategies.

5. Conclusions

This study furthers research into genetic modifiers in CCM1 disease by studying genetic markers in candidate oxidative stress-related gene families at multiple levels of information: superfamilies, families, and individual genetic markers. Our results suggest that genetic variability within CYP4, CYP8, CYP19, CYP24, CYP27, CYP46, CYP51 and the MMP Stromelysins families may influence the severity of CCM1 disease and could serve as predictors of CCM1 disease outcomes. These findings most probably reflect the important role of the identified CYP and MMP enzymes in vascular homeostasis through the regulation of eicosanoid and cholesterol metabolism, and extracellular matrix remodeling, respectively, suggesting that these biological processes contribute to the heterogeneity in the presentation and natural history of CCM disease.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.freeradbiomed.2016.01.008.

References

[1] R.E. Clatterbuck, et al., Ultrastructural and immunocytochemical evidence that an incompetent blood-brain barrier is related to the pathophysiology of cavernous malformations, J. Neurol. Neurosurg. Psychiatry 71 (2) (2001) 188–192.
[2] J. Gault, et al., Pathobiology of human cerebrovascular malformations: basic mechanisms and clinical relevance, Neurosurgery 55 (1) (2004) 1–16 (discussion 16-7).
[3] S. Batra, et al., Cavernous malformations: natural history, diagnosis and treatment, Nat. Rev. Neurol. 5 (12) (2009) 659–670.
[4] D.D. Cavalcanti, et al., Cerebral cavernous malformations: from genes to

proteins to disease, J. Neurosurg. 116 (1) (2012) 122–132.
[5] C. Denier, et al., Genotype–phenotype correlations in cerebral cavernous malformations patients, Ann. Neurol. 60 (5) (2006) 550–556.
[6] D. Rigamonti, Cavernous Malformations of the Nervous System, Cambridge University Press, 2011.
[7] H. Choquet, et al., Association of cardiovascular risk factors with disease severity in cerebral cavernous malformation type 1 subjects with the common Hispanic mutation, Cerebrovasc. Dis. 37 (1) (2014) 57–63.
[8] H. Choquet, et al., Polymorphisms in inflammatory and immune response genes associated with cerebral cavernous malformation type 1 severity, Cerebrovasc. Dis. 38 (6) (2014) 433–440.
[9] G. Boulday, et al., Tissue-specific conditional CCM2 knockout mice establish the essential role of endothelial CCM2 in angiogenesis: implications for human cerebral cavernous malformations, Dis. Models Mech. 2 (3–4) (2009) 168–177.
[10] Y. He, et al., Stabilization of VEGFR2 signaling by cerebral cavernous malformation 3 is critical for vascular development, Sci. Signal. 3 (116) (2010).
[11] K.J. Whitehead, et al., The cerebral cavernous malformation signaling pathway promotes vascular integrity via Rho GTPases, Nat. Med. 15 (2) (2009) 177–184.
[12] K.J. Whitehead, et al., CCM1 is required for arterial morphogenesis: implications for the etiology of human cavernous malformations, Development 131 (6) (2004) 1437–1448.
[13] X.J. Zheng, et al., CCM3 signaling through sterile 20-like kinases plays an essential role during zebrafish cardiovascular development and cerebral cavernous malformations, J. Clin. Investig. 120 (8) (2010) 2795–2804.
[14] G. Boulday, et al., Developmental timing of CCM2 loss influences cerebral cavernous malformations in mice, J. Exp. Med. 208 (9) (2011) 1835–1847.
[15] A. Fischer, et al., Cerebral cavernous malformations: from CCM genes to endothelial cell homeostasis, Trends Mol. Med. 19 (5) (2013) 302–308.
[16] C.C. Gibson, et al., Strategy for identifying repurposed drugs for the treatment of cerebral cavernous malformation, Circulation 131 (3) (2015) 289–299.
[17] S. Chrissobolis, et al., Oxidative stress and endothelial dysfunction in cerebrovascular disease, Front Biosci. (Landmark Ed.) 16 (2011) 1733–1745.
[18] L. Goitre, et al., Molecular crosstalk between integrins and cadherins: do reactive oxygen species set the talk? J. Signal. Transduct. 2012 (2012) 807682.
[19] L.R. Freeman, J.N. Keller, Oxidative stress and cerebral endothelial cells: regulation of the blood–brain-barrier and antioxidant based interventions, Biochim. Biophys. Acta 1822 (5) (2012) 822–829.
[20] F.M. Faraci, Protecting against vascular disease in brain, Am. J. Physiol. Heart Circ. Physiol. 300 (5) (2011) H1566–H1582.
[21] T. Fukai, M. Ushio-Fukai, Superoxide dismutases: role in redox signaling, vascular function, and diseases, Antioxid. Redox Signal. 15 (6) (2011) 1583–1606.
[22] L. Goitre, et al., KRIT1 loss of function causes a ROS-dependent upregulation of c-Jun, Free Radic Biol Med. 68 (2014) 134–147.
[23] M. Ushio-Fukai, Compartmentalization of redox signaling through NADPH oxidase-derived ROS, Antioxid. Redox Signal. 11 (6) (2009) 1289–1299.
[24] L. Goitre, et al., KRIT1 regulates the homeostasis of intracellular reactive oxygen species, PLoS One 5 (7) (2010) e11786.
[25] M. Fidalgo, et al., Adaptor protein cerebral cavernous malformation 3 (CCM3) mediates phosphorylation of the cytoskeletal proteins ezrin/radixin/moesin by mammalian Ste20-4 to protect cells from oxidative stress, J. Biol. Chem. 287 (14) (2012) 11556–11565.
[26] A. Moglia, et al., Evaluation of the bioactive properties of avenanthramide analogs produced in recombinant yeast, Biofactors 41 (1) (2015) 15–27.
[27] K.H. Jung, et al., Proteomic analysis reveals KRIT1 as a modulator for the antioxidant effects of valproic acid in human bone-marrow mesenchymal stromal cells, Drug Chem. Toxicol. 38 (3) (2015) 286–292.
[28] S. Marchi, et al., Defective autophagy is a key feature of cerebral cavernous malformations, EMBO Mol. Med. 7 (11) (2015) 1403–1417.
[29] E. Trapani, S.F. Retta, Cerebral cavernous malformation (CCM) disease: from monogenic forms to genetic susceptibility factors, J. Neurosurg. Sci. 59 (3) (2015) 201–209.
[30] T.J. Guzik, et al., Functional effect of the C242T polymorphism in the NAD(P)H oxidase p22phox gene on vascular superoxide production in atherosclerosis, Circulation 102 (15) (2000) 1744–1747.
[31] C.L. Heslop, et al., Combined polymorphisms in oxidative stress genes predict coronary artery disease and oxidative stress in coronary angiography patients, Ann. Hum. Genet. 76 (2012) 435–447.
[32] J.E. Klaunig, et al., Oxidative stress and oxidative damage in chemical carcinogenesis, Toxicol. Appl. Pharmacol. 254 (2) (2011) 86–99.
[33] L. Lyrenas, E. Wincent, L. Forsberg, U. De Faire, R. Morgenstern, The potential impact of polymorphism on oxidative stress status, in: Redox-Genome Interactions in Health and Disease, CRC Press, 2003.
[34] S.L. Cheng, C.J. Yu, P.C. Yang, Genetic polymorphisms of cytochrome p450 and matrix metalloproteinase in chronic obstructive pulmonary disease, Biochem. Genet. 47 (7–8) (2009) 591–601.
[35] S.F. van Eeden, D.D. Sin, Oxidative stress in chronic obstructive pulmonary disease: a lung and systemic process, Can. Respir. J. 20 (1) (2013) 27–29.
[36] D.W. Nebert, K. Wikvall, W.L. Miller, Human cytochromes P450 in health and disease, Philos. Trans. R. Soc. Lond. B: Biol. Sci. 368 (1612) (2013) 20120431.
[37] D.W. Nebert, T.P. Dalton, The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis, Nat. Rev. Cancer 6 (12) (2006) 947–960.
[38] R.C. Zangar, D.R. Davydov, S. Verma, Mechanisms that regulate production of reactive oxygen species by cytochrome P450, Toxicol. Appl. Pharmacol. 199 (3) (2004) 316–331.
[39] P. Shahabi, et al., Human cytochrome P450 epoxygenases: variability in expression and role in inflammation-related disorders, Pharmacol. Ther. 144 (2) (2014) 134–161.
[40] E. Hedlund, J.A. Gustafsson, M. Warner, Cytochrome P450 in the brain; a review, Curr. Drug Metab. 2 (3) (2001) 245–263.
[41] R.L. Haining, M. Nichols-Haining, Cytochrome P450-catalyzed pathways in human brain: metabolism meets pharmacology or old drugs with new mechanism of action? Pharmacol. Ther. 113 (3) (2007) 537–545.
[42] C. Ghosh, et al., Pattern of P450 expression at the human blood–brain barrier: roles of epileptic condition and laminar flow, Epilepsia 51 (8) (2010) 1408–1417.
[43] J.D. Imig, et al., Cytochrome P450 eicosanoids and cerebral vascular function, Expert Rev. Mol. Med. 13 (2011) e7.
[44] M.K. Donnelly, et al., Genetic markers in the EET metabolic pathway are associated with outcomes in patients with aneurysmal subarachnoid hemorrhage, J. Cereb. Blood Flow Metab. 35 (2) (2015) 267–276.
[45] D. Gebremedhin, S. Gopalakrishnan, D.R. Harder, Endogenous events modulating myogenic regulation of cerebrovascular function, Curr. Vasc. Pharmacol. 12 (6) (2014) 810–817.
[46] S. Gu, et al., Association between genetic polymorphisms of cytochrome P450 2C19 and the risk of cerebral ischemic stroke in Chinese, BMC Med. Genet. 15 (2014) 83.
[47] C.R. Lee, et al., CYP2J2 and CYP2C8 polymorphisms and coronary heart disease risk: the Atherosclerosis Risk in Communities (ARIC) study, Pharmacogenet. Genom. 17 (5) (2007) 349–358.
[48] U.M. Zanger, M. Schwab, Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation, Pharmacol. Ther. 138 (1) (2013) 103–141.
[49] J. Haorah, et al., Oxidative stress activates protein tyrosine kinase and matrix metalloproteinases leading to blood-brain barrier dysfunction, J. Neurochem. 101 (2) (2007) 566–576.
[50] F.G. Spinale, Matrix metalloproteinases: regulation and dysregulation in the failing heart, Circ. Res. 90 (5) (2002) 520–530.
[51] K. Kameda, et al., Correlation of oxidative stress with activity of matrix metalloproteinase in patients with coronary artery disease. Possible role for left ventricular remodelling, Eur. Heart J. 24 (24) (2003) 2180–2185.
[52] Y. Chen, et al., Soluble endoglin modulates aberrant cerebral vascular remodeling, Ann. Neurol. 66 (1) (2009) 19–27.
[53] J. Tang, et al., Mmp-9 deficiency enhances collagenase-induced intracerebral hemorrhage and brain injury in mutant mice, J. Cereb. Blood Flow Metab. 24 (10) (2004) 1133–1145.
[54] P.J. Kelly, et al., Oxidative stress and matrix metalloproteinase-9 in acute ischemic stroke: the Biomarker Evaluation for Antioxidant Therapies in Stroke (BEAT-Stroke) study, Stroke 39 (1) (2008) 100–104.
[55] M. Garcia-Alloza, et al., Matrix metalloproteinase inhibition reduces oxidative stress associated with cerebral amyloid angiopathy in vivo in transgenic mice, J. Neurochem. 109 (6) (2009) 1636–1647.
[56] D.D. Thomas, et al., Signaling and stress: the redox landscape in NOS2 biology, Free Radic. Biol. Med. 87 (2015) 204–225.
[57] S. Yoon, et al., Genetic analysis of MMP3, MMP9, and PAI-1 in Finnish patients with abdominal aortic or intracranial aneurysms, Biochem. Biophys. Res. Commun. 265 (2) (1999) 563–568.
[58] C. Huai, et al., Allelic variation of the MMP3 promoter affects transcription activity through the transcription factor C-MYB in human brain arteriovenous malformations, PLoS One 8 (3) (2013) e57958.
[59] B. Sun, et al., The rs9509 polymorphism of MMP-9 is associated with risk of hemorrhage in brain arteriovenous malformations, J. Clin. Neurosci. 19 (9) (2012) 1287–1290.
[60] Y. Zhao, et al., The rs522616 polymorphism in the matrix metalloproteinase-3 (MMP-3) gene is associated with sporadic brain arteriovenous malformation in a Chinese population, J. Clin. Neurosci. 17 (12) (2010) 1568–1572.
[61] N. Lamblin, et al., Polymorphisms in the promoter regions of MMP-2, MMP-3, MMP-9 and MMP-12 genes as determinants of aneurysmal coronary artery disease, J. Am. Coll. Cardiol. 40 (1) (2002) 43–48.
[62] H. Li, et al., Association of a functional polymorphism in the MMP-3 gene with Moyamoya Disease in the Chinese Han population, Cerebrovasc. Dis. 30 (6) (2010) 618–625.
[63] R. Al-Shahi Salman, et al., Hemorrhage from cavernous malformations of the brain: definition and reporting standards. Angioma Alliance Scientific Advisory Board, Stroke 39 (12) (2008) 3222–3230.
[64] T.J. Hoffmann, et al., Next generation genome-wide association tool: design and coverage of a high-throughput European-optimized SNP array, Genomics 98 (2) (2011) 79–89.
[65] M. Florczak-Rzepka, et al., Matrix metalloproteinases in human spontaneous intracerebral hemorrhage: an update, Cerebrovasc. Dis. 34 (4) (2012) 249–262.
[66] L. Yadav, et al., Matrix metalloproteinases and cancer-roles in threat and therapy, Asian Pac. J. Cancer Prev. 15 (3) (2014) 1085–1091.
[67] C.C. Lin, et al., Functional polymorphisms in matrix metalloproteinases-1,-3,-9 are associated with arteriovenous fistula patency in hemodialysis patients, Clin. J. Am. Soc. Nephrol. 5 (10) (2010) 1805–1814.
[68] Z.A. Corbin, et al., White matter hyperintensity volume correlates with matrix metalloproteinase-2 in acute ischemic stroke, J. Stroke Cerebrovasc. Dis.

23 (6) (2007) 1300–1306.
[69] W.Q. Niu, Y. Qi, Matrix metalloproteinase family gene polymorphisms and risk for coronary artery disease: systematic review and meta-analysis, Heart 98 (20) (2012) 1483–1491.
[70] D.W. Huang, et al., The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists, Genome Biol. 8 (9) (2007).
[71] C.S. Ferguson, R.F. Tyndale, Cytochrome P450 enzymes in the brain: emerging evidence of biological significance, Trends Pharmacol. Sci. 32 (12) (2011) 708–714.
[72] A. Kalsotra, H.W. Strobel, Cytochrome P450 4F subfamily: at the crossroads of eicosanoid and drug metabolism, Pharmacol. Ther. 112 (3) (2006) 589–611.
[73] P. Christmas, Role of cytochrome P450s in inflammation, Adv. Pharmacol. 74 (2015) 163–192.
[74] N. Sehgal, et al., Cytochrome P4504f, a potential therapeutic target limiting neuroinflammation, Biochem. Pharmacol. 82 (1) (2011) 53–64.
[75] Y.C. Fang, et al., Induction of prostacyclin/PGI2 synthase expression after cerebral ischemia–reperfusion, J. Cereb. Blood Flow Metab. 26 (4) (2006) 491–501.
[76] T. Nakayama, Prostacyclin synthase gene: genetic polymorphisms and prevention of some cardiovascular diseases, Curr. Med. Chem. Cardiovasc. Hematol. Agents 3 (2) (2005) 157–164.
[77] J.A. Ospina, D.N. Krause, S.P. Duckles, 17Beta-estradiol increases rat cerebrovascular prostacyclin synthesis by elevating cyclooxygenase-1 and prostacyclin synthase, Stroke 33 (2) (2002) 600–605.
[78] A. Meliton, et al., KRIT1 mediates prostacyclin-induced protection against lung vascular permeability induced by excessive mechanical forces and TRAP6, Am. J. Respir. Cell Mol. Biol. (2015).
[79] S.P. Duckles, D.N. Krause, Cerebrovascular effects of oestrogen: multiplicity of action, Clin. Exp. Pharmacol. Physiol. 34 (8) (2007) 801–808.
[80] D.N. Krause, S.P. Duckles, D.A. Pelligrino, Influence of sex steroid hormones on cerebrovascular function, J. Appl. Physiol. (1985) 101 (4) (2006) 1252–1261.
[81] C. Stirone, et al., Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels, Mol. Pharmacol. 68 (4) (2005) 959–965.
[82] G. Lorbek, M. Lewinska, D. Rozman, Cytochrome P450s in the synthesis of cholesterol and bile acids-from mouse models to human diseases, FEBS J. 279 (9) (2012) 1516–1533.
[83] G. Poli, F. Biasi, G. Leonarduzzi, Oxysterols in the pathogenesis of major chronic diseases, Redox Biol. 1 (2013) 125–130.
[84] E.G. Lund, et al., Knockout of the cholesterol 24-hydroxylase gene in mice reveals a brain-specific mechanism of cholesterol turnover, J. Biol. Chem. 278 (25) (2003) 22980–22988.
[85] D.W. Russell, et al., Cholesterol 24-hydroxylase: an enzyme of cholesterol turnover in the brain, Annu. Rev. Biochem. 78 (2009) 1017–1040.
[86] T.M. Hughes, et al., Markers of cholesterol metabolism in the brain show stronger associations with cerebrovascular disease than Alzheimer's disease, J. Alzheimers Dis. 30 (1) (2012) 53–61.
[87] T. Sakaki, et al., Metabolism of vitamin D3 by cytochromes P450, Front. Biosci. 10 (2005) 119–134.
[88] I. Schuster, Cytochromes P450 are essential players in the vitamin D signaling system, Biochim. Biophys. Acta 1814 (1) (2011) 186–199.
[89] H.J. Ting, Y.F. Lee, Vitamin D and oxidative stress, in: A.F. Gombart (Ed.), Vitamin D: Oxidative Stress, Immunity, and Aging, CRC Press, 2012, pp. 131–150.
[90] P.E. Norman, J.T. Powell, Vitamin D and cardiovascular disease, Circ. Res. 114 (2) (2014) 379–393.
[91] I. Van Hove, et al., Matrix metalloproteinase-3 in the central nervous system: a look on the bright side, J. Neurochem. 123 (2) (2012) 203–216.
[92] G. Bruno, et al., Vascular extracellular matrix remodeling in cerebral aneurysms, J. Neurosurg. 89 (3) (1998) 431–440.
[93] Y. Yang, G.A. Rosenberg, Blood–brain barrier breakdown in acute and chronic cerebrovascular disease, Stroke 42 (11) (2011) 3323–3328.
[94] M. Fujimura, et al., Expression of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMP) in cerebral cavernous malformations: immunohistochemical analysis of MMP-2, -9 and TIMP-2, Acta Neurochir. (Wien) 149 (2) (2007) 179–183 (discussion 183).
[95] L.Y. Geer, et al., The NCBI bioSystems database, Nucl. Acids Res. 38 (2010) D492–D496.
[96] R. Singh, et al., Chronic oxidative stress sensitizes hepatocytes to death from 4-hydroxynonenal by JNK/c-Jun overactivation, Am. J. Physiol. Gastrointest. Liver Physiol. 297 (5) (2009) G907–G917.
[97] S.L. Miksys, R.F. Tyndale, Drug-metabolizing cytochrome P450s in the brain, J. Psychiatry Neurosci. 27 (6) (2002) 406–415.
[98] Y. Wang, et al., Gene regulation of CYP4F11 in human keratinocyte HaCaT cells, Drug Metab. Dispos. 38 (1) (2010) 100–107.
[99] M. Norlin, et al., Androgen receptor-mediated regulation of the anti-atherogenic enzyme CYP27A1 involves the JNK/c-jun pathway, Arch. Biochem. Biophys. 506 (2) (2011) 236–241.
[100] M. Miodovnik, et al., The inflammatory response of keratinocytes and its modulation by vitamin D: the role of MAPK signaling pathways, J. Cell. Physiol. 227 (5) (2012) 2175–2183.
[101] M. De Gobbi, et al., A regulatory SNP causes a human genetic disease by creating a new transcriptional promoter, Science 312 (5777) (2006) 1215–1217.
[102] N. von Ahsen, M. Oellerich, The intronic prothrombin 19911 A > G polymorphism influences splicing efficiency and modulates effects of the 20210G > A polymorphism on mRNA amount and expression in a stable reporter gene assay system, Blood 103 (2) (2004) 586–593.
[103] S.C. Li, P. Tang, W.C. Lin, Intronic microRNA: discovery and biological implications, DNA Cell Biol. 26 (4) (2007) 195–207.
[104] F. Cunningham, et al., Ensembl 2015, Nucl. Acids Res. 43 (D1) (2015) D662–D669.
[105] P. Dammann, et al., Two-hit mechanism in cerebral cavernous malformation? A case of monozygotic twins with a CCM1/KRIT1 germline mutation, Neurosurg. Rev. 36 (3) (2013) 483–486.
[106] D.W. Nebert, D.W. Russell, Clinical importance of the cytochromes P450, Lancet 360 (9340) (2002) 1155–1162.
[107] I. Fleming, et al., Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries, Circ. Res. 88 (1) (2001) 44–51.
[108] M. Ingelman-Sundberg, et al., Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects, Pharmacol. Ther. 116 (3) (2007) 496–526.
[109] D.H. Kim, et al., A Promoter polymorphism (rs17222919, -1316T/G) of ALOX5AP is associated with intracerebral hemorrhage in Korean population, Prostaglandins Leukot. Essent. Fatty Acids 85 (3–4) (2011) 115–120.
[110] C.G. Harrod, H.H. Batjer, B.R. Bendok, Deficiencies in estrogen-mediated regulation of cerebrovascular homeostasis may contribute to an increased risk of cerebral aneurysm pathogenesis and rupture in menopausal and postmenopausal women, Med. Hypotheses 66 (4) (2006) 736–756.
[111] M.A. Jamous, et al., Role of estrogen deficiency in the formation and progression of cerebral aneurysms. Part I: experimental study of the effect of oophorectomy in rats, J. Neurosurg. 103 (6) (2005) 1046–1051.
[112] S. Galluzzi, et al., Association of blood pressure and genetic background with white matter lesions in patients with mild cognitive impairment, J. Gerontol. A Biol. Sci. Med. Sci. 63 (5) (2008) 510–517.
[113] A.N. Garcia, et al., Cyp46 polymorphisms in Alzheimer's disease: a review, J. Mol. Neurosci. 39 (3) (2009) 342–345.
[114] M. Norlin, et al., Androgen receptor-mediated regulation of the anti-atherogenic enzyme CYP27A1 involves the JNK/c-jun pathway, Arch. Biochem. Biophys. 506 (2) (2011) 236–241.
[115] M.J. Nunes, et al., Okadaic acid inhibits the trichostatin A-mediated increase of human CYP46A1 neuronal expression in a ERK1/2-Sp3-dependent pathway, J. Lipid Res. 53 (9) (2012) 1910–1919.
[116] V. Leoni, C. Caccia, Oxysterols as biomarkers in neurodegenerative diseases, Chem. Phys. Lipids 164 (6) (2011) 515–524.
[117] G. Jones, D.E. Prosser, M. Kaufmann, Cytochrome P450-mediated metabolism of vitamin D, J. Lipid Res. 55 (1) (2014) 13–31.
[118] M. Fornage, et al., Family-based association study of matrix metalloproteinase-3 and -9 haplotypes with susceptibility to ischemic white matter injury, Hum. Genet. 120 (5) (2007) 671–680.
[119] M.J. Golden, et al., Increased number of white matter lesions in patients with familial cerebral cavernous malformations, AJNR Am. J. Neuroradiol. 36 (5) (2015) 899–903.
[120] S. Efroni, C.F. Schaefer, K.H. Buetow, Identification of key processes underlying cancer phenotypes using biologic pathway analysis, PLoS One 2 (5) (2007) e425.
[121] C.C. Wu, L.C. Strong, S. Shete, Effects of measured susceptibility genes on cancer risk in family studies, Hum. Genet. 127 (3) (2010) 349–357.