Published in final edited form as:

Pharmacogenet Genomics. 2015 January ; 25(1): 41–47. doi:10.1097/FPC.0000000000000100.

PharmGKB summary: very important pharmacogene information for CYP4F2

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Keywords

warfarin; vitamin K; phyloquinone; menaquinone; MK-4; vitamin E; α -tocopherol; arachidonic acid; eicosanoids; pharmacogenomics

Introduction

Cytochrome p450, family 2, subfamily F, polypeptide 2 (CYP4F2) catalyzes the NADPH-dependent oxidation of the terminal carbon of long and very long-chain fatty acids, the side chains of vitamin K (K₁, K₂) and vitamin E (tocopherols and tocotrienols), arachidonic acid (AA), and leukotriene B₄ (LTB₄). CYP4F2 localizes to the endoplasmic reticulum of cells. Although it is predominantly expressed in the liver and kidneys, there is some evidence that CYP4F2 is expressed in human enteric microsomes [1, 2]. In the liver and kidneys, CYP4F2 catalyzes the synthesis of 20-hydroxyeicosatetraenoic acid (20-HETE) from AA. Hepatic CYP4F2 also regulates the bioavailability of vitamin K and vitamin E, catalyzes the inactivation of LTB₄, and the bioactivation of the anti-malarial drug pafuramidine. Although initial studies of CYP4F2 were focused on its role as a regulator of LTB₄ and 20-HETE, current investigations focus on how variants of *CYP4F2* affect warfarin drug dosing and safety. A fully interactive version of this brief review can be accessed at: <http://www.pharmgkb.org/gene/PA27121>.

Substrates of CYP4F2

Fatty Acids

Fatty acids (FA) can be degraded by α , β , and ω -oxidation of their carbon chains. The preferred pathway of fatty acid degradation is β -oxidation in the mitochondria and peroxisomes. Very long chain fatty acids (VLCFAs), which are fatty acids with more than 22 carbons in their chain, cannot be β -oxidized and must be chain-shortened before they can be oxidized by the mitochondria. CYP4F2 contributes to FA oxidation by catalyzing the ω -

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Conflict of Interest

The authors report no conflicts of interest.

oxidation and chain shortening of VLCFAs [3, 4]. Under normal physiologic conditions, ω -hydroxylation contributes somewhere between 4-15% of FA metabolism, but there is evidence supporting an increasingly larger role for CYP4F2 in FA metabolism during periods of fasting, upon administration of lovastatin, and as a rescue pathway in disorders of peroxisomal β -oxidation [4-6].

Eicosanoids

AA is a precursor of the eicosanoids, which are signaling molecules that mediate the inflammation and immune response. Acute inflammation at the site of injury or infection protects the body from pathogens, but prolonged inflammation damages healthy cells and tissue; thus inflammation must be tightly controlled. LTB₄ is a pro-inflammatory eicosanoid that induces activation of polymorphonuclear leukocytes, monocytes, and fibroblasts, generation of superoxide, and the release of cytokines to attract neutrophils [7]. ω -oxidation of LTB₄ is catalyzed by multiple members of the CYP4F family, but in the liver CYP4F2 is the principle enzyme to ω -oxidize LTB₄ to 20-OH LTB₄, a far less potent eicosanoid metabolite [4, 8-10].

CYP4F2 also catalyzes the ω -oxidation of AA, which converts it to the signaling molecule 20-hydroxyeicosatetraenoic acid (20-HETE). 20-HETE promotes vasoconstriction by contributing to the myogenic response and by inducing alterations smooth muscle calcium homeostasis, which sensitize the smooth muscle to constrictor stimuli such as angiotensin II, phenylephrine and endothelin. In the nephron, 20-HETE is present in the proximal tubules, the thick ascending loop of Henle (TALH), and the glomerulus and it promotes natriuresis by acting on various ion transporters [11, 12]. Because 20-HETE is both vasoconstrictive and natriuretic its overall effect on arterial blood pressure depends on where it is acting, but the mechanism regulating its tissue-specific formation is still unclear.

Studies in rats and mice have shown that decreased levels of renal 20-HETE are associated with salt-sensitive hypertension whereas increased levels of renal 20-HETE are associated with other types of hypertension (e.g. spontaneous or androgen induced hypertension) [13, 14]. Multiple studies have reported that urinary 20-HETE is positively associated with hypertension in humans. If urinary 20-HETE reflects vascular 20-HETE levels it would provide a reasonable explanation for the positive association between urinary 20-HETE and increased blood pressure [15-17] or impaired vascular function [18]. Unfortunately, the relationship between urinary 20-HETE levels and CYP4F2 enzyme activity is equivocal. One study reported that a haplotype shown to increase *CYP4F2* expression (which is presumed to increase renal 20-HETE formation) was associated with increased urinary 20-HETE excretion and increased risk for hypertension [15]. A second study reported that a single nucleotide polymorphism (SNP) in *CYP4F2*, which has been shown to decrease 20-HETE formation *in vitro* [8], was also associated with increased urinary 20-HETE and increased blood pressure [17].

Blood pressure is a sexually dimorphic characteristic; on average men under the age of 60 have higher blood pressure than age-matched pre-menopausal women [19, 20]. A study in male Sprague-Dawley rats showed that administration of 5 α -dihydrotestosterone stimulated production of 20-HETE in the renal vasculature, which preceded a statistically significant

rise in systolic blood pressure. The rise in systolic blood pressure was abrogated upon administration of a 20-HETE antagonist [21]. Several studies have also reported sex-specific associations between certain genetic variants of *CYP4F2* with blood pressure, vascular function and risk of cerebral infarction [17, 18, 22, 23].

Vitamin E

Vitamin E is the name given to eight fat-soluble tocopherols and tocotrienols. Tocopherols are the most investigated form of vitamin E. Vitamin E is known as a free-radical scavenger that prevents cellular peroxidation of polyunsaturated fatty acids (PUFA). Vitamin E is especially important for protecting the cell membranes of neurons, and patients with perturbations in vitamin E absorption are at an increased risk of peripheral neuropathy and ataxia [24]. α -tocopherol is the most biologically active of the four isomers of tocopherol: α , β , γ , δ . Each isomer differs in both the placement, and the number of hydroxyl groups on their chromanol rings. Studies have shown that regardless of the isoform of vitamin E consumed, the α -tocopherol isoform is always the predominant isoform in plasma and tissue. Like LCFAs and VLCFAs, tocopherols are metabolized by chain shortening. [25-27]. Parker RS *et al.* were the first to report that the γ and δ tocopherols are preferentially catabolized over α -tocopherol, which promotes the retention of α -tocopherol in plasma and tissues. CYP4F2 is the only known enzyme to ω -hydroxylate vitamin E (tocotrienols as well as tocopherols), thus making it a critical modulator of circulating plasma vitamin E levels [4, 26, 28]

Vitamin K

Vitamin K refers to a group of fat-soluble derivatives of naphthoquinones, which differ in the number of double bonds present in their hydrocarbon chains. Plants synthesize vitamin K₁ (VK₁), also known as phyloquinone. VK₁ is highly concentrated in leaves where it acts as an electron acceptor in the light dependent reactions of photosynthesis. Menaquinones, also known as vitamin K₂, are the main form of vitamin K in animals. The most common form is menaquinone 4 (MK-4), which can be synthesized from VK₁, which animals can obtain by eating dark green leafy vegetables. Animals also obtain menaquinones from eating fermented food, and animal products [29]. Both types of vitamin K can act as co-factors for γ -glutamyl carboxylase (GGCX), which catalyzes the biochemical activation of proteins involved in blood clotting and bone mineralization. CYP4F2 ω -hydroxylates and inactivates vitamin K, which makes CYP4F2 an important negative regulator of vitamin K levels [30-32].

Anti-Parasitic Drugs

Pafuramidine (DB289) is a pro-drug of the anti-parasitic drug furamidine (DB75). Two studies identified CYP4F2 as one of the enzymes responsible for catalyzing the first-pass biotransformation of pafuramidine to furamidine in human liver microsomes and human enteric microsomes [1, 33].

The CYP4F2 Gene

CYP4F2 is part of a cluster of *CYP4F* genes on chromosome 19p13. The genetic architecture of all *CYP4F* genes is highly similar and the genes are thought to have evolved by duplication. Human *CYP4F2* has thirteen exons, twelve introns, and its open reading frame is encoded between exons two and thirteen [34]. The amino acid sequences of *CYP4F2* and *CYP4F3* are reported to be between 81-93% similar [34-36]. Analyses of the upstream 5' regions of *CYP4F2* show that there are putative RXR α and RAR α response elements (RARE), sterol regulatory element binding protein elements (SREBPE), NF- κ B and Myb response elements [15, 34, 37].

Hirani and colleagues (2008) developed a targeted polyclonal antibody of *CYP4F2* to quantify *CYP4F2* protein levels in human liver and kidney cortex samples [36]. With this antibody substantial inter-individual variability in *CYP4F2* expression from liver and kidney samples was discovered. Microsomal protein levels of *CYP4F2* ranged between 0-80.1 pmol/mg protein in liver and between 0-11.3 pmol/ mg protein in kidney cortex. Total hepatic microsomal immunoreactive *CYP4F* (e.g *CYP4F3b*, *CYP4F11*, *CYP4F12*) protein was present in all patients, even those without detectable *CYP4F2* protein. This evidence suggests that *CYP4F2* may be regulated in a different manner than other *CYP4F* family members [36].

Inducers and inhibitors of CYP4F2

The molecular mediators that regulate *CYP4F2* expression have only been elucidated in in vitro systems. Studies in hepatoblastoma cell lines (HepG2) have shown that retinoic acid regulates *CYP4F2* transcription through the RXR α and RAR α nuclear receptors. A study using a luciferase reporter system in HepG2 cells that were transiently transfected with the upstream *CYP4F2* regions -506/6 and -1726/6 revealed that co-transfection with human RXR α , and all-trans retinoic acid (ATRA), or 9-cis-retinoic acid (9cRA) stimulated reporter activity [37]. In contrast, co-transfection of either 9cRA or ATRA and RAR α , or RXR α /RAR α heterodimers attenuated the observed increase in promoter activity. The same study also identified several putative retinoic response elements (RARE) near *CYP4F2*, but none were confirmed RAREs. Although both retinoic acids induced promoter construct activity, only 9cRA was capable of inducing protein translation of *CYP4F2* [37]. Saturated fatty acids, such as lauric acid and stearic acid also induced *CYP4F2* expression in HepG2 cells [38].

The promoter region of *CYP4F2* contains multiple sterol regulatory element binding protein (SREBP) elements. SREBP-2 was found to mediate lovastatin-induced expression of *CYP4F2* in primary human hepatocytes, and in *SREBP-1a* transfected HepG2 cells SREBP-1a was found to mediate lovastatin-induced expression of *CYP4F2* [5]. None of these findings have been confirmed in clinical studies, although a few case studies provide some evidence that co-administration of lovastatin and warfarin is associated with longer prothrombin time in patients [39].

25-hydroxycholesterol and peroxisomal proliferators, such as clofibrate, ciprofibrate, WY14, 643, and PDFO, were shown to inhibit the lovastatin-mediated induction of *CYP4F2*

gene expression in HepG2 cells [5, 38]. The anti-fungal drug ketoconazole and sesamin, a molecule found in sesame oil, also inhibit ω -hydroxylation of tocopherols in rat and human liver microsomes, as well as in HepG2 cells. Studies with recombinant *CYP4F2* expressed in insect cells demonstrated that *CYP4F2* is enzymatically inhibited by both ketoconazole and sesamin [26, 27, 40].

Genetic variants of CYP4F2

Initially investigated due to its role in eicosanoid metabolism, rs2108622 was later discovered to have a small, albeit significant, role in affecting warfarin dose in several populations, most notably in Han Chinese and several different Caucasian populations [30, 41-44]. The rs2108622 (NT_011295.11:g.7253233C>T, V433M) polymorphism was initially reported in a screen of *CYP4F2* in an effort to discover SNPs associated with alterations in AA metabolism [8]. The T allele at rs2108622 is also associated with increased blood pressure [16, 17] and decreased vitamin E metabolism [28, 45].

A study in Han Chinese reported that a seven SNP haplotype (Haplotype I) in the proximal regulatory region of *CYP4F2* that included the A allele at rs3093105 (NC_000019.10:g.15897578A>C, W12G) was associated with increased urinary 20-HETE and increased risk of hypertension in case-control studies. Haplotype I was also transmitted more frequently to hypertensive offspring in family association studies [15]. In vitro assays showed that Haplotype I was associated with increased basal, as well as LPS-induced expression of *CYP4F2* when compared to Haplotype II, which included the C allele at rs3093105. Sequence analysis and functional studies showed that a second SNP in Haplotype II, rs3093098 (NC_000019.10:g.15897702A>G), eliminated a putative NK- κ B binding site, which provides a plausible explanation for the decrease in basal and LPS-induced expression associated with Haplotype II as compared to Haplotype I. A separate study in Japanese patients reported that when it is part of a haplotype with two other SNPs (rs1558139 A and rs2108622 C) the A allele at rs3093105 was protective against hypertension in men [23].

p.Val433Met (rs2108622)

The T allele at rs2108622, which has been designated as *3 by the CYP Allele Nomenclature Database (<http://www.cypalleles.ki.se>), replaces a valine residue with a methionine residue at position 433. Although this polymorphism does not affect mRNA transcription, or enzymatic activity of the protein, it does result in decreased protein levels. This could be due to a decreased rate of protein translation or an increased rate of protein degradation [31]. Functional in vitro assays show that the T allele decreases 20-HETE synthesis by 50-60% in human liver microsomes [8].

Pharmacogenomics

Warfarin

Warfarin is the most commonly prescribed oral anticoagulant in the world to prevent the occurrence of thromboembolic events, but achieving therapeutic dose of warfarin is difficult. There is high inter-individual variability of therapeutic dose between patients and warfarin

has a narrow therapeutic index. Too high of a dose increases a patient's risk of hemorrhage, but too low of a dose increases a patient's risk of thromboembolisms. A common cause of emergency room visits is attributed to errors in warfarin dosing [46, 47]. Multiple blood-clotting factors (II, VII, VIII, X, and proteins C, S, and Z) require post-translational carboxylation of their glutamate residues in order to become biologically active. Carboxylation of these glutamate residues is catalyzed by the enzyme γ -glutamyl carboxylase (GGCX), which requires vitamin K as a co-factor. An enzyme complex called vitamin K epoxide reductase (VKORC) ensures vitamin K bioavailability. By inhibiting a subunit of VKORC (VKORC1) warfarin inhibits the ability of GGCX to activate clotting factors. CYP4F2 metabolizes vitamin K and limits vitamin K availability.

A 2008 study in three separate Caucasian populations was the first to report that the T allele at rs2108622 in *CYP4F2* was associated with an increase in warfarin dose. The study calculated that each T allele at rs2108622 is associated with a 4-12% increase in warfarin dose, such that a patient with the TT genotype could require an approximate 1mg/day higher dose of warfarin compared to a patient with the CC genotype [41]. An independent study in Han Chinese confirmed an association between the T allele at rs2108622 and increased warfarin dose. The study also reported that the T allele was responsible for approximately 4% of the inter-individual variation in dose within Han Chinese [43]. Table 2 summarizes the results of several studies investigating the T allele at rs2108622 and its association with warfarin dose. A systematic review and meta-analysis stratified for common genetic variants in *CYP2C9* (*2 and *3) and *VKORC1* (-1639 G>A) and reported that patients carrying a T allele at rs2108622 required an 8.3% higher mean daily dose of warfarin than patients with the CC genotype [30]. The majority of the studies investigating associations between the T allele at rs2108622 and warfarin dose are of patients in the induction phase, but Roth *et al.* investigated whether *CYP2C9*, *VKORC1*, or *CYP4F2* genotypes were significantly associated with risk of bleeding events in long-term users of warfarin (the average duration of use was 3.4 and 3.7 years for cases and controls, respectively). In these patients the T allele at rs2108622 was significantly associated with an approximately 40% lower risk of bleeding events as compared to the C allele [48].

The T allele at rs2108622 in *CYP4F2* is found at a much lower frequency in populations of African descent as compared to populations of Caucasian or Asian descent, and studies investigating the effects of rs2108622 in non-Caucasian and non-Asian populations repeatedly report no association between the T allele and dose of warfarin [49-53]. The low frequency of the T allele at rs2108622 in some populations, combined with its small effect size may explain why so many studies in more ethnically diverse populations report no association between warfarin dose and presence of the T allele at rs2108622 (see Table 1 for allele frequencies of the T allele at rs2108622 in various ethnic groups). For example, a study in a multiethnic population investigated whether the inclusion of additional SNPs, including the T allele at rs2108622, enhanced the predictive power of existing warfarin dosing algorithms. The study compared the weekly doses of warfarin that were predicted by those algorithms to the actual weekly doses in patients that had reached a target international normalized ratio (INR) of between 2 and 3. The study concluded that inclusion of the rs2108622 genotype did not enhance the predictive power of any of the algorithms tested

[54-56]. A recent study investigated whether copy number variants (CNV) of *CYP4F2*, *CYP2C9*, or *VKORC1* might affect warfarin dose, but CNVs were so rare that they were deemed unlikely to contribute to variability in warfarin dose [57].

Acenocoumarol

Although warfarin is by far the most commonly prescribed oral anti-coagulant in the world acenocoumarol is more commonly prescribed in Europe. Like warfarin, acenocoumarol is a vitamin K antagonist and it is administered as a racemate. Unlike warfarin, however, the half-life of acenocoumarol is significantly shorter (T_{1/2} acenocoumarol is 2-8 hours and T_{1/2} warfarin is 32-43 hours) and it is the R-enantiomer of acenocoumarol that is the most biologically active, unlike the S-enantiomer of warfarin [58]. There are far fewer studies investigating how polymorphisms in vitamin K pathway genes affect acenocoumarol dose but the findings are similar to what is known about warfarin. In addition to various polymorphisms in *CYP2C9* and *VKORC1*, the T allele at rs2108622 also affects acenocoumarol dose. Two studies reported that patients that were carriers of the T allele had small but significant increases in acenocoumarol dose when compared to patients that were homozygous for the C allele. One of the two studies also reported that the T allele was protective against acenocoumarol overdosing in patients 3 months after starting therapy [58, 59].

Vitamin E

Two randomized clinical studies, one in adults and one in pediatric patients, concluded that supplements of the α -tocopherol form of vitamin E may improve liver histology in patients with non-alcoholic fatty liver disease (NAFLD) [60, 61]. Data from those studies, including rs2108622 genotype information, were also analyzed in a separate study to investigate whether the T allele was associated with improvement in patient prognosis or with baseline α -tocopherol levels before and after administration of α -tocopherol supplements. The study reported that the T allele was associated with increased baseline α -tocopherol levels in pediatric patients, but not in adult patients. The study also reported that, in patients given α -tocopherol, the T allele was associated with a significant increase in α -tocopherol levels when compared to patients with the C allele at 48 weeks, but the difference dissipated by 96 weeks. Overall, the T allele was not associated with improvement of hepatic steatosis in patients with NAFLD administered vitamin E [62, 63]. Finally, a genome-wide association study reported that the T allele at rs2108622 was associated with increased plasma α -tocopherol, but not γ -tocopherol levels [45].

Conclusion

CYP4F2 plays a critical role in the metabolism of arachidonic acid, and 20-hydroxyeicosatetraenoic acid, vitamin E and vitamin K. In patients of Asian and Caucasian descent the T allele at rs2108622 is significantly associated with a small increase in warfarin dose. The T allele is virtually absent in some Amerindian and African populations, and when compared to Asian and Caucasian populations the frequency of the T allele in admixed populations of African descent is low. There is no strong evidence of an association between the T allele at rs2108622 and warfarin dose in populations of African descent is

low. There is no strong evidence of an association between the T allele at rs2108622 and warfarin dose in populations of African descent. *In vitro* studies provide evidence that statins increase *CYP4F2* expression and that PPAR α agonists inhibit *CYP4F2* expression, although these findings have not been confirmed in clinical studies. Additional research is needed to discover whether *CYP4F2* metabolizes other important endogenous molecules and xenobiotics and whether other polymorphisms in *CYP4F2* contribute to inter-patient variability in warfarin dose and safety.

Acknowledgments

PharmGKB is supported by the NIH/NIGMS R24 GM61374.

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Table 1

Frequencies of the T allele at rs2108622 in different populations. Centre Etude Polyphormisme Humain/ Human Genome Diversity Project (CEPH/HGDP), Molecular Anthropology Laboratory, Canadian volunteers for University of Toronto at Mississauga (UT Mississauga), Patients from Instituto Nacional de Cardiologia de Laranjeiras, Rio de Janeiro, Brazil (INCL), Patients Volunteers from the New York Blood Center (NYBC), Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: <http://evs.gs.washington.edu/EVS/>) [July, 2014]

Population Reported	OMB	Sample Size	T allele frequency	Source	PMID
E. Asian	Asian	233	0.292	CEPH/HGDP	20555338
E. Asian	Asian	100	0.233	UT Mississauga	20555338
Central/ S. Asian	Asian	204	0.350	CEPH/HGDP	20555338
Central/ S. Asian	Asian	95	0.484	UT Mississauga	20555338
Asian	Asian	600	0.357	NYBC	20504253
European	White	159	0.297	CEPH/HGDP	20555338
European	White	121	0.240	UT Mississauga	20555338
Caucasian	White	600	0.447	NYBC	20504253
Brazilian white	White	184	0.30	INCL	20182420
Brazilian Admixed	White	112	0.23	INCL	20182420
European, White	White	4300	0.291	ESP	N/A
African	Black or African-American	108	0.070	CEPH/HGDP	20555338
Brazilian Black	Black or African-American	74	0.15	INCL	20182420
African-American	Black or African-American	600	0.302	NYBC	20504253
African-American	Black or African-American	2203	0.0971	ESP	N/A
Middle Eastern	Other	164	0.403	CEPH/HGDP	20555338
Ashkenazi Jewish	Other	1000	0.486	NYBC	20504253
Oceania	Other	31	0.613	CEPH/HGDP	20555338
Hispanic	Other	600	0.355	CEPH/HGDP	20555338
American	Other	64	0.098	CEPH/HGDP	20555338

Table 2

A chronology of association studies of rs2108622 and warfarin dose

Year	Title	Summary of Findings	PMID
2008	CYP4F2 genetic variant alters required warfarin dose	The T allele at rs2108622 is associated with increased warfarin dose in two of three independent Caucasian populations.	18250228
2009	CYP4F2 is a vitamin K1 oxidase: An explanation for altered warfarin dose in carriers of the V433M	Functional studies that demonstrate that CYP4F2 oxidizes, and inactivates vitamin K1; the T allele at rs2108622 is associated with a reduced capacity to oxidize vitamin K	19297519
2009	A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose	The T allele at rs2108622 is significantly associated with a higher mean warfarin dose in Swedish patients.	19300499
2010	CYP4F2 rs2108622: a minor significant genetic factor for warfarin dose in Han Chinese patients with mechanical heart valve replacement	The T allele at rs2108622 is significantly associated with a higher mean warfarin dose in Han Chinese patients. The genotype at rs2108622 contributes 4% to inter-individual variability in dosage within the Han Chinese population, but does not explain the difference in mean warfarin dose between Han Chinese and Caucasians.	20653676
2010	Impact of CYP4F2 rs2108622 on the Stable Warfarin Dose in an Admixed Population	The T allele at rs2108622 is not associated with warfarin dose in a Brazilian population, which includes people with European and African ancestry.	20182420
2010	Genetic and Clinical Predictors of Warfarin Dose Requirements in African Americans	The T allele at rs2108622 is not associated with warfarin dose in African-American patients. The study also confirms the low allele frequency in African-Americans as compared to Caucasians.	20072124
2011	Genetic and nongenetic factors associated with warfarin dose requirements in Egyptian patients	rs2108622 is not significantly associated with warfarin dose in an Egyptian population.	21228733
2011	Influence of CYP4F2 rs2108622 (V433M) on warfarin dose requirement in Asian patients	The frequency of the T allele at rs2108622 is higher in Indian and Caucasian populations compared to Chinese, Malay, and African-American populations. The T allele in CYP4F2 is significantly associated with a higher daily mean warfarin dose in patients of Asian descent.	21084764
2013	Responsiveness to low-dose warfarin associated with genetic variants of VKORC1, CYP2C9, CYP2C19, and CYP4F2 in an Indonesian population	rs2108622 is not significantly associated with warfarin dose in an Indonesian population.	22855348
2014	Genetic Risk Factors for Major Bleeding in Patients Treated with Warfarin in a Community Setting	rs2108622 is significantly associated with a decreased risk of major bleeding events in long-term users of warfarin (the average duration was apx. 3 years).	24503627