Prevalence of es2108622 (*CYP4F2*3*) Single Nucleotide Polymorphism – A Review

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Literature review

Abstract: *Cardiovascular diseases are known to be treated with anticoagulants lifelong. Warfarin is one of the most commonly used medications for anticoagulation despite causing serious side effects in some patients. Different single nucleotide polymorphisms (SNPs) that have a role in the cytochrome P450 system can also affect the metabolism, as well as dosing, of warfarin. The purpose of this review is to look into the prevalence of this SNP in the past research and screen for possible correlations with age, place of origin, family history of cardiovascular and cerebrovascular diseases, or other medical conditions possibly present in various populations. In total, 20 scientific articles falling under the inclusion criteria were reviewed and found usable, and the rest of the cases will be highly beneficial in the upcoming years to determine the role of the recently discovered CYP4F2 rs2108622 variant, as well as the previously known CYP2C9 and VKORC1 SNPs, in the variance of warfarin dose requirement. These findings may also point researchers in the right direction for qualifying and validating these genetic variants for use as GBs (genomic biomarkers) in the clinical and medical practice of treatment with warfarin.*

Keywords: SNP, rs210862 (*CYP4F2*3*) SNP, warfarin, vitamin K, cardiovascular disease, anticoagulation, INR.

1. Introduction

Throughout the patient's life, cardiovascular diseases are treated with anticoagulants. These diseases include transient ischemic attack (TIA), coronary artery bypass grafting (CABG), pulmonary thromboendarterectomy (PTE), rheumatic heart disease (RHD), atrial/aortic valve replacement (AVR), permanent pacemaker (PPM), percutaneous coronary intervention (PCI), cerebrovascular accident (CVA), left atrial (LA) clot, mitral valve replacement (MVR), atrial fibrillation (AF), deep vein thrombosis (DVT), percutaneous transvenous mitral commissurotomy (PTMC) as well as combined conditions. Anticoagulants interact at various stages of the coagulation cascade and are classified into two types: those that act directly by inhibiting enzymes and those that act indirectly by binding to antithrombin or preventing its synthesis in the liver. [1]. According to the types of anticoagulants, there are several available: low molecular weight heparin (LMWH), unfractionated heparin (UFH), low molecular weight heparin (LMWH), direct factor 10a Inhibitors and vitamin K-dependent antagonists, and direct thrombin inhibitors. Medical conditions, pat preferences, and risk stratification should be taken into consideration when choosing the appropriate anticoagulant. Atrial fibrillation, venous thromboembolism, and post-heart valve replacement are the most common medical conditions requiring anticoagulation treatment. Venous thromboembolism is important because it is often one of the first symptoms of several other medical conditions [2]. Warfarin, a vitamin K-dependent antagonist, is one of the most commonly used anticoagulants. Warfarin works by inhibiting the enzyme vitamin K epoxide reductase which is required for the gamma-carboxylation of vitamin K-dependent factors. The dosing limit is low, and the effect is highly influenced by various factors (including diet), which may lead to resistance to the treatment. Treatment with these anticoagulants requires regular monitoring with an International Normalized Ratio. This enables the usage of a standardized method of analysis and reporting the consequences of an oral anticoagulant (like warfarin) intake, specifically related to blood clotting. Results will vary according to medication the patient takes, age, and any additional health issues. The INR number should be between 2 and 3 if a patient takes an anticoagulant, but it could be different, depending on the patient's condition. Single nucleotide polymorphisms are recognized as the most popular molecular markers for genetic studies as they are a type of variation of a single base pair of polymorphism. SNPs have been found to associate with drug response, diseases, and other phenotypes [3].

*A. Variant rs2108622 of CYP4F2*3*

CYP4F2 is a cytochrome P450 enzyme. It participates in the -hydroxylation of arachidonic acid and vitamin E. Researchers discovered that CYP4F2 participates in VK1 metabolism, and that the rs2108622 polymorphism may affect VK1 oxidase activity. When investigating VK1 oxidase activity in human liver microsomes, the *CYP4F2* CC pool showed to have the highest activity. On the other hand, the *CYP4F2* TT pool demonstrated a decrease of 75%, and the CT pool had its activity on the intermediate level. The reason behind this is that the carriers of rs2108622 might have higher levels of VK1 oxidase, thus requiring a higher dose of warfarin. The rs2108622 polymorphism has been found to impact warfarin dose necessity and clarify roughly 2% - 7% of the variance. *CYP4F2* was also found to be a minor predictive of medication dose in a genome-wide association study (GWAS) involving 1,053 Swedish subjects. Those certain research results revealed that *CYP4F2* rs2108622 T carriers need to have a higher warfarin dose and that *CYP4F2* could be the 3rd hereditary predictive of warfarin daily dosage [4].

Studies chosen for this review are based on the connection of the rs2108622 single nucleotide polymorphism with warfarin dose, the reactions performed within the patient, and INR in populations. The goal is to investigate the prevalence of this single nucleotide polymorphism in previously published studies and detect possible correlations with age, place of origin, family history of cardiovascular and cerebrovascular diseases, or other medical conditions. It is very important to take all of these into consideration during diagnosis, as well as treatment.

2. Materials and methods

A. *Search Strategy*

Databases used in this review were BioMed Central (BMC), National Center for Biotechnology Information (NCBI), Journal of Human Genetics, PLoS Genetics, Future Medicine, and Science Direct. These databases were used due to a large number of free articles, and also a higher number of articles related to the topic. The search was firstly based on keywords related to the topic, namely rs2108622 SNP, *CYP4F2*, PCR, Warfarin, Vitamin K, Anticoagulation, INR, Cardiovascular diseases, and any combination of these.

B. *Inclusion and Exclusion Criteria*

Table 1 shows the inclusion and exclusion criteria used in this paper. Only studies written in the English language were included to avoid translational mistakes. Articles that were not dealing with previously mentioned keywords were excluded. Sources were manually and thoroughly analyzed to exclude duplicates and potentially biased articles.

TABLE 1. Inclusion and exclusion criteria.

Inclusion criteria	**Exclusion criteria**
Language: English	Any other language
Focusing on *CYP4F2* rs2108622, warfarin treatment and cardiovascular diseases, and connection between these (and other keywords listed above)	Papers incompletely or not include any of the selected keywords, especially *CYP4F2* rs2108622, warfarin treatment, and cardiovascular diseases
Peer-reviewed papers	Unreviewed articles, textbooks

3. Results and Discussion

Before implementing the inclusion and exclusion criteria, 34 studies were found to be in line with the topic according to keywords, but four were immediately excluded because they were inaccessible.

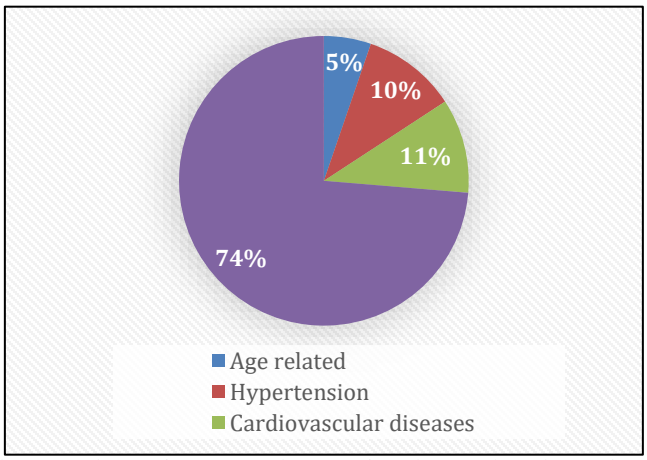

FIGURE 1. Summary of the number of papers chosen for the review based on the topic the studies were focusing on. It outlines the general organization of the review and findings.

After exclusion criteria were applied, five articles were excluded because they were published by unapproved sites (not scientific journals), and five were excluded because they were not based on warfarin maintenance or rs2108622 SNP. In total, 20 scientific articles falling under inclusion criteria were reviewed, meaning those published in the English language, and focusing on warfarin dosing in correlation with age, cardiovascular diseases, hypertension, and INR (Sakiene *et al*, 2016; Pawlowska *et al*, 2019; Karpinos *et al*, 2013; Bener *et al*, 2013; Zhang *et al*, 2017; Geng *et al*, 2019; Luo *et al*, 2015; Khosropanah *et al*, 2017; Banecka-Majkutewicz *et al*, 2012; Meng *et al*, 2015; Liao *et al*, 2016; Li *et al*, 2018; Li *et al*, 2012; Ross *et al*, 2010; Kumar *et al*, 2014; Sipeky *et al*, 2015; Krajčíová *et al*, 2014; Borgiani *et al*, 2009; Caldwell *et al*, 2008; and Takeuchi *et al*, 2009). Out of these, fourteen were related to warfarin dosing in populations, three of which included data on patients with cardiovascular diseases. Two papers were related to cardiovascular diseases, two with hypertension and one with age (Figure 1).

A. rs2108622 Single-Gene Polymorphism (CYP4F2) – Age-Related

Neurodegenerative disorder named age-related macular degeneration (AMD) seems to be a disorder that is the main cause of permanent blindness in people over the age of 65, especially in Western countries [5]. The amount of people with AMD is estimated to rise by roughly 50% by 2020, and the disease's responsibility is expected to increase with age. According to Pawlowska et al. (2019), the accumulation of oxidized lipids appears to play a central role in the growth of AMD [6].

Sakiene *et al* (2016) analyzed patients with exudative age-related molecular degradation and patients with early age-related macular degeneration. The experiment was done by DNA extraction (blood) and PCR reaction. The comparison of the rs2108622 genotype frequency by age groups did not reveal significant differences. The comparison between male and female carriers of the rs2108622 (people who have to die to diagnose AMD and the control group) did not reveal any significant differences, but males and females with AMD did. The analysis showed that the codominant variables inside the group of persons under the age of 65 were significant statistically. It is conceivable that gene-environment interactions influenced the genotype distribution of rs2108622 in patients with exudative AMD and control subjects [5].

B. Correlation CYP4F2 (rs2108622) Gene Polymorphism and Hypertension

The most common cardiovascular disease is hypertension [7]. Hypertension has recently been identified as a complex multifactorial illness caused by interactions between countless genetics and the environment [8], [9]. A study found that rs2108622, rs1558139 and rs2108622 single nucleotide polymorphisms on the gene *CYP4F2* are linked to hypertension, with the rs1558139 polymorphism being extremely powerful in males. These findings are also supported by six studies, three of which looked into the rs1558139 polymorphism and six of which looked into the rs2108622 polymorphism [10].

Luo *et al* (2015) included 4 studies that contain a total of 1878 patients with hypertension and 1512 healthy control subjects. The study, which contained 4 independent case-control studies, has shown that the *CYP4F2* gene rs2108622 polymorphism was not linked to an increased risk of high blood pressure [11].

C. Correlation Between Cardiovascular Diseases and CYP4F2 Gene Rs2108622 Polymorphism

Warfarin has a wide range of applications, including pulmonary embolism, stroke, and preventative measures of thromboembolic events, as well as atrial fibrillation, coronary dysfunction, and prosthetic valve placement. Due to the FDA's Adverse Event Reporting System, warfarin has been one of the 10 leading drugs with the most serious side effects, particularly

during the initial phase of treatment [12]. Figure 2 depicts the cardiovascular diseases included in this study: ischemic stroke, cardiac ablation, valve replacement, and cardiovascular patients in general.

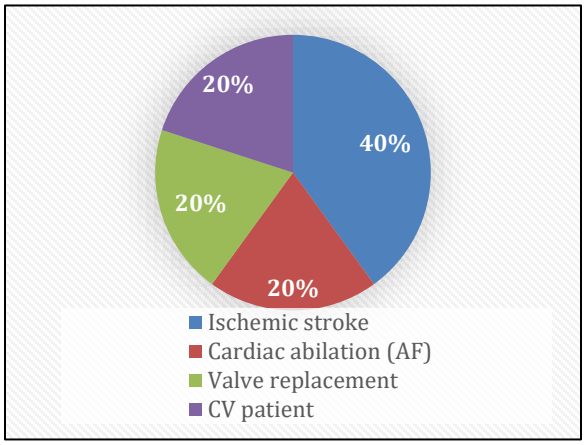

FIGURE 2. Summary of the number of CV disease-related papers chosen for the review based on the type of CV disease the studies were dealing with.

The figure outlines the general organization of one subtopic discussed in the review. Ischemic stroke is caused by the interaction of genetics and the environment, and many genetic factors can affect its pathogenesis. Of 100% possibility to have any of strokes it is 80% to have Ischemic stroke (IS) [13].

Six studies were performed and included a total of 2,187 cases and 7,556 controls, and the correlation between *CYP4F2* V433M polymorphisms and susceptibility to ischemic stroke was evaluated truly by the Recessive model. The methodology they used is Q statistic test methods and I^2 quantitative assessment for evaluating the heterogeneity size between the studies. On the one hand, the study can only combine the crude OR values to examine the association because it is impossible to collect the OR (odds ratio) value of each study after adjusting for age and sex. As a result, there may be a discrepancy between the test and real-life association intensity. However, due to the small number of papers included, it is not possible to analyze the connection in the subgroup by race [14].

Another study took 396 patients with ischemic stroke and 378 controls were genotyped for rs9333025, rs3093135, rs2269231, and rs2108622. Generalized multifactor dimensionality reduction (GMDR) methodologies were used to investigate gene-gen interactions. The GMDR analysis revealed that rs2108622 and rs9333025 had a strong gene–gene interaction. Polymorphisms: rs9333025 GG and rs2108622 GG genotypes, this gene–gene interaction predicted a significantly higher risk of ischemic stroke [15].

One of the most represented heard conditions is atrial fibrillation (AF). Research has been conducted within the Chinese population in comparison with African and Caucasian populations. According to the 2010 Chinese Census, China's population of AF cases aged 35 years is 5.26 million, and the number of AF ablation procedures is quickly increasing. AF catheter ablation is a surgery that necessarily requires rest for three weeks, and warfarin therapy for three months. Warfarin dosage is presented with the INR and carefully regulated due to ethnic and individual dosing variances.

Jiao Li *et al* used a total of 222 patients from West China Hospital in their study (82 males and 140 females). Their demographic data, such as body weight, constituent warfarin dosage, age, height, was meticulously documented, and blood samples were taken for DNA isolation. Methodology they used was DNA extraction and PCR reaction. The genotyping results performed

low frequency of variant rs2108622 *CYP4F2* gene of all T allele carriers. The TT and CT carriers required a significantly higher dose of warfarin in *CYP4F2* rs2108622 genotype patients. Sichuan Chinese patients were present a low frequency of warfarin dose which can be the main cause of sensitivity to warfarin and their requirements in warfarin dosage in compared with Caucasians [16].

Li *et al* (2012) studied 352 patients after heart valve replacement surgery Patients' warfarin dosing was considered to obtain an INR of 1.8 to 2.5. They investigated for SNPs in *CYP4F2* in these patients and looked into their relationship with warfarin dosing. The following information was collected for each patient: (1) general information, (2) drug, (3) surgical history, (4) medical history. DNA samples deployed were isolated from blood. Methodology conducted involved DNA extraction and PCR. The study also includes 352 patients, 228 of these have a CYP4F2 wild-type homozygous CC genotype, 104 had a heterozygous CT genotype, and 20 had a mutant TT genotype. The results show that the warfarin dose necessity increased substantially in Chinese people who have at least one T allele versus those who are homozygous for the C allele [17].

In the study performed by Khosropanah et al. (2017), 226 cases were selected from the 230 participants, with 152 subjects classified into group A (dosage of warfarin is 5 mg/day) and 74 cases classified into group B (dosage of warfarin is above 5 mg/day). The study's findings show that when Iranians have at least one T allele, their warfarin dose requirement increases significantly when compared to those who are homozygous for the C allele [12]. One of the limitations of such studies involving a wide range of diseases, duration of warfarin therapy, and age of patients participating in the study is that it may have to affect statistical analysis methods.

D. Warfarin Dosage Response Related Pharmacogenetics in populations

Because there is little possibility of over-or under-anticoagulation warfarin dosing is to hold prothrombin time 2 – 3 of the international normalized ratio (INR). Earlier studies have shown that two genes have interacted with the vitamin K-dependent clotting pathway, *VKORC1* and *CYP2C9,* account for an additional 30–54 percent of the variant in dosing warfarin [18]. South Indians, Roma and Hungarians, Slovaks, Chinese, Italians, Caucasians, Asians, Africa, Swedish, and Koreans were all studied.

Kumar *et al* (2014) reported the mean daily requirement dose of warfarin to be 4.7 ± 2.1 mg/day in the South Indian population with increased warfarin therapy in patients with rs2108622 SNP [19].

Sipeky *et al* (2015) found that members of the Roma population have an elevated chance for higher mean warfarin dose requirement in comparison with the Hungarian population, besides a decreased risk of major bleeding events in long-term warfarin use [20]. Another study showed that polymorphisms in the genes CYP4F2 have a smaller effect on warfarin pharmacogenetics than in VKORC1 and *CYP2C9* and which have a meaningful impact on personal reaction to warfarin dosages in the Slovak population [21]. Lastly, scientists reported warfarin maintenance in the Italian population. Consumers included just patients with CV disorders, and it is clear from these observational studies that the quantity of required warfarin dose modifications was reduced, and patients were outside of the target INR range [22]. The possible medical advantage of CYP4F2 genotyping differs by the racial group due to the difference in the frequency of the foundational gene variants between many substantial racial groups. The minimal allele frequency for CYP4F2 is roughly 30% in Asians and Caucasians while being only 7% in African populations. As a result, the African population's tends stable dose of warfarin is expected to be lower than that of Asians and Caucasians [23].

Takeuchi *et al* (2009) strongly indicate that the 3rd gene, *CYP4F2* (rs2108622) affects warfarin dose in the Swedish population, with an INR of 3.0-4.0 [24]. According to Ross *et al* (2010), gender, age, INR, BSA, and *CYP2C9, VKORC1,* and *CYP4F2* polymorphisms all influence warfarin dosage requirements in the Korean population. The most recent variation suggested affecting warfarin dosing was also knowns as a non-synonymous - rs2108622 SNP (C/T polymorphism) located within the *CYP4F2* gene. A recent genome-wide association (GWA) study

on patients in the Swedish population examined the relationship of rs2108622 with a dosage of warfarin after controlling for the effects of *CYP2C9* and *VKORC1* [18].

After analyzing the studies, it can be concluded that the rs2108622 SNP is one of the three main factors for determining the dosage of warfarin, which may be detected with enzyme restriction. DNA source was blood for all the studies and considering the patient's conditions, just one group of investigators have included a healthy positive control group, while others included patients with cardiovascular disorders. It is of utmost importance to refer to a control group in order to "calibrate" the results, and probably lead to more comprehensive conclusions and possible comparisons. On the other hand, the information obtained and organized in this review increases comprehensiveness.

4. Conclusion

Usable cases will be incredibly beneficial in the coming few years to determine the role of the recently discovered *CYP4F2* rs2108622 single nucleotide polymorphism, as well as the previously known *CYP2C9* and *VKORC1* SNPs, in the variance of requirement dose of warfarin. The findings of these studies may also point researchers in the right direction for qualifying and validating these genetic variants for use as GBs (genomic biomarkers) in the clinical and medical practice of treatment with warfarin.

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