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CYP2C9 and VKORC1-1639 genetic polymorphisms involved in warfarin pharmacogenetics and pharmacodynamics in Latin American population

MSc. Sara Negaresh
Pharmaceutical Chemistry
MSc. Environmental Management
Universidad Nacional Autónoma de Nicaragua, Managua
https://orcid.org/0000-0002-1196-8522
snegaresh@unan.edu.ni

PhD. Marianela Corriols
PhD in Public Health
Faculty of Medical Sciences
Universidad Nacional Autónoma de Nicaragua, Managua
https://orcid.org/0000-0002-3955-230X
marianelacorriols@gmail.com

PhD. José René Silva Arrechavala
PhD in Molecular Biology
Faculty of Medical Sciences
Universidad Nacional Autónoma de Nicaragua, Managua
https://orcid.org/0000-0002-8106-5775
rsilva@unan.edu.ni

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ABSTRACT

Warfarin is the most common anticoagulant used worldwide for prevention and treatment of thromboembolic events. Polymorphisms in the CYP2C9 and VKORC1 -1639 genes have been associated with variability in the response to warfarin in several populations. The aim of this research is to determine the frequency of

these polymorphisms in Latin American population. The identification of the presence of polymorphisms can guarantee the safest and most effective use of warfarin by predicting the appropriate dose for each patient.

We included eleven studies with frequency data of genetic polymorphisms in 2,830 Latin American patients. The allelic variants CYP2C9 * 1 / * 2 and CYP2C9 * 1 / * 3 were more frequent in the study population, with 13.15% and 6.93% respectively. The allelic variant * 3 / * 3 was present in 0.17%. As for the polymorphisms of the VKORC1 -1639 gene frequencies of 49.11%, 33% and 17% were reported for the GA, GG and AA variants respectively.

Frequency of the allelic variants in the genes CYPC9 and VKORC1, in the Latin American population, allows to establish starting points to suggest the genotypes that should be evaluated before the indication of the warfarin dose. Comparing the frequency of polymorphisms in the CYP2C9 and VKORC1 -1639 genes in the Latin American population with other populations, similarities are observed with the populations of origin.

INTRODUCTION

The variability of response to drugs in individuals or populations, from a pharmacogenetic point of view, depends on variations in the DNA sequence known as polymorphisms (Arribas, 2010). Knowing the effect of a given genetic polymorphism on the pharmacokinetics and pharmacodynamics of a drug, it is possible to predict the appropriate dose, prevent adverse reactions and ensure the safest and most effective use of the drug.

Most pharmacogenetic studies have been conducted in Europe, North America, and Asia. Little is known about pharmacogenetic markers in Latin American populations. These populations tend to have a wide variety of racial mixtures, of African, Amerindian, and European origin, so extrapolation of pharmacogenetic results from other populations with Latin American populations would not be correct. This reality suggests the need for pharmacogenetic characterization studies in populations of this region (Céspedes, 2016).

Warfarin is an oral anticoagulant widely used for the prevention and treatment of thromboembolic events (Gaikward , 2013; Kaye, 2017). The use of warfarin reduces cases of stroke in patients by approximately 60% (Li, 2015; Tsai, 2017; Miklosz, 2018). The management of anticoagulant therapy with warfarin becomes complex due to the wide variability of inter- and intra-individual response of patients (Parra, 2015; Al-Eitan, 2018). Inappropriate warfarin dosing significantly increases the risk of thromboembolism, bleeding, hospitalization, and death in patients.

Warfarin dose adjustments are usually necessary and are based on measurement of prothrombin time (PT) in the blood and INR (International Normalized Ratio) calculation.

A high INR value predisposes to an increased risk of bleeding, while an INR value below the therapeutic range, between 2 and 3, indicates that the warfarin dose is insufficient to protect the patient from thromboembolic events (Kaye, 2017).

Many clinical and environmental factors such as age, sex, race, body mass index, comorbidities (mainly kidney or liver disease), polymedication (Zhang, 2016), tobacco use, diet, rapid vitamin K metabolism, as well as genetic mutations affect warfarin dose requirements (Bryk, 2018). Genetic polymorphisms in the genes of the 2C9 subunit of the cytochrome P450 complex (CYP2C9) and the 1 subunit of the vitamin K epoxide reductase complex (VKORC1) have been associated with 40% to 60% variation in the interindividual response to warfarin (Flockhart, 2008; Dean, 2012; Tavares, 2018; Liu, 2017; Razavi, 2017; Wattanachai, 2017; Liu, 2017).

Warfarin is a racemic mixture of S-warfarin and R-warfarin. S-warfarin is five times more potent than R-warfarin and is responsible for the anticoagulant effect in patients (Galvez, 2018). Two amino acid variants, $Arg_{144}Cys$, which result from the substitution of C_{430} by T in exon 3 (CYP2C9*2), and $Ile_{359}Leu$, produced by the substitution of A_{1075} by C in exon 7 (CYP2C9*3) reduce the catalytic activity of the enzyme compared to the normal genetic variant (native gene) CYP2C9*1. The catalytic activity of the enzyme resulting from the expression of the CYP2C9*3 gene is much lower than in CYP2C9*2 and CYP2C9*1 carriers (Dong, 2018). S-warfarin clearance *in vivo* is reduced by 66% and 90% in heterozygous and homozygous subjects for the CYP2C9*3 allele, respectively, compared to homozygous CYP2C9*1 subjects (Yoon, 2001). Patients with the allelic variants CYP2C9*2 and CYP2C9*3 have been shown to require lower doses of warfarin (Dilge, 2016; Natarajan, 2013; Benavides, 2015).

The enzyme vitamin K epoxy reductase participates in the reduction of vitamin K (essential cofactor for the activation of γ-glutamyl carboxylase that catalyzes the carboxilation of glutamic acid residues in many proteins involved in blood coagulation, factor II, VII, IX, and X) (Moyer, 2009) activating it for incorporation into the coagulation cascade (vitamin K regeneration, oxidation, and reduction cycle). The gene for the subunit 1 of the vitamin K epoxy reductase complex, VKORC1, is responsible for the expression of this enzyme. Warfarin exerts its anticoagulant effect through inhibition of the genetic products of VKORC1 (Cullell, 2018). Carriers of VKORC1 -1639 GG require higher doses of warfarin compared to carriers of GA and AA variants (Cavallari, 2011).

Despite several pharmacogenetic studies that explain the variability of response to warfarin in different races, African and Latin American populations have not been significantly represented. It has been shown that warfarin dose variation is greater in populations of mixed descent, so Latinos and African Americans tend to be at greater risk for inadequate response

to warfarin that could result in cases of intracranial hemorrhage from suboptimal warfarin management (Golwala,2016; Shen, 2007).

The characterization of these populations in terms of relevant biomarkers, already identified in pharmacogenetic studies worldwide, will validate the utility of these tests in the clinical management of the patient, promoting the safest and most effective use of this drug.

The objective of this study is to identify the frequency of CYP2C9 and VKORC1 -1639 genetic polymorphisms in the Latin American population to suggest the genotype that should be evaluated before the prescription of warfarin.

DEVELOPMENT

Search strategy

The bibliographic search of scientific articles was carried out in the period from December 2017 to October 2018. The databases consulted were PubMed, HINARI and SciELO. The search was limited to all studies published in English and Spanish, in the period from 2001 to 2018.

The keywords used for the search were: warfarin, pharmacogenetics, genetic polymorphisms, CYP2C9 gene, VKORC1 gene, Latin America, adverse reactions, intracranial hemorrhage and thromboembolism.

Study selection criteria

The selection of scientific articles was made based on the following inclusion criteria: patients who received treatment with warfarin, Latin American patients, patients with CYP2C9 and VKORC1 genetic polymorphisms; and reporting of polymorphism frequencies in CYP2C9 and VKORC1 genes.

Statistical Analysis

The statistical analysis of the data was done with Minitab 17 software. For data analysis of CYP2C9 gene polymorphisms, Grubbs contrast was applied to identify anomalous data and Anderson Darling normality test was applied to determine if the data followed a normal distribution with a 95% confidence level.

For the VKORC1 -1639 gene genetic polymorphism data, only the Grubbs test was applied. Due to the sample size (seven items) a conclusive application of the normality test is not possible.

The Hardy-Weinberg balance deviation was evaluated separately for each polymorphism within each study group using the X^2 test with a degree of freedom and a significance level of 0.050 (Limdi, 2010; Cabrero).

Selection of studies

We found 402 scientific articles referred to the pharmacogenetics of warfarin, of these 382 were excluded because they do not include Latin American population. Twenty articles were identified in Latin American population, of these, only eleven met the criteria for inclusion, the remaining nine articles (Tavares, 2018; Liu, 2017; Valentin, 2014; Guerrero, 2009; Isaza, 2010; Raggio, 2005; Claudio-Campos, 2015; Parra, 2015; Villagra, 2010) were excluded (Figure 1). The Latin American countries with the largest number of publications are Puerto Rico (six articles; two included) and Brazil (five articles; three included), followed by Mexico (two articles; both included), Chile (two articles; both included), Colombia and Argentina (one article per country; both included). Of the selected articles, nine were published in English and two in Spanish.

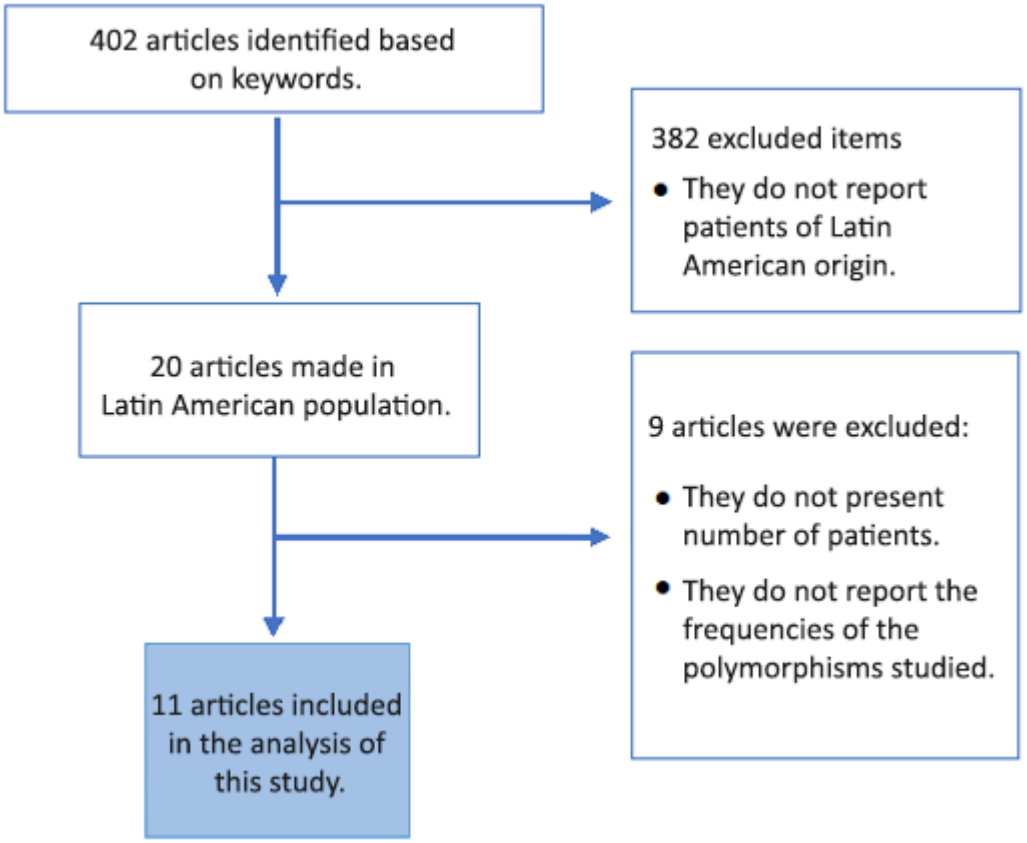


Figure 1. Study selection diagram

Characteristics of the study

The 2,830 patients included were of Latin American origin. Indications for warfarin were reported in only five studies, the most frequent being atrial fibrillation (AF), valve prosthesis (PV), thromboembolism (TE), deep vein thrombosis (DVT) and stroke (CVA).

Of the eleven articles, all studied the genetic polymorphisms of CYP2C9 (2,830 patients), seven (1,236 patients) presented the polymorphisms of the VKORC1 -1639 gene and seven (1,219 patients) analyzed the two genotypes CYP2C9 and VKORC1 -1639.

Table 1 summarizes the characteristics of the studies. *(see table on next page)*

Table 1. Data from the studies analyzed

No.	Study	Location	Warfarin indication	Number of patients	Age	Genotypic frequency								
						CYP2C9 % (n)						VKORC1 -1639 % (n)		
						*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	AA	GA	GG
1	Perini et.al. (2009)	Brasil, mixed descent	-	331	-	72.2 % (239)	14.5 % (48)	10.9 % (36)	0.9 % (3)	0.9 % (3)	0.6 % (2)	-	-	-
2	Botton, et.al (2011)	Brasil, Spanish descent	FA PV TE ACV TF	279	62.6 (18-88)	65.2 % (182)	22.9 % (64)	9.0 % (25)	1.1 % (3)	1.8 % (5)	0 (0)	13.6 % (38)	46.2% (129)	40.2% (112)
2	Miranda et.al (2011)	Chile	-	24	25 (18-55)	79.2 % (19)	16.7 % (4)	(0)	4.2 % (1)	(0)	(0)	-	-	-
4	Valentina et.al (2012)	Puerto Rico, mixed descent	FA TVPEP AC-VRVAAIT-FCCRVMB BABF IM, EVA EACI	103 (101)	67.2	59 % (61)	25 % (26)	5 % (5)	3 % (3)	6 % (6)	0 (0)	14.7 % (15)	52 % (54)	33 % (34)
5	Scibona et.al (2012)	Argentina	-	101	35 (21-67)	56 % (57)	23 % (24)	4 % (5)	13 % (14)	1 % (1)	0 (0)	20 % (21)	60 % (60)	19 % (20)

No.	Study	Location	Warfarin indication	Number of patients	Age	Genotypic frequency								
						CYP2C9 % (n)						VKORC1 -1639 % (n)		
						*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	AA	GA	GG
6	Castelan et.al (2013)	México, mestizos	-	947	58	83 % (788)	8.7 % (82)	6.8 % (64)	0.4 % (4)	0.7 % (7)	0.2 % (2)	-	-	-
7	Botton et.al (2014)	Brasil, Spanish descent	FA. PV, TE, ACV, TF	198	60.5 (28-97)	66.7 % (132)	33.3 % (66)				0 (0)	12.6 % (25)	50.5 % (100)	36.9 % (73)
8	Villegas et.al (2015)	México, mestizos	-	292	-	79 % (233)	12.9 % (38)	6.8 % (20)	0.3 % (1)	0	0	-	-	-
9	Benavides et.al (2015)	Chile	-	170	-	70.6 % (120)	17.1 % (29)	7.65 % (13)	1.17 % (2)	2.94 % (5)	0.58 % (1)	25.3 % (43)	49.4 % (84)	25.3 % (43)
10	Cifuentes et.al (2016)	Colombiamixed descent	PV, TVP, FA, EP, ACV, EC, SAFL, DV	130	66.2 (28-88)	77 % (100)	14.7 % (19)	7.7 % (10)	0.8 % (1)	0 (0)	0 (0)	35.4 % (46)	43.1 % (56)	21.5 % (28)
11	Ducongec et.al (2016)	Puerto Rico, Hispanic Caribbean	FA, TVP, EP	255 (240)	68.1 (31-94)	70 % (179)	14.1 % (36)	6.7 % (17)	0.8 (2)	2.4 % (6)	0 (0)	13 % (33)	48.6 % (124)	38.4 % (98)
	Total patients			2,830	-	1,978	370	195	34	33	5	221	607	408

Notes to table 1.

AF-Auricular Fibrillation; PV-Valve Prosthesis; TE-Thromboembolism; TF-Thrombophilia; PE-Pulmonary Embolism; EC-Coronary Disease; SAFL-Anti-Phospholipid Syndrome; DV-Ventricular Dilation; DVT-Depth Venous Thrombosis; PE-Pulmonary Embolism RVA-Arrotic Valve Replacement; TIA-Temporary Ischemic Attack; FCC-Congestive Heart Failure; BVMB-Bioprostatic Mitral Valve Replacement; MI-Myocardial Infarction; ALS-Aortic Valve Disease; CAD-Internal Carotid Arterial Stenosis.

Valentine[a]- Two patients in this study tested positive for the polymorphisms CYP2C9 *1/*5 and *2/*5 not taken into account in this study, therefore, the total population for this polymorphism was 101 patients, indicated in parentheses ( ) in the table.

Botton[b]- The data of the CYP2C gene polymorphisms reported in this study were not taken into account in the analysis because they do not specifically present the alleles found.

Duconge[c]- Fifteen patients in this study presented other polymorphisms in the CYP2C9 gene that were not taken into account in this study, therefore, the total population for this polymorphism was 240 patients, indicated in parentheses ( ) in the table.

Polymorphisms in the CYP2C9 gene

The allele frequency of CYP2C9 in the Latin American populations studied is shown in Table 1. The native allele CYP2C9*1/*1 homozygous presents a frequency between 56-83 %, being the Mexican mestizo population the one that presented the highest frequency (83%) and the Argentinean population the lowest (56%).

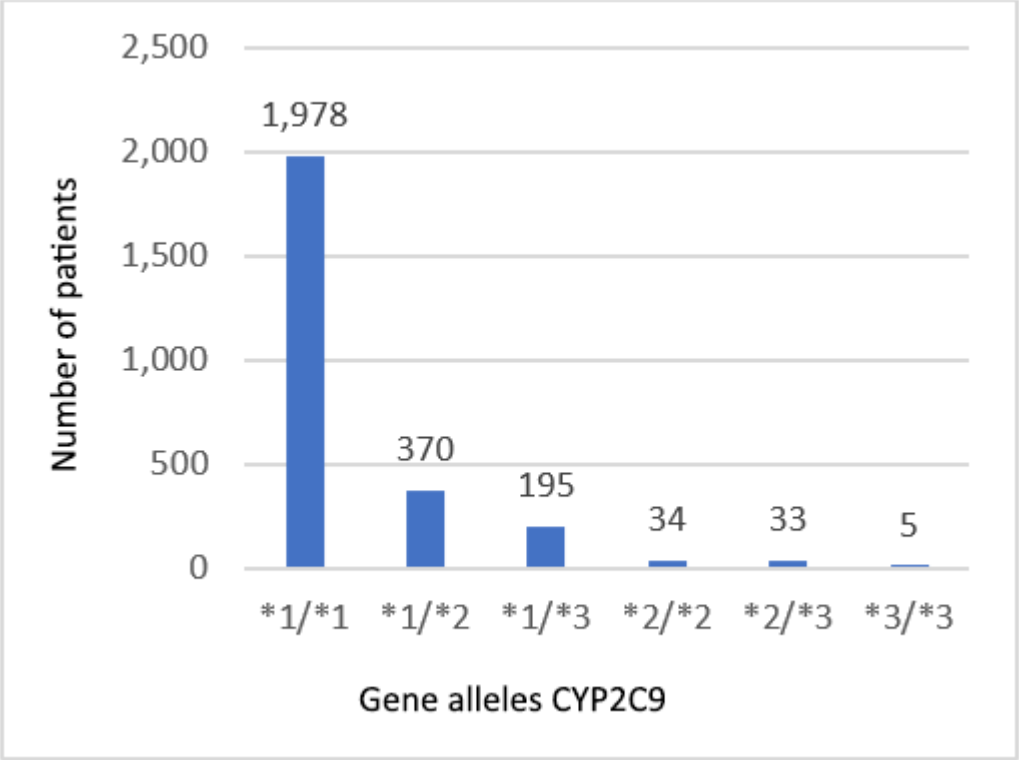
The populations of mixed origin present different frequencies in relation to the populations of Spanish origin. If the Brazilian population of mixed origin is compared with the population of Spanish origin, differences can be observed between the values of genotype frequency CYP2C9 up to twice the value, as occurs with the allele *2/*3 which presents a frequency of 0.9% and 1.8% for each population (Table 1).

The CYP2C9*1/*2 and CYP2C9*1/*3 alleles presented frequencies between 12.9 %-25 % and 4 %-10.9 % respectively. Together with the native allele (CYP2C9*1/*1) these frequency values are the highest compared to the rest of the alleles studied.

The frequency values found in this study for the CYP2C9*2/*2 and CYP2C9*2/*3 alleles range from 0.3 % to 13 % and 0.7 %-6.0 %, respectively. The homozygous allele CYP2C9*2/*2 was representative in all the studies analyzed (34 patients). The heterozygous allele CYP2C9*2/*3 was reported in nine studies with a total of 33 patients.

The *2/*2 and *2/*3 polymorphisms of patients from Argentina and Puerto Rico with mixed descent presented an atypical frequency with respect to the rest of the population studied, 13% and 6% respectively. This means that the distribution of data for these polymorphisms is not normal when applying the Anderson Darling test (Annex 1).

As for the *3/*3 polymorphism, a very low frequency was found in the population, five patients out of a total of 2,813. The frequency of distribution of polymorphic alleles of the CYP2C9 gene is shown in Graph 1.

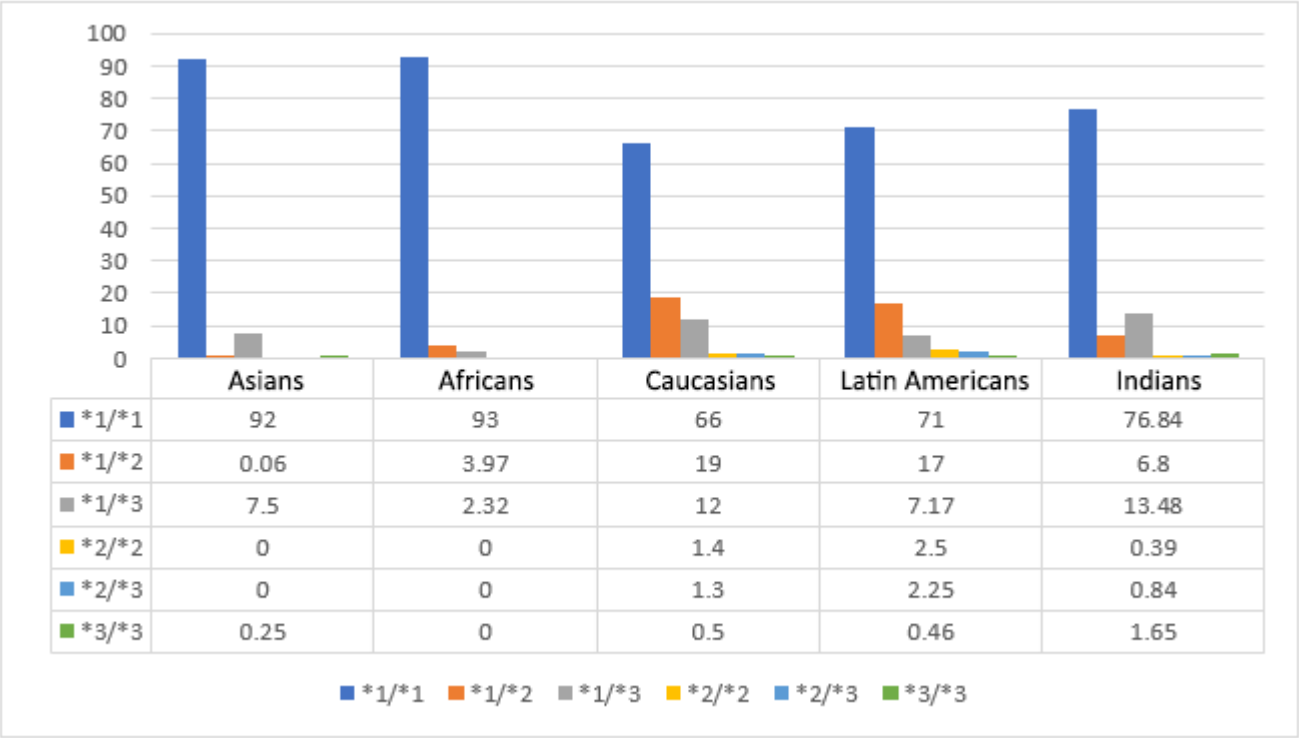


Graph 1. Frequency of polymorphisms of the CYP2C9 gene in the studied population.

The statistical analysis of the frequency of polymorphisms in the populations studied, applying Grubbs contrast to identify outliers and the Anderson Darling statistician for the normality test determined that for the alleles *1/*1, *1/*2 and *1/*3 there are no outliers and the data follow a normal distribution (Annex 1).

The comparison of the frequency of polymorphisms in the CYP2C9 genes in Latin American populations with respect to other populations in the world is shown in Graph 2. The frequency of the CYP2C9 *1/*2 and CYP2C9 *1/*3 alleles (17% and 7.17 %) in the studied population is similar to the results obtained in populations of Caucasian origin with frequencies of 19% and 12% respectively (Kliegman; Zhang, 2016).

The *2/*2 and *2/*3 variants in the studied population present a frequency of 2.5% and 2.25%, values that differ from the Asian, African, Caucasian and Indian populations, whose frequencies are in a range of 0 % to 1.4%. The variant *3/*3 is presented in a frequency similar to that observed in the Caucasian population, 0.46 % and 0.5 % respectively.



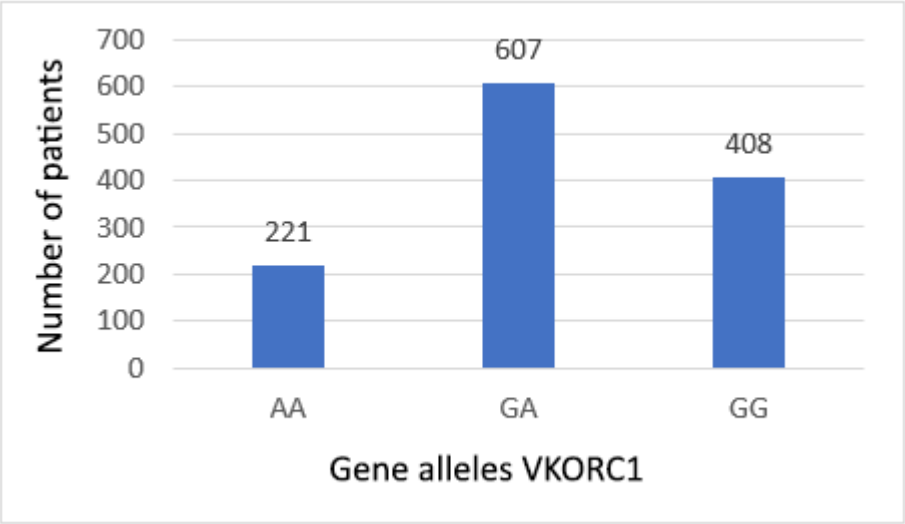
	Asians	Africans	Caucasians	Latin Americans	Indians
*1/*1	92	93	66	71	76.84
*1/*2	0.06	3.97	19	17	6.8
*1/*3	7.5	2.32	12	7.17	13.48
*2/*2	0	0	1.4	2.5	0.39
*2/*3	0	0	1.3	2.25	0.84
*3/*3	0.25	0	0.5	0.46	1.65

Graph 2. Frequency comparison of CYP2C9 genotypes in different populations.

The frequency values of the Asian, African, Caucasian and Indian populations were obtained from Zhang's study, 2016; in the case of the Asian population, the average value of the Han Chinese, Japanese and Korean populations was calculated. The data for the Latin American population are the average values of the data reported in the 11 articles under study.

Genetic polymorphisms in the VKORC1 -1639 gene

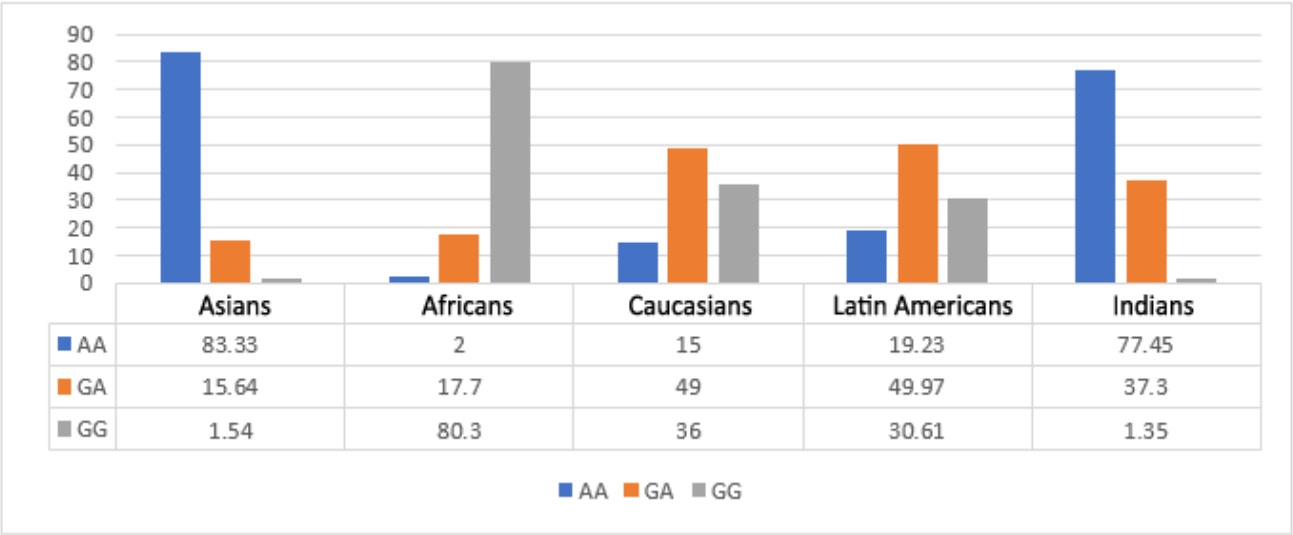
Graph 3 shows the frequencies of polymorphisms of the VKORC1 -1639 gene found in the Latin American population. *(see graph 3 on next page)*



Graph 3. Frequency of VKORC gene polymorphisms -1639

The most frequent polymorphism of the VKORC1 -1639 gene was the heterozygous GA allele with 49.11 % (607 patients), followed by the GG allele with 33 % (408 patients) and the AA allele with 17.88 % (221 patients). No abnormal data were identified in the population studied when applying the Grubbs statistic (Annex 1).

Graph 4 shows the comparison of the frequency of the GA variant of the VKORC1 -1639 gene in the population studied with the frequency observed in the population of Caucasian origin, which present very close values of 49.97 % and 49 % respectively. In relation to the frequencies of the native gene GG and the variant AA, the values of both populations are distributed in close ranges from 30 % to 36 % and 15 % to 19 % for each one. The frequencies of these variants in the Asian, African and Indian populations do not present similarities with the studied population.



	Asians	Africans	Caucasians	Latin Americans	Indians
AA	83.33	2	15	19.23	77.45
GA	15.64	17.7	49	49.97	37.3
GG	1.54	80.3	36	30.61	1.35

Graph 4. Frequency comparison of VKORC1-1639 genotypes in various populations.

One of the limitations when studying the genetic polymorphisms CYP2C9 and VKORC1 -1639 in Latin American population, in the ethnic diversity of this region. The genotypic frequencies of CYP2C9 and VKORC1 -1639 vary depending on the ethnic origin. Since the Latin American population is a mixed population that has experienced diverse migratory processes throughout its history, an increase in heterozygous character within these populations is expected. The migratory characteristics of each country have influenced the diversity of racial mixtures throughout the region.

Although articles have been published on the frequency of CYP2C9 and VKORC1 -1639 polymorphisms in Latin America, no comparative analysis of these populations with other population groups has been carried out. In that sense, this study represents the first effort to compile existing information allowing to determine the current state and identify knowledge gaps as the non-existent information on the frequency of these polymorphisms in the Central American region.

In this study it was found that the frequency of the alleles CYP2C9 *1/*2 and CYP2C9 *1/*3 in the Latin American population is similar to the results obtained in populations of Caucasian origin (Kliegman; Zhang, 2016). Limdi et al (2008) report a frequency of 19% and 12% in the Caucasian population. In the Latin American population these two variants are more frequent than the *2/*2, *2/*3 and *3/*3 variants, so they should be taken into account in pharmacogenetic analyses for the adjustment of warfarin doses.

The frequency of the *2/*2 and *2/*3 variants in the Latin American population differs from the Asian, African, Caucasian and Indian populations. According to Seng et al. (2003), Daneshjou (2013) and Gaikward et al. (2013) both the Asian and African populations have zero frequencies for the *2/*2 allele. A similar phenomenon occurs in the population of Indian origin, with frequencies of 0.39% reported. The high values of these variants reported in the Latin American population are due to frequencies of 13% for the *2/*2 allele identified in the Argentine population, 6.0% and 2.94% for the *2/*3 allele reported in the Puerto Rican (mixed descent) and Chilean populations respectively.

Since these three populations present particularly different values compared to other ethnic groups, the *2/*2 and *2/*3 variants should be evaluated in pharmacogenetic studies of the region since they may represent a specific ethnic characteristic of the Latin American population.

The homozygous variant *3/*3 has a similar mean frequency to the frequency reported in the Caucasian population. According to Zhang J., et al. (2016), patients with this rare allelic variant require much lower doses of warfarin compared to carriers of the native *1/*1 allele; if

they received the same dose they would be at greater risk of bleeding compared to carriers of the native allele.

As for the VKORC1 -1639 gene, the frequencies of the GA, AA and GG variants presented in the population coincide with the Caucasian population, however, when compared with the results obtained in Asian, African and Indian populations, important differences are observed. The Asian and Indian populations present the highest frequencies of AA and the lowest frequencies of GG located in the extremes, while the African population is the only one to present the highest frequency of GG, 2.6 times more than the Latin American population.

This analysis allows establishing a dose prediction criterion based on the close offspring of the patients assessed during the consultation. According to Flockhart D. et al (2008) patients who present phenotypic traits of African or Asian descent can be classified in individuals with low sensitivity (high frequency of GG) and high sensitivity (high frequency of AA) to warfarin, this indicates a dose increase of 35% for the first case and a dose decrease of 32% for AA carriers. This is in line with Hosseinkhani Z., et al (2018), which states that individuals with mutations (AA or AG) require lower doses of warfarin compared to those without mutations (GG).

In nine of the ten studies analyzed, the genotypic distributions for CYP2C9 and VKORC1 -1639 were in Hardy-Weinberg equilibrium within each group, detecting a significant deviation in the Argentine population, which presents an outlier value possibly due to the reduced sample size (Annex 2).

The implications of this study are focused on putting into perspective the pharmacogenetic studies carried out in the region and their contributions in reducing the gaps in knowledge regarding genetic variants to help direct the transfer of pharmacogenetic knowledge to the Latin American population that has been underestimated in global pharmacogenetic studies.

CONCLUSIONS

The frequency of allelic variants in the genes CYPC9 and VKORC1 in the Latin American population, allows establishing a starting point to indicate the genotypes that should be evaluated prior to the prescription of warfarin. Thus, the *1/*1, *1/*2, *1/*3 variants of the CYP2C9 gene and the GA, GG and AA variants of the VKORC1 -1639 gene must be evaluated in the Latin American population when presented at high frequencies. As for similarities found with other populations, the frequency of polymorphisms in the CYP2C9 and VKORC1 -1639 genes in the Latin American population shows a behavior similar to that reported in populations of Caucasian origin.

A particular characteristic was identified in the Latin American population in relation to the frequency of the *2/*2 and *2/*3 alleles. These are presented with distant frequencies in

comparison with other ethnic groups and could be a specific characteristic of this population that should be studied in greater depth in future research.

REFERENCES

Al-Eitan, L. N., Almasri, A. Y., & Khasawneh, R. H. (2018). Impact of CYP2C9 and VKORC1 Polymorphisms on Warfarin Sensitivity and Responsiveness in Jordanian Cardiovascular Patients during the Initiation Therapy. Genes, 9(12), 578. https://doi.org/10.3390/genes9120578

Arribas IA. (2010). Farmacogenética y variabilidad interindividual en la respuesta a los medicamentos. Academia de Farmacia "Reino de Aragón. http://www.academiadefarmaciadearagon.es/docs/documentos/documento21.pdf

Benavides, F., Grossman, N., Poggi, H., Nieto, E., Bertrán, A., Araos, D., Vásquez, M., Ibarra, I., Cáceres, F., Espinoza, K., Lagos, M., & Repetto M, G. (2015). Efecto de las variantes de VKORC1 y CYP2C9 sobre la dosis de anticoagulantes orales en individuos chilenos Revista medica de Chile, 143(11), 1369–1376. https://doi.org/10.4067/S0034-98872015001100001

Botton, M. R., Bandinelli, E., Rohde, L. E., Amon, L. C., & Hutz, M. H. (2011). Influence of genetic, biological and pharmacological factors on warfarin dose in a Southern Brazilian population of European ancestry. British journal of clinical pharmacology, 72(3), 442–450. https://doi.org/10.1111/j.1365-2125.2011.03942.x

Bryk, A. H., Wypasek, E., Plens, K., Awsiuk, M., & Undas, A. (2018). Bleeding predictors in patients following venous thromboembolism treated with vitamin K antagonists: Association with increased number of single nucleotide polymorphisms. Vascular pharmacology, 106, 22–27. https://doi.org/10.1016/j.vph.2018.02.002

Cabrero J. y Camacho P. Fundamentos de Genética de Poblaciones. http://sesbe.org/sites/sesbe.org/files/recursos-sesbe/fundamentos_GdeP.pdf

Castelán-Martínez, O. D., Hoyo-Vadillo, C., Sandoval-García, E., Sandoval-Ramírez, L., González-Ibarra, M., Solano-Solano, G., Gómez-Díaz, R. A., Parra, E. J., Cruz, M., & Valladares-Salgado, A. (2013). Allele frequency distribution of CYP2C9 2 and CYP2C9 3 polymorphisms in six Mexican populations. Gene, 523(2), 167–172. https://doi.org/10.1016/j.gene.2013.03.128

Cavallari, L. H., Shin, J., & Perera, M. A. (2011). Role of pharmacogenomics in the management of traditional and novel oral anticoagulants. Pharmacotherapy, 31(12), 1192–1207. https://doi.org/10.1592/phco.31.12.1192

Céspedes-Garro, C., Naranjo, M. G., Rodrigues-Soares, F., LLerena, A.,

Duconge, J., Montané-Jaime, L. K., Roblejo, H., Fariñas, H., Campos, M. L., Ramírez, R., Serrano, V., Villagrán, C. I., & Peñas-LLedó, E. M. (2016). Pharmacogenetic research activity in Central America and the Caribbean: a systematic review. Pharmacogenomics, 17(15), 1707–1724. https://doi.org/10.2217/pgs-2016-0053

Cifuentes, R., Murillo, J. y Avella, E. (2016). Predicción a la sensibilidad a la warfarina con base en polimorfismos de los genes VKORC1 y CYP2C9 en pacientes colombianos. Biomédica, 36, 91-100. https://www.revistabiomedica.org/index.php/biomedica/article/view/2795

Claudio-Campos, K., Orengo-Mercado, C., Renta, J. Y., Peguero, M., García, R., Hernández, G., Corey, S., Cadilla, C. L., & Duconge, J. (2015). Pharmacogenetics of healthy volunteers in Puerto Rico. Drug metabolism and personalized therapy, 30(4), 239–249. https://doi.org/10.1515/dmpt-2015-0021

Cullell, N., Carrera, C., Muiño, E., Torres, N., Krupinski, J., & Fernandez-Cadenas, I. (2018). Pharmacogenetic studies with oral anticoagulants. Genome-wide association studies in vitamin K antagonist and direct oral anticoagulants. Oncotarget, 9(49), 29238–29258. https://doi.org/10.18632/oncotarget.25579

Daneshjou, R., Tatonetti, N. P., Karczewski, K. J., Sagreiya, H., Bourgeois, S., Drozda, K., Burmester, J. K., Tsunoda, T., Nakamura, Y., Kubo, M., Tector, M., Limdi, N. A., Cavallari, L. H., Perera, M., Johnson, J. A., Klein, T. E., & Altman, R. B. (2013). Pathway analysis of genome-wide data improves warfarin dose prediction. BMC genomics, 14 Suppl 3(Suppl 3), S11. https://doi.org/10.1186/1471-2164-14-S3-S11

Dean, L. (2012). Warfarin Therapy and VKORC1 and CYP Genotype. In V. M. Pratt (Eds.) et. al., Medical Genetics Summaries. National Center for Biotechnology Information (US).

Dilge Taşkın, B., Kula, S., Ergün, M. A., Altun, D., Olguntürk, R., Tunaoğlu, F. S., Oğuz, A. D., & Gürsel, T. (2016). The effect of CYP2C9 and VKORC1 genetic polymorphisms on warfarin dose requirements in a pediatric population. Anatolian journal of cardiology, 16(10), 791–796. https://doi.org/10.14744/AnatolJCardiol.2015.6150

Dong, A. N., Tan, B. H., Pan, Y., & Ong, C. E. (2018). Cytochrome P450 genotype-guided drug therapies: An update on current states. Clinical and experimental pharmacology & physiology, 45(10), 991–1001. https://doi.org/10.1111/1440-1681.12978

Duconge, J., Cadilla, C. L., Windemuth, A., Kocherla, M., Gorowski, K., Seip, R. L., Bogaard, K., Renta, J. Y., Piovanetti, P., D'Agostino, D., Santiago-Borrero, P. J., & Ruaño, G. (2009). Prevalence of combinatorial CYP2C9 and VKORC1

genotypes in Puerto Ricans: implications for warfarin management in Hispanics. Ethnicity & disease, 19(4), 390–395. https://www.ncbi.nlm.nih.gov/pubmed/20073138

Flockhart, D. A., O'Kane, D., Williams, M. S., Watson, M. S., Flockhart, D. A., Gage, B., Gandolfi, R., King, R., Lyon, E., Nussbaum, R., O'Kane, D., Schulman, K., Veenstra, D., Williams, M. S., Watson, M. S., & ACMG Working Group on Pharmacogenetic Testing of CYP2C9, VKORC1 Alleles for Warfarin Use (2008). Pharmacogenetic testing of CYP2C9 and VKORC1 alleles for warfarin. Genetics in medicine : official journal of the American College of Medical Genetics, 10(2), 139–150. https://doi.org/10.1097/GIM.0b013e318163c35f

Gaikward, T., Ghosh K, Kulkarni B, Kulkarni V, Ross C. and Shetty S. (2013). Influence of CYP2C9 and VKORC1 gene polymorphism on warfarin dosage, over anticoagulation and other adverse outcomes in Indian Population. European Journal of Pharmacology, 710:80-84. https://www.sciencedirect.com/science/article/abs/pii/S0014299913002951

Galvez, J. M., Restrepo, C. M., Contreras, N. C., Alvarado, C., Calderón-Ospina, C. A., Peña, N., Cifuentes, R. A., Duarte, D., Laissue, P., & Fonseca, D. J. (2018). Creating and validating a warfarin pharmacogenetic dosing algorithm for Colombian patients. Pharmacogenomics and personalized medicine, 11, 169–178. https://doi.org/10.2147/PGPM.S170515

Golwala, H., Jackson, L. R., 2nd, Simon, D. N., Piccini, J. P., Gersh, B., Go, A. S., Hylek, E. M., Kowey, P. R., Mahaffey, K. W., Thomas, L., Fonarow, G. C., Peterson, E. D., Thomas, K. L., & Outcomes Registry for Better Informed Treatment for Atrial Fibrillation (ORBIT-AF) Registry (2016). Racial/ethnic differences in atrial fibrillation symptoms, treatment patterns, and outcomes: Insights from Outcomes Registry for Better Informed Treatment for Atrial Fibrillation Registry. American heart journal, 174, 29–36. https://doi.org/10.1016/j.ahj.2015.10.028

Guerrero, F. (2009). Farmacogenética de Warfarina. Revista Médica Sanitas, 12(2): 8-12. http://www.gbcbiotech.com/laboratorio/assets/farmacogenetica-de-warfarina.pdf

Hosseinkhani, Z., Sadeghalvad, M., Norooznezhad, F., Khodarahmi, R., Fazilati, M., Mahnam, A., Fattahi, A., & Mansouri, K. (2018). The effect of CYP2C9*2, CYP2C9*3, and VKORC1-1639 G>A polymorphism in patients under warfarin therapy in city of Kermanshah. Research in pharmaceutical sciences, 13(4), 377–384. https://doi.org/10.4103/1735-5362.235165

Isaza, C, Henaao, J. y Beltrán, L. (2010). Resistencia y Sensibilidad a Warfarina. Investigaciones ANDINA, 12(20): 100.

Kaye, J. B., Schultz, L. E., Steiner, H. E., Kittles, R. A., Cavallari, L. H., & Karnes, J. H. (2017). Warfarin Pharmacogenomics in Diverse Populations. Pharmacotherapy, 37(9), 1150–1163. https://doi.org/10.1002/phar.1982

Kliegman, R., Stanton, B., y Gene, J. Farmacogenética, farmacogenómica y el concepto de medicina personalizada. Nelson. Tratado de pediatría. Edición 19. Capítulo 56: 1-12. http://www.studentconsult.es/ficheros/booktemplate/9788480869591/files/056_0_contbb.pdf

Li, J., Wang, L., Hu, J., & Xu, G. (2015). Warfarin use and the risks of stroke and bleeding in hemodialysis patients with atrial fibrillation: A systematic review and a meta-analysis. Nutrition, metabolism, and cardiovascular diseases : NMCD, 25(8), 706–713. https://doi.org/10.1016/j.numecd.2015.03.013

Limdi, N. A., Arnett, D. K., Goldstein, J. A., Beasley, T. M., McGwin, G., Adler, B. K., & Acton, R. T. (2008). Influence of CYP2C9 and VKORC1 on warfarin dose, anticoagulation attainment and maintenance among European-Americans and African-Americans. Pharmacogenomics, 9(5), 511–526. https://doi.org/10.2217/14622416.9.5.511

Limdi, N. A., Wadelius, M., Cavallari, L., Eriksson, N., Crawford, D. C., Lee, M. T., Chen, C. H., Motsinger-Reif, A., Sagreiya, H., Liu, N., Wu, A. H., Gage, B. F., Jorgensen, A., Pirmohamed, M., Shin, J. G., Suarez-Kurtz, G., Kimmel, S. E., Johnson, J. A., Klein, T. E., Wagner, M. J., … International Warfarin Pharmacogenetics Consortium (2010). Warfarin pharmacogenetics: a single VKORC1 polymorphism is predictive of dose across 3 racial groups. Blood, 115(18), 3827–3834. https://doi.org/10.1182/blood-2009-12-255992

Liu, N., Irvin, M. R., Zhi, D., Patki, A., Beasley, T. M., Nickerson, D. A., Hill, C. E., Chen, J., Kimmel, S. E., & Limdi, N. A. (2017). Influence of common and rare genetic variation on warfarin dose among African-Americans and European-Americans using the exome array. Pharmacogenomics, 18(11), 1059–1073. https://doi.org/10.2217/pgs-2017-0046

Liu, R., Cao, J., Zhang, Q., Shi, X. M., Pan, X. D., & Dong, R. (2017). Clinical and genetic factors associated with warfarin maintenance dose in northern Chinese patients with mechanical heart valve replacement. Medicine, 96(2), e5658. https://doi.org/10.1097/MD.0000000000005658

Miklosz, J., Kalaska, B., & Mogielnicki, A. (2018). Pharmacogenetic considerations of anticoagulant medication. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society, 69(4), 10.26402/jpp.2018.4.01. https://doi.org/10.26402/jpp.2018.4.01

Miranda, C., Roco, A., Garay, J., Squicciarini, V., Tamayo, E., Agúndez, J., et.al. (2011). Determinación del polimorfismo de CYP2C9*2 y su relación con la farmacocinética de acenocumarol en voluntarios sanos. Revista Chilena de cardiología, 30, 218-224. https://scielo.conicyt.cl/pdf/rchcardiol/v30n3/art05.pdf

Moyer, T. P., O'Kane, D. J., Baudhuin, L. M., Wiley, C. L., Fortini, A., Fisher, P. K., Dupras, D. M., Chaudhry, R., Thapa, P., Zinsmeister, A. R., & Heit, J. A. (2009). Warfarin sensitivity genotyping: a review of the literature and summary of patient experience. Mayo Clinic proceedings, 84(12), 1079–1094. https://doi.org/10.4065/mcp.2009.0278

Natarajan, S., Ponde, C. K., Rajani, R. M., Jijina, F., Gursahani, R., Dhairyawan, P. P., & Ashavaid, T. F. (2013). Effect of CYP2C9 and VKORC1 genetic variations on warfarin dose requirements in Indian patients. Pharmacological reports : PR, 65(5), 1375–1382. https://doi.org/10.1016/s1734-1140(13)71496-8

Parra, E. J., Botton, M. R., Perini, J. A., Krithika, S., Bourgeois, S., Johnson, T. A., Tsunoda, T., Pirmohamed, M., Wadelius, M., Limdi, N. A., Cavallari, L. H., Burmester, J. K., Rettie, A. E., Klein, T. E., Johnson, J. A., Hutz, M. H., & Suarez-Kurtz, G. (2015). Genome-wide association study of warfarin maintenance dose in a Brazilian sample. Pharmacogenomics, 16(11), 1253–1263. https://doi.org/10.2217/PGS.15.73

Perini, J. A., Vargens, D. D., Santana, I. S., Moriguchi, E. H., Ribeiro-Dos-Santos, A. K., Tsutsumi, M., & Suarez-Kurtz, G. (2009). Pharmacogenetic polymorphisms in Brazilian-born, first-generation Japanese descendants. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas, 42(12), 1179–1184. https://doi.org/10.1590/s0100-879x2009001200010

Raggio V, Neira P, Esperón P, Lorenzo M and Stoll M. (2005). Respuesta terapéutica inadecuada a la warfarina en pacientes genéticamente susceptibles. Revista médica Uruguay, 21:242-246. http://www.scielo.edu.uy/pdf/rmu/v21n3/v21n3a11.pdf

Razavi, F. E., Zarban, A., Hajipoor, F., & Naseri, M. (2017). The allele frequency of CYP2C9 and VKORC1 in the Southern Khorasan population. Research in pharmaceutical sciences, 12(3), 211–221. https://doi.org/10.4103/1735-5362.207202

Scibona, P., Redal, M. A., Garfi, L. G., Arbelbide, J., Argibay, P. F., & Belloso, W. H. (2012). Prevalence of CYP2C9 and VKORC1 alleles in the Argentine population and implications for prescribing dosages of anticoagulants. Genetics and molecular research : GMR, 11(1), 70–76. https://doi.org/10.4238/2012.January.9.8

Seng, K., Gin, G., Sangkar, V. and Phipps, E. (2003). Frequency of Cytochrome P450 2C9 (CYP2C9) alleles in three ethnic groups in Malaysia. Asia Pacific Journal of Molecular Biology and biotechnology,11(2), 83-91

Shen, A. Y., Yao, J. F., Brar, S. S., Jorgensen, M. B., & Chen, W. (2007). Racial/ethnic differences in the risk of intracranial hemorrhage among patients with atrial fibrillation. Journal of the American College of Cardiology, 50(4), 309–315. https://doi.org/10.1016/j.jacc.2007.01.098

Tavares, L. C., Marcatto, L. R., Soares, R., Krieger, J. E., Pereira, A. C., & Santos, P. (2018). Association Between ABCB1 Polymorphism and Stable Warfarin Dose Requirements in Brazilian Patients. Frontiers in pharmacology, 9, 542. https://doi.org/10.3389/fphar.2018.00542

Tsai, C., Marcus, L. Q., Patel, P., & Battistella, M. (2017). Warfarin Use in Hemodialysis Patients With Atrial Fibrillation: A Systematic Review of Stroke and Bleeding Outcomes. Canadian journal of kidney health and disease, 4, 2054358117735532. https://doi.org/10.1177/2054358117735532

Valentin, I. I., Vazquez, J., Rivera-Miranda, G., Seip, R. L., Velez, M., Kocherla, M., Bogaard, K., Cruz-Gonzalez, I., Cadilla, C. L., Renta, J. Y., Feliu, J. F., Ramos, A. S., Alejandro-Cowan, Y., Gorowski, K., Ruaño, G., & Duconge, J. (2012). Prediction of warfarin dose reductions in Puerto Rican patients, based on combinatorial CYP2C9 and VKORC1 genotypes. The Annals of pharmacotherapy, 46(2), 208–218. https://doi.org/10.1345/aph.1Q190

Valentín, I. I., Rivera, G., Nieves-Plaza, M., Cruz, I., Renta, J. Y., Cadilla, C. L., Feliu, J. F., Seip, R. L., Ruaño, G., & Duconge, J. (2014). Pharmacogenetic association study of warfarin safety endpoints in Puerto Ricans. Puerto Rico health sciences journal, 33(3), 97–104. https://pubmed.ncbi.nlm.nih.gov/25244877/

Villagra, D., Duconge, J., Windemuth, A., Cadilla, C. L., Kocherla, M., Gorowski, K., Bogaard, K., Renta, J. Y., Cruz, I. A., Mirabal, S., Seip, R. L., & Ruaño, G. (2010). CYP2C9 and VKORC1 genotypes in Puerto Ricans: A case for admixture-matching in clinical pharmacogenetic studies. Clinica chimica acta; international journal of clinical chemistry, 411(17-18), 1306–1311. https://doi.org/10.1016/j.cca.2010.05.021

Villegas-Torres, B., Sánchez-Girón, F., Jaramillo-Villafuerte, K., Soberón, X., & Gonzalez-Covarrubias, V. (2015). Genotype frequencies of VKORC1 and CYP2C9 in native and Mestizo populations from Mexico, potential impact for coumarin dosing. Gene, 558(2), 235–240. https://doi.org/10.1016/j.gene.2014.12.068

Wattanachai, N., Kaewmoongkun, S., Pussadhamma, B., Makarawate, P., Wongvipaporn, C., Kiatchoosakun, S., Vannaprasaht, S., & Tassaneeyakul, W. (2017). The impact of non-genetic and genetic factors on a stable warfarin dose in Thai patients. European journal of clinical pharmacology, 73(8), 973–980. https://doi.org/10.1007/s00228-017-2265-8

Yoon, Y. R., Shon, J. H., Kim, M. K., Lim, Y. C., Lee, H. R., Park, J. Y., Cha, I. J., & Shin, J. G. (2001). Frequency of cytochrome P450 2C9 mutant alleles in a Korean population. British journal of clinical pharmacology, 51(3), 277–280. https://doi.org/10.1046/j.1365-2125.2001.00340.x

Zhang, J., Chen, Z., & Chen, C. (2016). Impact of CYP2C9, VKORC1 and CYP4F2 genetic polymorphisms on maintenance warfarin dosage in Han-Chinese patients: A systematic review and meta-analysis. Meta gene, 9, 197–209. https://doi.org/10.1016/j.mgene.2016.07.002

Zhang, H., Ma, K., Liu, W., Yang, F., Liu, J., & Zhou, H. (2016). Impact of CYP2C19 gene polymorphism on warfarin maintenance doses in patients with non-valvular atrial fibrillation. Gene, 591(1), 80–84. https://doi.org/10.1016/j.gene.2016.06.046

ANNEXES

Annex 1. Grubbs statistic of the frequency of polymorphisms in the CYP2C9 gene

Gene	Variable	N	Mean	Standard deviation	Minimum	Maximum	G	P
CYP2C9	1*/1*	10	0.7112	0.0892	0.5600	0.8300	1.69	0.693
	1*/2*	10	0.1696	0.0517	0.0870	0.2500	1.60	0.913
	1*/3*	10	0.06455	0.02969	0.00000	0.10900	2.17	0.101
	2*/2*	10	0.0257	0.0387	0.0030	0.1300	2.70	0.000
	2*/3*	10	0.01574	0.01861	0.00000	0.06000	2.38	0.026
	3*/3*	10	0.001380	0.002463	0.000000	0.006000	1.88	0.382
VKORC1-1639	AA	7	0.1923	0.0850	0.1260	0.3540	1.90	0.129
	GA	7	0.4997	0.0530	0.4310	0.6000	1.89	0.137
	GG	7	0.3061	0.0860	0.1900	0.4020	1.35	1.000
*P >0.05 the H_0 is accepted, there is no abnormal data presence; P<0.05 the H_0 is rejected there is an anomalous data presence.								

Annex 2. Handy-Weinberg´s balance X^2 values calculated

N	Study	Place	X^{2a}		
			CYP2C9		VKORC1 -1639
			*1/*1 *1/*2 *2/*2	*1/*1 *1/*3 *3/*3	AA GA GG
1	Perini et.al (2009)	Brasil, descendencia mixta	0.11707 <3.84 H_0 is accepted	0.2494 <3.84 H_0 is accepted	-
2	Rodrigues et.al (2011)	Brasil, descendencia española	1.0137 <3.84 H_0 is accepted	0.8546 <3.84 H_0 is accepted	0.0078 <3.84 H_0 is accepted
3	Cifuentes et.al (2016)	Colombia, descendencia mixta	0.0086 <3.84 H_0 is accepted	0.2494 <3.84 H_0 is accepted	1.9233 <3.84 H_0 is accepted

N	Study	Place	X^{2a}		
			CYP2C9		VKORC1 -1639
			*1/*1 *1/*2 *2/*2	*1/*1 *1/*3 *3/*3	AA GA GG
4	Valentin et.al (2012)	Puerto Rico, descendencia mixta	0.01260 <3.84 H_0 is accepted	0.1023 <3.84 H_0 is accepted	0.7526 <3.84 H_0 is accepted
5	Duconge et.al (2016)	Puerto Rico, hispano caribeños	0.0161 <3.84 H_0 is accepted	0.4029 <3.84 H_0 is accepted	0.4107 <3.84 H_0 is accepted
6	Castelan et.al (2013)	México, mestizos	1.3593 <3.84 H_0 is accepted	0.3348 <3.84 H_0 is accepted	-
7	Villegas et.al (2015)	México, mestizos	0.1754 <3.84 H_0 is accepted	0.4286 <3.84 H_0 is accepted	-
8	Benavides et.al (2015)	Chile	0.02713 <3.84 H_0 is accepted	0.8999 <3.84 H_0 is accepted	0.02353 <3.84 H_0 is accepted
9	Miranda et.al (2011)	Chile	1.3605 <3.84 H_0 is accepted	-	-
10	Scibonab et.al (2012)	Argentina	12.6308 > 3.84 H_0 is rejected	0.1094 <3.84 H_0 is accepted	3.5790 > 3.84 H_0 is rejected

[a] The value of X^2 was calculated with X^2 with a degree of freedom and a 0.050 level of significance of (X^2 tab=3.84). When comparing the values of X^2 calculated with X^2 tabulated the H_0 is accepted indicating that the population is in the balance of Hardy-Weinberg, for one to nine studies.

[b] The values of X^2 obtained in this research shows an atypical behavior which is probably due to the size of the sample.