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The Influence of Ethnicity on Warfarin Dosage Requirements in the Chilean Population

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ABSTRACT

Background: Vitamin K antagonists are drugs that are widely prescribed around the world and their use has helped improve the prognosis of patients with thromboembolic disease. However, a high interindividual variability has been observed in dosage requirements to reach the desired anticoagulation range that could be due to environmental and genetic factors. Studies suggest that ethnicity influences coumarin response, supporting the observed differences in dose requirements across various populations. Studies using mitochondrial DNA (mtDNA) markers have suggested that the Chilean population has a predominantly Amerindian genetic pool.
Objective: To evaluate the influence of ethnicity, defined by the presence of Amerindian mtDNA haplogroups, on the variability in therapeutic response to warfarin in the Chilean population.
Methods: A total of 191 patients treated with warfarin were included in this study. Analysis of the mitochondrial genome for detecting the presence of Amerindian mtDNA haplogroups was performed using polymerase chain reaction and polymerase chain reaction restriction fragment length polymorphism techniques. The evaluation of warfarin requirements according to each haplogroup was performed by ANOVA with a 95% CI and assuming statistical significance at $P < 0.05$.
Results: Based on the presence of an mtDNA haplogroup, 91% of the Chilean population had an Amerindian background. There were no significant differences in warfarin dosage requirements among the different Amerindian haplogroups ($P = 0.083$).
Conclusions: The presence of Amerindian mtDNA haplogroup does not influence warfarin dosage requirements in the Chilean population.

Introduction

Anticoagulant therapy has improved the prognosis of patients with a variety of conditions, including prevention and/or treatment of venous thrombosis, pulmonary embolism, and thromboembolic complications associated with atrial fibrillation and mechanical prosthetic heart valves. However, there is wide interindividual variability in dose requirements to achieve the desired anticoagulation range. A variety of both genetic and environmental factors contribute to this phenomenon. These include body weight; age; vitamin K intake; drug interactions; allelic variants in cytochrome P450 2C9 and vitamin K epoxide reductase complex, subunit 1 (VKORC1); and ethnicity. Several studies have shown the influence of cytochrome P450 2C9 and VKORC1 variability in dosage requirements, explaining a significant proportion of variation in drug response.[1] In Latin American populations, there is little evidence regarding the frequency of occurrence of the polymorphisms and variability in the response to oral anticoagulant therapy. Guzman et al[2] provide some background in the Chilean population when they describe the frequency of variant VKORC1 1639 G > A, associating with lower requirements of warfarin, but this association was not observed for genotype CYP2C9*2. In addition, factors such as age and body weight independently influence dose requirements. Furthermore, several

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Table 1
Clinical and demographic characteristics of Chilean patients treated with warfarin.

Characteristic	Men (n = 77)	Women (n = 114)
Age, y*	65 (13.0)	66 (13.4)
Height, m*	1.70 (0.0)	1.56 (0.0)
Weight, kg*	79.6 (15.3)	68 (14.2)
Hypertension†	47 (61)	66 (57.9)
Diabetes mellitus†	11 (14,2)	10 (8.7)
Dyslipidemia†	12 (15.5)	26 (22.8)

* Values are given as mean (SD).
† Values are given as n (%).

studies suggest that ethnicity is an important factor that may influence the dosage requirements of the antagonists of vitamin K. A clear example of this is the lower requirements of coumarin in Asian patients (between 30% and 40%) compared with white populations,[3] a factor that could influence dose requirements of antagonists of vitamin K.[4] Because of the value that has been given to ethnicity as a factor that might influence dosage requirements of coumarin, mitochondrial DNA (mtDNA) is an appropriate tool to assess this variable, given its unique features. It has a high rate of variation in sequence (5–10 times greater than that in nuclear DNA) and it behaves as haploid gene inheritance via maternal parent over generations and not does not undergo genetic recombination. Its sequence has been completely known since 1981; it comprises 16,569 base pairs in 37 gene segments. The mtDNA noncoding is concentrated in the area called loop D, with approximately 1,100 base pairs, which shows a high degree of polymorphism in sequence.[5,6]

Available scientific evidence suggests that ethnicity is a determining factor in oral anticoagulant dosage requirements.[7] For example, Asian populations show requirements between 30% and 40% lower than white populations, whereas African populations require significantly higher dosages than white populations.[8]

Studies on population genetics show that the Chilean population has a mainly Amerindian genetic background, determined by using mitochondrial genome markers named Amerindian mtDNA haplogroups.[9] Interestingly, in a recent study conducted on patients treated with atorvastatin, a high frequency of Amerindian mtDNA haplogroups was described, showing that haplogroup B individuals had higher levels of total cholesterol after treatment,[10] suggesting that the presence of this mitochondrial genome marker may define a lower response to treatment using this lipid-lowering drug.

According to the evidence that shows the mainly Amerindian component of the Chilean population and the absence of evidence that shows the influence of ethnicity on warfarin response in the Amerindian population, the aim of our study was to evaluate the potential association between the presence of Amerindian mtDNA haplogroups and the variability in warfarin dosage requirements.

Materials and Methods

Population under study

A total of 191 patients older than age 18 years (77 men with a mean [SD] age of 65 [13] years and 114 women with a mean [SD] age of 66 [13] years) and being treated at 2 medical centers in the cities of Santiago and Temuco, Chile, were included in the study. The group was patients receiving oral anticoagulant treatment using warfarin with a low international normalized ratio (between 2 and 3) in at least the previous 2 medical checks. Patients younger than age 18 years and patients with chronic liver disease were excluded from the study.

All patients included in the study signed an informed consent form, in accordance with the basic criteria for biomedical research listed in the Declaration of Helsinki. After signing the informed consent form, each patient was surveyed to gather relevant clinical epidemiologic information for the study, as well as to measure anthropometric parameters such as weight and size to calculate body mass index.

Amerindian mtDNA haplogroup characterization

Venous blood anticoagulated with EDTA was extracted using standard methods and the genomic DNA was extracted using whole blood using the standardized methodology described by Salazar et al.[11]

The Amerindian mtDNA haplogroup characterization was conducted in accordance with the method described.[12] Amplification reactions were carried out in a final volume of 25 μL with 50 ng genomic DNA, 100 mM of each primer, 200 mM of each of the 4 deoxyribonucleotides, 1 unit of Taq DNA polymerase, and polymerase chain reaction buffer (50 mM potassium chloride, 2 mM magnesium chloride, 20 mM ammonium sulfate, and 75 mM Tris-hydrogen chloride; pH 9.0). To determine presence or absence of the 3 polymorphic restriction sites typical in the haplogroups A, C, and D of the Amerindian population, digestion with specific endonucleases *Hae III, Hinc II*, and *Alu I*, respectively, was conducted.

Finally, the restriction fragments were evaluated by electrophoresis in 3% agarose gel stained with GelRed (Biotium Inc, Hayward, California). Regarding the marker that defines the B haplogroup and consists of deleting 1 of the 2 9-base pair repetitions located in the V intergenic region, visualization was conducted by electrophoresis in 3% agarose gel directly from the polymerase chain reaction products obtained. All the gels were analyzed by 2 people and 20% of the analyses were randomly repeated for internal control measures.

Statistical Analysis

Amerindian mtDNA haplogroup frequencies were estimated/calculated directly. To evaluate the differences in warfarin dosage requirements considering the presence of an Amerindian mtDNA haplogroup, an ANOVA test was conducted, with a 95% CI and considering a statistical significance value of $P < 0.05$.

Results

Clinical and demographic characteristics of the patients included in this study are shown in **Table 1**. The mean age of the patients evaluated was similar between men and women, at 66 years and 65 years, respectively. Also it should be noted that of the

Table 2
Amerindian mitochondrial DNA haplogroup frequency in patients treated with warfarin.

	Patient	
Haplogroup	n	%
A	14	7.3
B	60	31.4
C	56	29.3
D	44	23.1
NA	17	8.9
Total	191	100

NA = Not Amerindian.

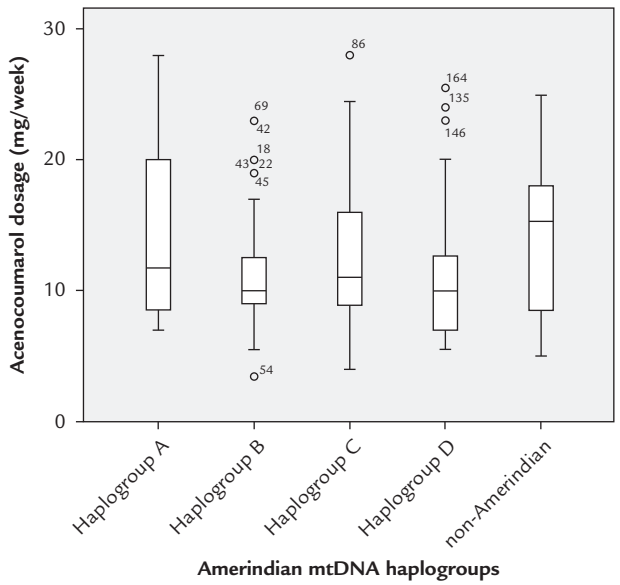

Fig. Warfarin (Acenocoumarol) dosage according to Amerindian mitochondrial DNA haplogroups in Chilean patients treated with an oral anticoagulant. The horizontal line shows the mean, the box covers the percentiles 25% to 75%, and the interquartile range. The vertical line above and below the boxes shows the minimum and maximum values. Each atypical value is shown inside a circle. The warfarin dosages were not significantly different among the Amerindian mitochondrial DNA haplogroups.

surveyed chronic diseases the most predominant in both groups was high blood pressure.

Frequency distribution Amerindian haplogroups in patients treated with warfarin is shown in **Table 2**. In general terms, the most prevalent haplogroups—B and C—are found in 31% and 29.3% of the population, respectively; 91% of the population included in this study showed 1 of the 4 Amerindian mtDNA haplogroups described.

Warfarin dosage requirements according to the presence of the different Amerindian mtDNA haplogroups are shown in the **Figure**. Results show that there were no significant differences in warfarin dosage requirements according to the presence of 1 of the 4 Amerindian haplogroups analyzed ($P = 0.083$).

Discussion

Coumarins are widely prescribed around the world, showing a significant variability in therapeutic response as a consequence of genetic and environmental factors. In our study, we evaluated the potential influence of ethnicity, defined by population markers of the mitochondrial genome, on warfarin dosage requirements in Chilean patients being treated at 2 public hospitals in the cities of Santiago and Temuco.

Results show that the Amerindian component is predominant in the population that was included in our study, which correlates with previous data that show a high frequency of Amerindian mtDNA haplogroups in Chile.[10,12,13] Furthermore, the distribution of mtDNA haplogroups in the population is not homogenous; the frequency of distribution of haplogroups C and D progressively increases toward the south of the country.

When we evaluated the warfarin dosage requirements of any of the Amerindian haplogroups, no significant differences in dosage requirements among the different Amerindian haplogroups was observed. In this sense, the presence of any Amerindian mtDNA haplogroup does not independently influence warfarin dosage requirements in the Chilean population. Ours is the first study to evaluate the potential association between the presence of mtDNA haplogroups and therapeutic response to coumarins in a population with an Amerindian genetic background.

Although our results show that the presence of a specific ethnic marker of the mitochondrial genome does not influence warfarin dosage requirements in the Chilean population, they do suggest that ethnicity could define different frequencies for certain polymorphisms, which would at least partly explain the variability in vitamin K antagonist requirements observed in various populations around the world.

Recently, Scott et al[14] provided evidence of the global difference in the frequency of variants in *VKORC1,* showing a significant difference among the populations studied ($P < 0.0001$). Similarly, in a study that included individuals from various ethnic backgrounds, the allele frequency of the *VKORC1 -1639 G > A* variant was 0.108, 0.667, 0.406, 0.436, and 0.467 in the African American, Asian, white, Hispanic, and Jewish populations, respectively.[15] **Table 3** shows the population differences observed for single-nucleotide polymorphisms and haplotypes classically described in the *VKORC1* gene.

We have recently shown that the *VKORC1 -1639 G > A* allele variant has an increased frequency (0.52) in the Chilean population, showing that this genetic marker defines significantly lower requirements of warfarin.[16] Based on this, it is possible to suggest that the presence of dissimilar variant frequencies that significantly influence coumarin dosage requirements could partly explain the variability observed in the therapeutic response to vitamin K antagonists in various populations. In this sense, the increased frequency of the *VKORC1* – 1639 G > A observed in a population with an Amerindian genetic background could explain the lower warfarin dosage requirements in the Chilean population.

Table 3
Allele frequency of single-nucleotide polymorphism and haplotypes in the *VKORC1* gene in different populations around the world.

Variant	Population	n	Allele frequency	Reference
VKORC1 -1639 G > A rs17878363	African American	600	0.108	Scott et al[14]
	Asian	204	0.667	Scott et al[14]
	White	212	0.406	Scott et al[14]
	Hispanic	202	0.436	Scott et al[14]
	Chilean		0.52	Guzmán et al[2]
VKORC1 1173 C > T rs9934438	Asian	132	0.848	Nakai et al[18]
		24	0.958	Geisen et al[17]
	European	200	0.415	Geisen et al[17]
	African	23	0.130	Geisen et al[17]
Group A Haplotype (H1, H2)	European	119	0.37	Rieder et al[19]
	African	96	0.14	Rieder et al[19]
	Asian	120	0.89	Rieder et al[19]
Group B Haplotype (H7, H8, H9)	European	119	0.58	Rieder et al[19]
	Africana	96	0.46	Rieder et al[19]
	Asian	120	0.10	Rieder et al[19]

This could be extrapolated to other Latin American populations that share this characteristic.

Conclusions

Results of our study show that there is an increased frequency of Amerindian mtDNA haplogroups in the population included in this study, which shows the predominance of this genetic background in the Chilean population. However, the variation of the Amerindian mtDNA haplogroups does not influence the warfarin dosage requirements in the group studied.

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Valeska Subiabre were responsible for literature search, figure creation, data collection, study design, data interpretation, writing; Neftali Guzman and Ivan Palomo were responsible for study design, data interpretation, writing; Luis Gonzalez, Eduardo Retamal and Hugo Henriquez were responsible for data collection.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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