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ORIGINAL ARTICLE

The Effect of Demographic Factors and VKORC1 1639 G>A Genotypes on Estimated Warfarin Maintenance Dose in Iranian Patients Under Warfarin Therapy

Fariba Rad[1] · Mohsen Hamidpour[2] · Akbar Dorgalaleh[3,4] · Behzad Poopak[5]

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Abstract Warfarin is an anticoagulant that inhibits vitamin K-dependent clotting factors including factor (F) II, FVII, FIX and FX. Different factors can change the effect of this anticoagulant in clinic. Therefore we assessed impact of VKORC1 -1639 G>A polymorphism and demographic factors on required maintenance dose in Iranian patients under warfarin therapy. The study population included 95 patients with a mean age of 61.3 ± 12.6 years. Target INR range of 2–3 was considered for these patients. The frequency of VKORC1 -1639 G>A polymorphism was assessed by polymerase chain reaction-restriction length polymorphism (PCR-RFLP). Finally the obtain data were analyzed by SPSS software. Our study revealed that 30.5%, 49.5%, and 20% of the patients had VKORC1 (G/G), (G/A), and (A/A) genotypes, respectively. Carriers of VKORC1 G/G genotype required a higher warfarin dose as compared to A/A carriers (4.48 ± 1.32 and 2.7 ± 1.16 mg/day, respectively; $P < 0.01$). In addition, patients with higher age required lower warfarin therapeutic dose ($r = -0.3$, $P < 0.01$). It seems that -1639 G>A polymorphism and demographic variables had significant effects on warfarin maintenance dose in Iranian patients under warfarin therapy.

Keywords Warfarin · Vitamin K epoxide reductase complex subunit 1 · Bleeding · Thrombosis

✉ Behzad Poopak
bpoopak@yahoo.com

Fariba Rad
fariba_rad23@yahoo.com

Mohsen Hamidpour
mohsenhp@sbmu.ac.ir

Akbar Dorgalaleh
dorgalaleha@gmail.com

[1] Cellular and Molecular Research Center, Yasuj University of Medical Sciences, Yasuj, Iran

[2] Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Department of Hematology and Blood Transfusion, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran

[4] Iranian Comprehensive Hemophilia Center, Tehran, Iran

[5] School of Paramedicine, Tehran Medical Branch of Islamic Azad University, P.O. Box 19395-1495, Tehran, Iran

Introduction

Warfarin is one of the most widely used oral anticoagulant. It is prescribed for prevention of thromboembolism in patients with deep vein thrombosis, atrial fibrillation, atrial cardiac arrhythmias, heart valve disease, prosthetic heart valve replacement and orthopedic patients [1].

Warfarin is a racemic mixture and the asymmetric carbon of warfarin (C9) produces two enantiomeric forms: R-warfarin and S-warfarin. Available warfarin consists of 50% R-warfarin and 50% S-warfarin which are differentially metabolized [2, 3]. The anticoagulant activity of R-warfarin is 3–5 times less than S-warfarin [3, 4].

Gamma-carboxylation of vitamin K-dependent clotting factors (factor (F) II, FVII, FIX and FX) is essential for proper blood clotting process. The reduced form of vitamin K and oxygen are required for gamma-carboxylase activity in order to add a carbon dioxide molecule to the side chain of glutamic-acid in vitamin K-dependent clotting factors. During carboxylation, the reduced vitamin K is oxidized to vitamin K 2, 3-epoxide from. The reduced vitamin K is

regenerated by vitamin K epoxide reductase to be used in another cycle of above pathway. Warfarin with inhibition of vitamin K epoxide reductase, interferes with gamma-carboxylation of vitamin K-dependent clotting factors [5, 6]. Polymorphism of -1639 (-1639 G>A) in vitamin K epoxide reductase complex subunit 1 (*VKORC1*) gene promoter is associated with decreased warfarin dosing in white and Asian populations [7]. The clinical and environmental factors including body weight, age, diet, drug, vitamin K intake, and social habits such as smoking or alcohol consumption have effects on warfarin dose requirement [8–10]. Determination of precise warfarin dose for each patient is crucial, because an under-dosing result in thrombosis and over-dosing is associated with bleeding. It has been reported that the risk of severe bleeding during warfarin therapy ranges from ~ 1 to ~ 4% [11]. International normalized ratio (INR) is a laboratory index for assessment the efficacy and safety of warfarin therapy; however, it has only 69% success rate in predicting the correct warfarin maintenance dose. Based on underling disorder, the INR has kept within the 2–3. If INR be higher than upper limit of the therapeutic range, the risk of hemorrhage would be increased, whereas lower than treatment range is associated with thrombosis risk [12–15]. In 2007, the Food and Drug Administration (FDA) approved genetic testing for determination of warfarin dose and initiated the update of warfarin labeling with genetic information [16]. Several studies have shown that *VKORC1* genotypes can affect warfarin therapeutic dose. Considering importance of *VKORC1* genotyping in deterring warfarin dosage, FDA has recommended the analysis labeling of *VKORC1* in patients to reduce the risk of warfarin therapy [17–20]. The polymorphisms in *VKORC1* gene resulted in elevated plasma warfarin level; therefore, patients who have at least one mutant allele should take a lower dose in comparison to patients with *VKORC1* gene wild type (WT) genotype [21].

In the present study we assessed effect of *VKORC1* 1639 G>A gene polymorphism on warfarin dose requirement in patients under warfarin therapy.

Patients and Methods

The study was conducted on 95 Iranian patients under warfarin therapy at the Moddares Hospital. All patients were received warfarin therapy to achieve an INR of 2–3. The study was approved by Shahid Beheshti University of Medical Sciences ethical committee and a written consent was obtained from all participants in the study. Initially all patients were interviewed by an expert in order to obtain clinical and demographic information such as age, gender, dietary habit (including the use of nutritional supplements, alcohol, and coffee consumption) and weight. Patients taking medications that interfere with warfarin were excluded from the study. Then, 8 mL of peripheral blood was collected into K_2EDTA and sodium citrate for molecular and coagulation studies. Genomic DNA was extracted from white blood cells by genomic DNA extraction kit (Fermentas, life Sciences). Quantity and quality of extracted DNA were determined by BioPhotometer (Eppendorf, Hamburg, Germany) and gel agarose, respectively. Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) was used to detect the VKORC1 1639 G>A polymorphism as described previously [1]. PCR reactions were done in a final volume of 25, 2.5 μL PCR buffer 10X (Genet bio), $MgCl_2$ 0.25 mM (Genet bio), 0.5 μL deoxynucleoside triphosphate (dNTP) (Genet bio), specific forward and reverse primers 1 μL (Macrogen), 1U Taq DNA polymerase (Genet bio), 100 ng of genomic DNA. The region containing VKORC1 1639 G>A polymorphism was amplified by: forward 5′ GCCAGCAGGAGAGGGAAATA 3′ and reverse 5′ AGTTTGGACTACAGGTGCCT 3′ primers. The amplified PCR products were digested by 1U Msp1 restriction enzyme (Thermo Fisher Scientific Inc., USA). PCR products were electrophoresed on a 6.5% polyacrylamide gel and stained with silver nitrate ($AgNO_3$). The PCR product sizes after restriction enzyme digestion were 123 bp and 167 base pairs (bp).

INR was calculated by the following formula.

$$\mathrm{INR} = \left(\frac{\mathrm{PT\ patient}}{\mathrm{PT\ \ control}}\right)^{\mathrm{International\ normalized\ index\ (ISI)}}$$

Results were reported as mean ± standard deviation (SD) for quantitative variables and percentages for categorical variables. Unpaired t-test and regression were used for statistical analysis/used. Statistical significance was based on two-sided design-based tests evaluated at the 0.05 level of significance. All the statistical analyses were performed by SPSS software.

Results

The study population included 95 patients with mean age of 61.3 ± 12.6 years. 47.3% (n = 45) and 52.7% (n = 50) of them were female and male, respectively. The mean weight was 68.8 ± 11.3 kg. Heart attack and stroke as well as hypertension are the main causes of warfarin therapy in the study population (Table 1).

Target range 2–3 INR was considered for these patients. The daily dose of warfarin administered was 3.84 ± 1.72 mg (minimum 1.25, maximum 10 mg), which achieved a stable anticoagulant effect following long-time

Table 1 Indications for warfarin therapy in the study population (n = 95)

Indications	Mean value
Atrial fibrillation	4.2% (n = 4)
Heart attack and stroke	25.26% (n = 24)
Heart valve replacement	23.16% (n = 22)
Hypertension	25.26% (n = 24)
Others	22.1% (n = 21)

warfarin therapy at the department of cardiovascular Medicine, at the Moddares hospital.

Although there is no correlation between warfarin dose and patients sex ($P = 0.06$), women required a higher warfarin dose (4.2 ± 1.9 vs 3.4 ± 1.4 mg/day, respectively). Simple correlation analysis of the data revealed that warfarin dose was negatively correlated with age ($r = -0.35$, $P < 0.001$) and younger patients required higher daily warfarin dosages than older patients (Fig. 1). Meanwhile, our data showed that weight significantly increases warfarin dose requirement ($r = 0.4$, $P < 0.001$) (Fig. 2).

VKORC1 1639 G>A genotype results were shown in Fig. 3. 30.53, 49.47 and 20% of the patients had WT *VKORC1* (G/G), (G/A), and (A/A) genotypes, respectively. Carriers of WT *VKORC1* (G/G) required a higher warfarin dose in comparison with (A/A) carriers (4.48 ± 1.32 mg/day vs 2.7 ± 1.16 mg, respectively; $P < 0.01$). The daily administered dose of warfarin and mean INR values in our patients are shown in Table 2.

Based on age, the patients have been categorized into 3 groups (< 50, 50–60 and > 60 years) and the relationship between each age group with mutation types of *VKORC1* have been studied. There is no significant relationship between them, but it has observed that warfarin dose requirement for having INR 2–3 is more in < 50 years group and in > 60 is less than others.

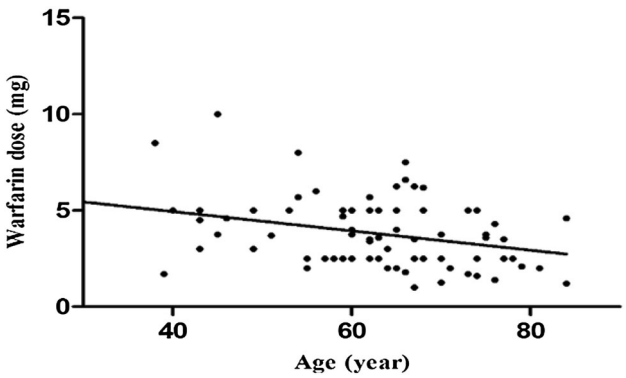

Fig. 1 Correlation plots of the statistical analysis between warfarin dose and age. Our data revealed that warfarin dose was negatively correlated with age ($r = -0.35$, $P < 0.001$) and younger patients required higher daily warfarin dosages than older patients

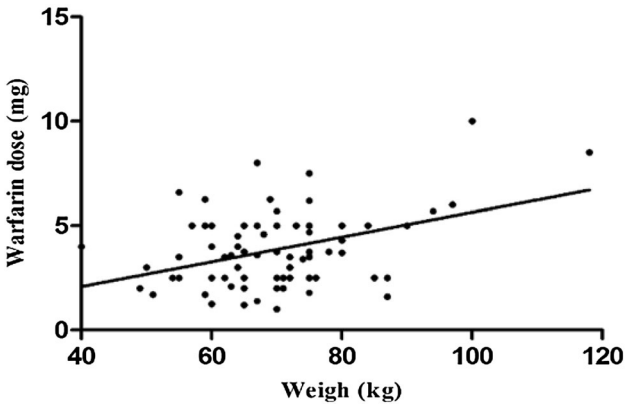

Fig. 2 Correlation plots of the statistical analysis between warfarin dose and weight. Simple correlation analysis of the data revealed that weight significantly increases warfarin dose requirement ($r = 0.4$, $P < 0.001$)

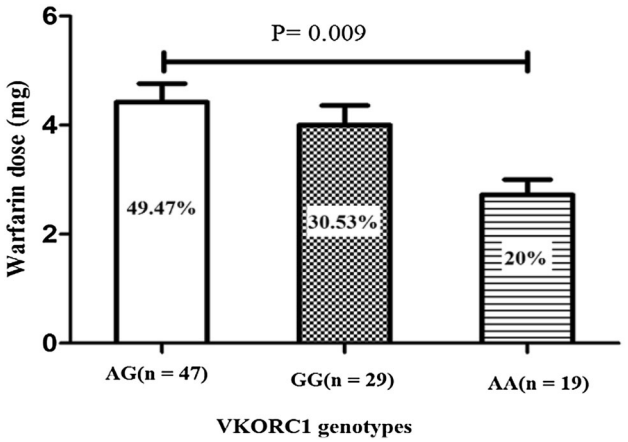

Fig. 3 Distribution of *VKORC1* 1639 G>A genotypes in the study population

Table 2 The daily administered dose of warfarin and average of INR in the study population with different VKORC1 genotypes

VKORC1 genotype	Warfarin dose (mg/day)	INR
GG	4.48 ± 1.32	2.13
GA	4.01 ± 1.95	2.41
AA	2.7 ± 1.16	2.02

Discussion

Bleeding is a common side effect of warfarin therapy, especially during the initiation phase of treatment. Warfarin dosing is a challenging process; because lower and

higher doses can be accompanied by thrombosis and bleeding risk, respectively. The appropriate warfarin dose may be varies from patient to patient and can be affected by several factors including sex, age and weight as well as dietary intake and genetic factors [14, 21, 22].

In the current study, the relationship between age, gender and weight with warfarin dose was investigated. In addition, the effect of *VKORC1* 1639 G>A gene polymorphism on warfarin dose was assessed.

We found that there is no statistically significant relationship between gender and warfarin dose, however, our result showed that women required a higher daily dose than men to obtain a therapeutic INR (4.2 ± 1.9 vs 3.4 ± 1.4 mg/day, respectively). While, it was same for both in other studies that showed a strong relationship between warfarin dose and gender [23–25].

Age and weight were the only demographic factors that significantly affected warfarin dosage ($P < 0.001$). There is a negative correlation between warfarin does and age ($r = -35$, $P < 0.001$). In other hand, the older patients required lower daily warfarin dose than younger ones. Our data were in agreement with a number of studies. The same results have been reported by Whitley et al., Garcia et al., and Khoury and Sheikh-Taha [8, 23, 24, 26, 27]. Wynne et al. [26] in their study on 104 patients, reported similar results. They indicated an inverse relationship between warfarin dosage and age at the beginning of treatment ($r = -0.30$, $P = 0.002$) [30].

Our data indicated a positive relationship between weight and warfarin dose ($r = 0.4$, $P < 0.001$). While, Blann et al. [27] found no correlation between weight and warfarin dose.

Genetic variations are the most important factors that can affect warfarin therapeutic dose. Several studies have shown that VKORC1 genotype is an important factor that can affect the warfarin dose. The pattern of gene polymorphism can be varied in different populations. *VKORC1* -1639 G>A, and 1173 C>T gene polymorphisms are related to variation in warfarin dose in different ethnic groups [8, 21, 22, 24].

In our study, VKORC1 G/A genotype was higher than others. This result was similar with other studies in UK, French, and Iran, but differs from the African-American, Chinese populations and a part of Iranian population [7, 28, 29]. In those Iranian patients, A/A (51%) genotype was the most common and this different with our population may be due to different in ethnicity of study population.

Higher frequency of the WT genotype of VKORC1 1639 G>A polymorphism was observed among African-Americans (73%) compared to Caucasians and Asians. Therefore, VKORC1 1639 G>A polymorphism is relatively common in African-Americans and Chinese people. The frequency of *VKORC1* 1639 G>A genotypes in the UK and European populations is relatively similar to our study population [7, 28, 30–32].

As previously mentioned, our patients with the (A/A) allele required the lowest warfarin dose than heterozygous carriers (G/A) and homozygous carriers (G/G). This is probably due to negative effect A allele on rate of VKORC1 enzyme synthesis [15, 28, 33].

Moreover, an association between VKORC1 genotype and warfarin dosage also was observed in our patients. Carriers of WT VKORC1 (G/G) required a higher warfarin dose as compared to (A/A) carriers.

Our study showed that VKORC1 genotype and somewhat age and weight are important factors and can affect warfarin dose. The diversity in therapeutic dosage of warfarin and the related adverse consequences partly is due to difference in this important gene polymorphism. Therefore VKORC1 1639 G>A genotyping can be performed previous to beginning of warfarin therapy in order to determine a precise and safe therapeutic dose.

Conclusions

It seems that demographic variables and VKORC1 1639 G>A genotypes can be used for determination of a safe dose in Iranian patients under warfarin therapy.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicting interests. Designed the research, analyzed the data, performed the research and wrote the paper, review and final approval of the version to be published.

References

1. Sconce EA, Khan TI, Wynne HA, Avery P, Monkhouse L, King BP et al (2005) The impact of CYP2C9 and VKORC1 genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. Blood 106(7):2329–2333
2. Juurlink DN (2007) Drug interactions with warfarin: what clinicians need to know. Can Med Assoc J 177(4):369–371
3. Zuo Z, Wo SK, Lo CM, Zhou L, Cheng G, You JH (2010) Simultaneous measurement of S-warfarin, R-warfarin, S-7-hydroxywarfarin and R-7-hydroxywarfarin in human plasma by liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal 52(2):305–310
4. Radwan MA, Bawazeer GA, Aloudah NM, AlQuadeib BT, Aboul-Enein HY (2012) Determination of free and total warfarin concentrations in plasma using UPLC MS/MS and its application to a patient samples. Biomed Chromatogr 26(1):6–11

5. Schurgers LJ, Teunissen KJ, Hamulyák K, Knapen MH, Vik H, Vermeer C (2007) Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. Blood 109(8):3279–3283
6. Li T, Chang C-Y, Jin D-Y, Lin P-J, Khvorova A, Stafford DW (2004) Identification of the gene for vitamin K epoxide reductase. Nature 427(6974):541–544
7. Takahashi H, Wilkinson GR, Nutescu EA, Morita T, Ritchie MD, Scordo MG et al (2006) Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra-and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans. Pharmacogenet Genomics 16(2):101–110
8. Whitley HP, Fermo JD, Chumney EC, Brzezinski WA (2007) Effect of patient-specific factors on weekly warfarin dose. Ther Clin Risk Manag 3(3):499
9. Dang M-TN, Hambleton J, Kayser SR (2005) The influence of ethnicity on warfarin dosage requirement. Ann Pharmacother 39(6):1008–1012
10. Walenga J, Adiguzel C (2010) Drug and dietary interactions of the new and emerging oral anticoagulants. Int J Clin Pract 64(7):956–967
11. White RH, Beyth RJ, Zhou H, Romano PS (1999) Major bleeding after hospitalization for deep-venous thrombosis. Am J Med 107(5):414–424
12. Flockhart DA, O'Kane D, Williams MS, Watson MS, Gage B, Gandolfi R et al (2008) Pharmacogenetic testing of CYP2C9 and VKORC1 alleles for warfarin. Genet Med 10(2):139–150
13. Morgan CL, McEwan P, Tukiendorf A, Robinson PA, Clemens A, Plumb JM (2009) Warfarin treatment in patients with atrial fibrillation: observing outcomes associated with varying levels of INR control. Thromb Res 124(1):37–41
14. Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S et al (2009) The largest prospective warfarin-treated cohort supports genetic forecasting. Blood 113(4):784–792
15. Schwarz UI, Ritchie MD, Bradford Y, Li C, Dudek SM, Frye-Anderson A et al (2008) Genetic determinants of response to warfarin during initial anticoagulation. N Engl J Med 358(10):999–1008
16. Lesko L (2007) Personalized medicine: elusive dream or imminent reality? Clin Pharmacol Ther 81(6):807–816
17. Rost S, Fregin A, Ivaskevicius V, Conzelmann E, Hörtnagel K, Pelz H-J et al (2004) Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature 427(6974):537–541
18. D'Andrea G, D'Ambrosio RL, Di Perna P, Chetta M, Santacroce R, Brancaccio V et al (2005) A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Blood 105(2):645–649
19. Fa Takeuchi, McGinnis R, Bourgeois S, Barnes C, Eriksson N, Soranzo N et al (2009) A genome-wide association study confirms VKORC1, CYP2C9, and CYP4F2 as principal genetic determinants of warfarin dose. PLoS Genet 5(3):e1000433
20. Huang S-W, Chen H-S, Wang X-Q, Huang L, Xu D-L, Hu X-J et al (2009) Validation of VKORC1 and CYP2C9 genotypes on interindividual warfarin maintenance dose: a prospective study in Chinese patients. Pharmacogenet Genomics 19(3):226–234
21. Zhu Y, Shennan M, Reynolds KK, Johnson NA, Herrnberger MR, Valdes R et al (2007) Estimation of warfarin maintenance dose based on VKORC1 (-1639 G>A) and CYP2C9 genotypes. Clin Chem 53(7):1199–1205
22. Duconge J, Cadilla CL, Windemuth A, Kocherla M, Gorowski K, Seip RL et al (2009) Prevalence of combinatorial CYP2C9 and VKORC1 genotypes in Puerto Ricans: implications for warfarin management in Hispanics. Ethn Dis 19(4):390
23. Khoury G, Sheikh-Taha M (2014) effect of age and sex on warfarin dosing. Clin Pharmacol Adv Appl 6:103
24. Garcia D, Regan S, Crowther M, Hughes RA, Hylek EM (2005) Warfarin maintenance dosing patterns in clinical practice: implications for safer anticoagulation in the elderly population. Chest J 127(6):2049–2056
25. Yildirim E, Erol K, Birdane A (2014) Warfarin dose requirement in Turkish patients: the influences of patient characteristics and polymorphisms in CYP2C9, VKORC1 and factor VII. Hippokratia 18(4):319–327
26. Wynne H, Kamali F, Edwards C, Long A, Kelly P (1996) Effect of ageing upon warfarin dose requirements: a longitudinal study. Age Ageing 25(6):429–431
27. Blann A, Hewitt J, Siddiqui F, Bareford D (1999) Racial background is a determinant of average warfarin dose required to maintain the INR between 2.0 and 3.0. Br J Haematol 107(1):207–209
28. Azarpira N, Namazi S, Hendijani F, Banan M, Darai M (2010) Investigation of allele and genotype frequencies of CYP2C9, CYP2C19 and VKORC1 in Iran. Pharmacol Rep 62(4):740–746
29. Samiee SM, Yeganeh SM, Paryan M, Rezvan H, Mostafavi E, Pasalar P (2014) Polymorphism detection of VKORC1 and CYP2C9 genes for warfarin dose adjustment by real-time PCR. Thrita 3(1):e14033
30. Buzoianu AD, Trifa AP, Mureşanu DF, Crişan S (2012) Analysis of CYP2C9* 2, CYP2C9* 3 and VKORC1-1639 G>A polymorphisms in a population from South-Eastern Europe. J Cell Mol Med 16(12):2919–2924
31. Yang L, Ge W, Yu F, Zhu H (2010) Impact of VKORC1 gene polymorphism on interindividual and interethnic warfarin dosage requirement—a systematic review and meta analysis. Thromb Res 125(4):e159–e166
32. Scott SA, Edelmann L, Kornreich R, Desnick RJ (2008) Warfarin pharmacogenetics: CYP2C9 and VKORC1 genotypes predict different sensitivity and resistance frequencies in the Ashkenazi and Sephardi Jewish populations. Am J Hum Genet 82(2):495–500
33. Scibona P, Redal M, Garfi L, Arbelbide J, Argibay P, Belloso W (2012) Prevalence of CYP2C9 and VKORC1 alleles in the Argentine population and implications for prescribing dosages of anticoagulants. Genet Mol Res 11(1):70–76