The Cytochrome 4F2 rs2108622 Genetic Variant among Unrelated Arab Jordanian Volunteers

Yazun Bashir Jarrar*[*1], Lama Hamadneh [1], Danya Iqtait [1], Rana Sadieh [1], Abdul Qader Al-Bawab [1], Malek Zihlif [2]

Abstract

Background: The *rs2108622* genetic variant in *CYP4F2* gene is associated with cardiovascular diseases and response to warfarin treatment. **Objective**: To determine the frequency of *CYP4F2rs2108622* alleles and genotypes among Jordanian population. **Methods**: The genomic DNA was extracted from 90 healthy Jordanian volunteers and the frequency of *CYP4F2 rs2108622* was determined by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism assays (RFLP). **Results**: The frequency (95% confidence interval) of *rs2108622 C* and *T* allele was 0.55 (0.48-0.62) and 0.45 (0.38- 0.52), respectively. The *CYP4F2rs2108622* genotype frequencies among healthy Jordanian volunteers were wild CC (0.27, 95% CI 0.18-0.36), heterozygote CT (0.55, 95% CI 0.45-0.65) and homozygote TT (0.18, 95% CI 0.10-0.26). The frequency of *rs2108622C>T* was high among the Jordanian population which was similar to Middle Eastern populations but higher than European Caucasians, Africans and Asians. **Conclusion:** Jordanian ethnic population has high frequency of *CYP4F2rs2108622* genetic variant which may play a major role in the predisposition to cardiovascular diseases and inter-individual variation in warfarin response among Jordanians.

Keywords: CYP4F2, genetic variant, Jordanian.

(J Med J 2017; Vol. 51 (2):69-76)

Received	***Accepted***
June 20, 2016	*Feb. 7, 2017*

Introduction

Cytochrome 4F2 (CYP4F2) is monooxygenase enzyme localized in the endoplasmic reticulum of cells. It metabolizes mainly endogenous lipid soluble compounds[(1)]. The CYP4F2 influences the blood vessel and platelet function through oxidizing of arahidonic acid and leukotriene-B4 to 20-hydroxyeicosatetraeonic acid and 20-hydroxyleukotriene-B4, respectively[(1,2)]. In addition, CYP4F2 oxidizes saturated and unsaturated fatty acids such as lauric and palmitic acids[(3)]. Furthermore, CYP4F2 is involved in vitamin K metabolism and hence affects blood coagulation and warfarin activity. Therefore, CYP4F2 plays a role in cardiovascular homeostasis[(4, 5)].

1. Department of Pharmacy, Faculty of Pharmacy, AlZaytoonah University of Jordan, Amman, Jordan.
2. Department of Pharmacology, Faculty of Medicine, University of Jordan, Amman, Jordan.
**** Correspondence should be addressed to:***
Yazun Bashir Jarrar
Assistant Professor of Pharmacology, Department of Pharmacy, Faculty of Pharmacy, AlZaytoonah University of Jordan.
E-mail: Yazun.jarrar@zuj.edu.jo, yznjrr@gmail.com

The expression of CYP4F2 is different in body tissues. CYP4F2 is predominantly expressed in the liver, kidney and human platelets[6], while it couldn't be detected in human megakaryoytes[7].

The CYP4F2 is encoded by *CYP4F2* gene located on chromosome 19 at position 13.12. The *CYP4F2* gene is 20096 base pair (bp) size with 13 exons and 12 introns[8-10)1]. Genetic variants on *CYP4F2* gene had been reported to affect CYP4F2 function and on the cardiovascular homeostasis. It is reported that 1347 C>T polymorphism (rs2108622) in the 11th exon region of *CYP4F2* gene were associated with cardiovascular diseases, such as hypertension[4,5]. Many studies showed that differences in *CYP4F2* genotype account for approximately 1-7% of the inter-individual variability in warfarin response[11]. The exonic non-synonymous *CYP4F2*rs2108622 genetic variant showed high influence on warfarin response[12]. This *CYP4F2rs2108622* genetic variant is characterized by the substitution of 1393 cytosine to thymine on exon 11 which replaces valine to methionine at position 433 on CYP4F2 amino acid sequence. This amino acid substitution reduces *CYP4F2* metabolic activity[1].

There is an inter-ethnic variation in the frequency of *CYP4F2rs2108622* among different ethnic groups. It was found that the frequency of *CYP4F2rs2108622 T* allele is higher among Caucasians (0.27) and Asians (0.26) than African- Americans (0.07) [14]. This difference may affect the susceptibility to cardiovascular disease and warfarin response among different ethnic groups. Among Middle Eastern countries, the frequency of *CYP4F2rs2108622* among Egyptian population was high (0.42) in comparison with other Caucasian and African ethnic groups[13].

Although *CYP4F2rs2108622* genetic variant plays a role in cardiovascular homeostasis and warfarin response, no study on *CYP4F2rs2108622* was done on Jordanian population. Therefore, the aim of the present study was to find the frequency of warfarin sensitive *CYP4F2rs2108622* genetic variant among healthy unrelated Jordanian population.

Method

Sample collection

A total of 90 healthy Jordanian volunteers (30 males and 60 females, aged 23± 3 years) agreed to participate in the study and signed an informed consent. This study was approved by the Institutional Review Board (IRB) committee of Al-Zytoonah University in Jordan with registration number 13-10-2016. From each volunteer, 2-3 ml of venous blood were obtained in EDETA tubes. Individuals were unrelated and healthy volunteers, as judged by the physical examination with no history of chronic diseases. The present study included only Jordanians with Arabian and excluded non-Arabian ancestries (Circassian, Kurd, Chechens and Armans) through questioning of the volunteers regarding direct paternal and maternal ethnicity. Most of them were from *Al-*Zytoonah university students and workers.

DNA extraction

DNA was extracted using Wizard DNA extraction kit (Promega, Madison, WI, USA) according to the manufacturer's method. Briefly, leukocytes were isolated from whole blood through 3000 rpm centrifugation and then incubated with Cell Lysis Solution (Promega, Madison, WI, USA). After that, the cell nucleus pellet was lysed through different lysis solution; Nuclei Lysis Solution (Promega, Madison, WI, USA). Then, the proteins in the samples were precipitated. Then, the genomic DNA was precipitated by

isopropanol and washed with 70% ethanol. Lastly, the DNA was dissolved in nuclease free water and was stored at -20˚C until used.

Amplification of *CYP4F2* gene fragment

The *CYP4F2* gene fragment in the DNA samples was amplified using polymerase chain reaction (PCR) with the following reaction system : a sample of 100 ng of genomic DNA was reconstituted in a total 50 µl reaction mixture containing 1 µl of 10 mM dNTPs; 2 µl of 25mM $MgCl_2$; 10µl of 10X Taq polymerase buffer, 1 unit of *Taq DNA polymerase* and 10 pmole from each of the following forward (ATCAACCCGTTCCCACCT) and reverse (CGTCTGGGAGCACAATGT) PCR primer sequences[14].

The PCR mixture was heated to 94°C for 5 min, and then incubated in 35 thermal cycles of: denaturation step at 94°C for 50 seconds, annealing step at 55 °C for 40 seconds and elongation step at 72°C for 50 seconds. Then, the PCR product was completed by incubating it at 72°C for 7 min as a final step of elongation.

Restriction fragment length polymorphism

A sample of 1 µg of DNA from PCR product was subjected to *PvuII* restriction enzyme analysis (New England Bio Labs, England). Breifly, 1 µl of *PvuII-HF*, 5 µl (x1) of 10x NEBuffer was added to 10 µl PCR product then incubated at 37°C for overnight to detect the genetic variation (*rs2108622*). The *PvuII* restriction enzyme cut the PCR product of the *CYP4F2rs2108622C* wild type to 2 fragments with size of 185 and 320 bps, while couldn't cut the *CYP4F2rs2108622T* mutant allele.

Cell digestion products were separated on 2% agarose gel for detection of rs2108622 variation after gel staining with ethidium bromide.

Statistical analysis

Chi-square (χ^2 test) was used to determine whether the *CYP4F2* allele distribution was in Hardy-Weinberg equilibrium. A p-value < 0.05 was used to reject the null hypothesis and z- core test was used for comparison of *CYP4F2* genotypes frequency among Jordanians with other populations.

Results

Figure 1 shows a representative gel electrophoresis, on 2% agarose gel, of amplified fragment of the coding region of *CYP4F2* gene using PCR. The size of the amplified PCR product was 505bp (Figure 1).

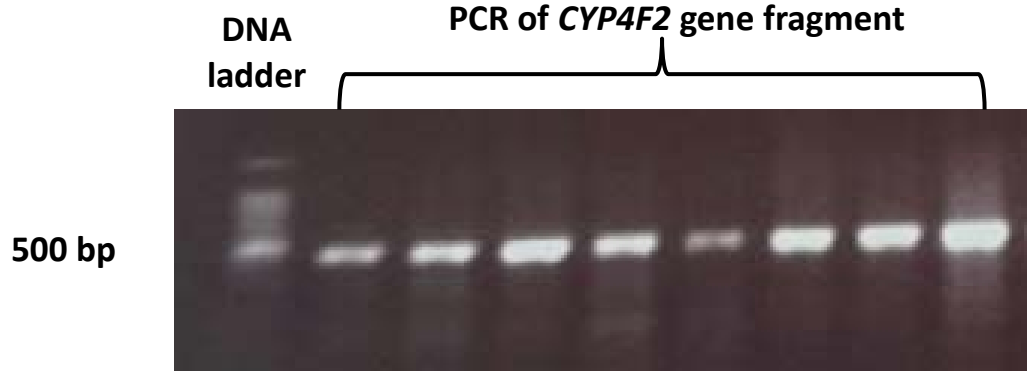

Figure 1: Gel electrophoresis of PCR products of *CYP4F2* gene amplification

The wild *CYP4F2rs2108622T* genotype was represented by PCR digested invariant allele bands (320 and 185 bp), while homozygote *CYP4F2rs2108622T* genotype was expressed by not-digested variant allele band (505 bp). The heterozygote *CYP4F2rs2108622T* genotype was represented by three bands (505 bp, 320 and 185 bp) on agarose gel electrophoresis as shown in Figure 2.

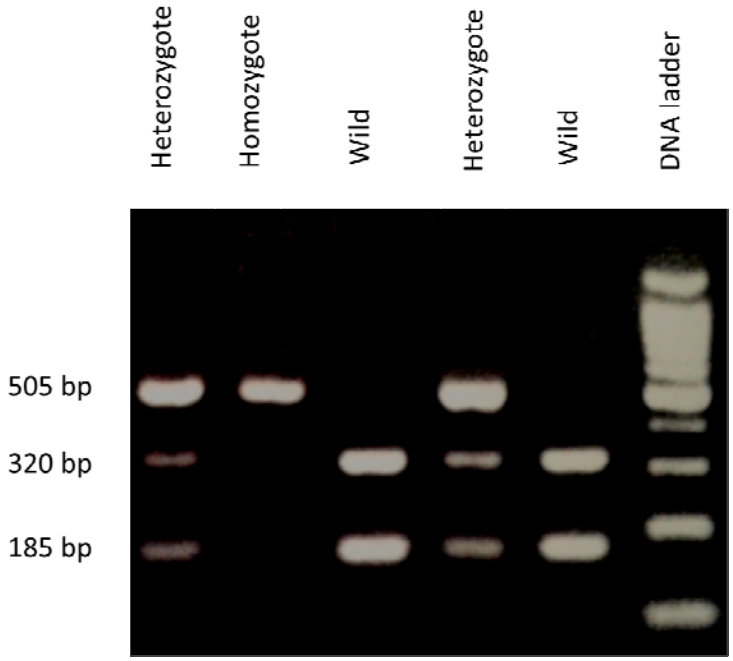

Figure 2: Represented gel-electrophoresis of *CYP4F2 rs2108622* genotype among sample of healthy Arab Jordanian volunteers

The *CYP4F2rs2108622T* allele was detected in 66 among 90 volunteers participated in this study. The frequency of *CYP4F2rs2108622 C* and *T* alleles among healthy unrelated Jordanians were 0.55 and 0.45, respectively (Table 1).

Table 1. The allele frequency of *CYP4F2rs2108622C > T* genetic variant among healthy Jordanian population

Allele	Frequency observed	95% confidence
C	0.55	0.48-0.62
T	0.45	0.38- 0.52
Total	1	

The *CYP4F2rs2108622* genotype frequencies among healthy Jordanian volunteers were wild CC (0.27), heterozygote CT (0.55), homozygote TT (0.18) as presented in Table 2. The data showed that heterozygote CT genotype is the most common genotype with frequency of 55% of the total *CYP4F2rs2108622* genotypes among these healthy volunteers. As shown on Table 2, all of *CYP4F2rs2108622* genotype frequencies were within Hardy-Weinberg equation.

Table 2. The *CYP4F2rs2108622* genotype frequency among healthy Jordanian population

Genotype	Observed number (frequency)	Predicted number (frequency)	χ^2 test †
Wild (C/ C)	24 (0.27)	27 (0.30)	>0.05
Heterozygote (C/T)	50 (0.55)	45 (0.50)	
Homozygote (T/T)	16 (0.18)	19 (0.20)	
Total	90 (1)		

† All of *CYP4F2rs2108622* genotype frequencies were within Hardy-Weinberg equation

In comparison with other ethnic populations, the current study found that the frequency of *CYP4F2rs2108622C > T* among Jordanians was similar to Middle Eastern Kuwaiti, Egyptian and Turkish populations, while it was higher than European Caucasians, African and Asian ethnic groups using z-test, p value <0.05 (Table 3).

Table 3. The comparison between *CYP4F2rs2108622* allele frequencies among healthy Jordanians with other ethnic populations

Ethnic group	*CYP4F2*rs2108622 C frequency	*CYP4F*2rs2108622 T Frequency	Different than Jordanian (χ^2 test)	Is there statistical difference in comparison with Jordanian population	Reference
European Caucasian	0.73	0.27	> 0.05	Yes	(14)
African-American	0.93	0.07	>0.05	Yes	(14)
Asian	0.74	0.26	>0.05	Yes	(14)
Oceania	0.39	0.61	>0.05	Yes	(14)
Hispanic	0.64	0.36	<0.05	NO	(14)
Middle Eastern					
Kuwait	0.60	0.40	<0.05	NO	(15)
Egyptian	0.58	0.42	<0.05	NO	(15)
Turkish	0.60	0.40	<0.05	NO	(17)
Jordanian	0.55	0.45	-	-	This study

Discussion

Genetic variants in *CYP4F2* gene alter cardiovascular homeostasis[16]. However, the frequency of the most common *CYP4F2* genetic variant *rs2108622* was reported in many ethnic populations, the frequency of *rs2108622* had not been screened among Jordanian population. To the best of our knowledge, the present study is the first study to investigate *CYP4F2rs2108622* among healthy Arab Jordanians. We showed that Jordanian population has high frequency of this genetic variant (45%), which may significantly affect warfarin response and susceptibility to cardiovascular disease among Jordanian ethnic population.

Since CYP4F2 is the major cytochrome responsible for synthesis of the vasoconstrictor and platelet activator 20-hydroxyeicosatetraenoic acid (20-HETE),

genetic variants on *CYP4F2* gene may affect 20-HETE production. The rs2108622 polymorphism in *CYP4F2* gene affected enzyme activity and decreased 20-HETE production[17]. Therefore, rs2108622 variant was associated with cardiovascular disease.

The cardiovascular diseases are major chronic diseases in Jordan and are considered one of the most common causes of death in the country by approximately 35% of total deaths[18]. As the frequency of *rs2108622* variant is high among Jordanian population, it is suggested to investigate the association of *rs2108622* variant with cardiovascular diseases in Jordan.

The size of the sample was relatively small, the statistical power of the test (beta = 80%) indicates that sample size of 88 volunteers is enough to study the high frequent rs2108622 variants among Caucasian populations. The present study used 90 samples from unrelated volunteers to determine the frequency of rs2108622. Therefore, the sample size of the current study was statistically enough to conclude that rs2108622 variant is high among Jordanian population.

Warfarin, is the most frequently prescribed anticoagulant drug with large inter-individual variability in response[16]. The CYP4F2 is vitamin K oxidase involved in the metabolism of vitamin K[23]. The *CYP4F2 rs2108622* is required in many medical algorithms in determining the dosage of warfarin to achieve the therapeutic efficacy and minim size serious warfarin toxicity, such as bleeding [19]. It is reported that patients with *CYP4F2rs2108622 T/T* genotype required approximately 1 mg/day more warfarin than patients with 2 *C/C* genotype[17].

The majority of Jordanian population are from Arabian tribes which came from the Arabian peninsula. However, the Jordanian population was mixed with other ethnic groups, such as Circassians, Armenians and Kurds, in addition to the Iraqi and Syrian refugees. In this study, we made sure that the volunteers were originally from the Arabian Jordanian tribes and excluded other mixed groups. However Arabs are considered as Caucasians, the results of this study showed that *CYP4F2rs2108622 C>T* frequency among Jordanians was similar to other Middle Eastern Arabian population like Kuwaiti, Egyptians and non-Arabian Turkish, but different than what was reported among European Caucasians. Therefore, *CYP4F2rs2108622* may contribute to the high prevalence of the cardiovascular diseases, such as hypertension, among Middle Eastern populations. Further studies are needed to explore the association of *CYP4F2rs2108622* with cardiovascular diseases among Jordanian and other Middle Eastern populations.

Conclusion

This study reported a high frequency of *CYP4F2rs2108622* polymorphism among Jordanians which may (20) contribute to the inter-individual variation in warfarin response and susceptibility to cardiovascular disease.

Acknowledgment

This work was supported by Deanship of Scientific Research at AlZaytoonah University of Jordan. Grand fund number is 13-10-2016.

The efficacy of collagen matrix has been demonstrated in animal models.[15-17] Chen and colleagues performed standard trabeculectomy on 17 rabbits, with their left eye receiving the collagen matrix implant and their right eye serving as surgical controls. During the first few days, the postoperative reduction in IOP (15%) was equal in both groups. Pressure had decreased to 55% below baseline values at day

28 in the treated eyes but had returned to preoperative levels by day 21 in the control eyes. Histological examination showed a prominent bleb in the treated eyes compared with scarring and limited bleb formation in the control eyes.[16]

In our retrospective series, the success rate of trabeculectomy using this collagen implant was fairly high (88%) over the course of follow up (averge: 7.9 months). Perez et al reported the outcomes on a larger series of 65 eyes and showed a significant mean IOP reduction (from 21.4± 9.2 to 12.3± 3.7 mmHg) over the period of follow up (36 months).[18]

These promising results could offer an alternative method for modulating wound healing following filtration surgery. However, further comparative studies against the use of antifibrotics are warranted.

Conclusion

The use of biodegradable collagen matrix implant appears to hold promise as a safe tool to increase success rate of glaucoma filtration surgery. Further studies are needed to evaluate the long term results and to compare the outcome and safety of using this collagen implant against the use of antifibrotics in trabeculectomy.

References

1. Snider NT, Walker VJ, Hollenberg PF. Oxidation of the endogenous cannabinoid arachidonoyl ethanolamide by the cytochrome P450 monooxygenases: physiological and pharmacological implications. Pharmacol Rev 2010; 62: 136-54.
2. Zhang, X. and J. P. Hardwick . Regulation of CYP4F2 leukotriene B4 omega-hydroxylase by retinoic acids in HepG2 cells. Biochem Biophys Res Commun 2000; 279: 864-71.
3. Stec DE, Roman RJ, Flasch A, Rieder MJ. Functional polymorphism in human CYP4F2 decreases 20-HETE production. Physiological Genomics. 2007; 30: 74-81.
4. Lasker JM, Chen WB, Wolf I, Bloswick BP, Wilson PD, Powell PK. Formation of 20-Hydroxyeicosatetraenoic Acid, a Vasoactive and Natriuretic Eicosanoid, in Human Kidney: ROLE OF CYP4F2 AND CYP4A11. J Bio Chem 2000; 275:4118-26.
5. Hardwick JP. Cytochrome P450 omega hydroxylase (CYP4) function in fatty acid metabolism and metabolic diseases. Biochem Pharmacol 2008; 75: 2263-75.
6. Edson KZ, Prasad B, Unadkat JD, Suhara Y, Okano T, Guengerich FP. Cytochrome P450-Dependent Catabolism of Vitamin K: ω-Hydroxylation Catalyzed by Human CYP4F2 and CYP4F11. Biochemistry 2013; 52: 8276-85.
7. Munshi A, Sharma V, Kaul S, Al-HazzaniA, Alshatwi AA, Shafi G. Association of 1347 G/A cytochrome P450 4F2 (CYP4F2) gene variant with hypertension and stroke. Mol Biol Rep 2012; 39: 1677-82.
8. Hirani V, Yarovoy A, Kozeska A, Magnusson RP, Lasker JM. expression of cyp4f2 in human liver and kidney: assessment using targeted peptide antibodies. Arch of Biochem Biophys 2008; 478: 59-68.
9. Jarrar YB, Cho SA, Oh KS, Kim DH, Shin JG, Lee SJ. Identification of cytochrome P450s involved in the metabolism of arachidonic acid in human platelets." Prostaglandins Leukot Essent Fatty Acids 2013; 89: 227-34.
10. Jarrar, Y. B., J. G. Shin, Lee SJ. Expression of arachidonic acid-metabolizing cytochrome P450s in human megakaryocytic Dami cells. In Vitro Cell Dev Biol Anim 2013; 49: 492-500.
11. Ding H, Cui G, Zhang L, Xu Y, Bao X. Association of common variants of CYP4A11 and CYP4F2 with stroke in the Han Chinese population. Pharmacogenet Genomics 2010; 20: 187-94.
12. Pautas E, Moreau C, Gouin-Thibault I, Golmard JL, Mahe I. Genetic factors (VKORC1, CYP2C9, EPHX1, and CYP4F2) are predictor variables for warfarin response in very elderly, frail inpatients. Clin Pharmacol Ther 2010; 87: 57-64.
13. Fung E, Patsopoulos NA, Belknap SM, O'Rourke DJ, Robb JF. Effect of genetic variants, especially CYP2C9 and VKORC1, on the pharmacology of warfarin. Semin Thromb Hemost 2012; 38: 893-904.
14. Alvarellos ML, Sangkuhl K, Daneshjou R, Whirl-Carrillo M, Altman RB, Klein TE.

PharmGKB summary: very important pharmacogene information for CYP4F2. Pharmacogenetics and Genomics. 2015; 25: 41-7.
15. Shahin MHA, Khalifa SI, Gong Y, Hammad LN, Sallam MTH, Shafey ME. Genetic and nongenetic factors associated with warfarin dose requirements in Egyptian patients. Pharmacogenet and Genomics 2011; 21:130-5.
16. Alsmadi O, John SE, Thareja G, Hebbar P, Antony D, Behbehani K. Genome at Juncture of Early Human Migration: A Systematic Analysis of Two Whole Genomes and Thirteen Exomes from Kuwaiti Population Subgroup of Inferred Saudi Arabian Tribe Ancestry. PLoS ONE 2014; 9:e99069.
17. Ozer M, Demirci Y, Hizel C, Sarikaya S, Karalti I, KasparC. Impact of genetic factors (CYP2C9, VKORC1 and CYP4F2) on warfarin dose requirement in the Turkish population. Basic Clin Pharmacol Toxicol 2013; 112: 209-14.
18. Meng C, Wang J, Ge WN, Tang SC, Xu GM. Correlation between CYP4F2 gene rs2108622 polymorphism and susceptibility to ischemic stroke. Int J Clinic Experiment Med 2015; 8: 16122-6.
19. Liu H, Zhao Y, Nie D, Shi J, Fu L, Li Y. Associationof a Functional Cytochrome P450 4F2 Haplotype with Urinary 20-HETE and Hypertension. J Am Soc Nephrol 2008; 19:714-21.
20. Hamed R. Takruri, Refat A. Alkurd. Intakes of Fats, Cholesterol, Fiber and Micronutrients as Risk Factors for Cardiovascular Disease in Jordan. Jo J Biol Sci 2014; 7: 119-26.
21. Bahou Y, Hamid H, Hadidi A. Ischaemic stroke in Jordan: a 2-year hospital-based study of subtypes and risk factors. East Mediterr Health J 2004; 10 (1-2): 138-46.
22. Li S, Zou Y, Wang X, Huang X, Sun Y. Warfarin dosage response related pharmacogenetics in Chinese population. PLoS ONE 2015; 10: e0116463.
23. McDonald MG, Rieder MJ, Nakano M, Hsia CK, Rettie AE. CYP4F2 is a vitamin K1 oxidase: An explanation for altered warfarin dose in carriers of the V433M variant. Mol Pharmacol 2009, 75: 1337-46.
24. Cen HJ, Zeng WT, Leng XY, Huang M, Chen X. CYP4F2 rs2108622: a minor significant genetic factor of warfarin dose in Han Chinese patients with mechanical heart valve replacement. Br J Clin Pharmacol 2010, 70: 234-40.

المتغير الجيني CYP4F2rs2108622 عند المتطوعين الغير أقارب العرب الأردنيين

يزن بشير جرار[1]، لمى حمادنة[1]، دانية اقطيط[1]، رنا سعدية[1]،عبدالقادر بواب[1]، مالك الزحلف[2]

1– قسم الصيدلة، كلية الصيدلة، جامعة الزيتونة الأردنية، عمان، الأردن.

2– قسم علم الأدوية، كلية الطب، الجامعة الأردنية، عمان، الأردن.

الملخص

الخلفية: هنالك علاقة بين المتغير الجيني CYP4F2rs2108622 مع أمراض القلب والأوعية الدموية والاستجابة علاج الوارفرين. **الهدف:** تحديد تردد تكرار النمط الجيني CYP4F2rs2108622 بين سكان الأردن. **الطرق البحثية:** تم استخراج الحمض النووي الجيني من 90 المتطوعين الأردنيين الأصحاء، وتم تحديد وتيرة rs2108622 CYP4F2 عن طريق تفاعل البلمرة المتسلسل واستخدام الإنزيمات القاطعة. **النتائج:** كان تكرار (فترة الثقة 95٪) من النمط الجيني rs2108622 وC T كانت 0.55 (0،48-0،62) و0.45 (0.38-0.52) على التوالي. كان تردد rs2108622C> T مرتفعا بين أفراد العينة على غرار سكان دول الشرق الأوسط الأخرى ولكن أعلى من القوقازيين الأوروبيين والأفارقة والآسيويين. **الخلاصة:** الأردنيون لديهم وتيرة عالية من المتغير الجيني CYP4F2rs2108622 الذي يمكن أن يلعب دوراً كبيراً في الإصابة بالأمراض القلبية الوعائية والاختلاف بين الأفراد في استجابة الوارفارين بين الأردنيين.

الكلمات الدالة: CYP4F2، المتغيرات الجينية، الأردنيين.