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Genetic Polymorphisms of VKORC1 in Han Population of Yunnan China*

LIU Jian-xing[1#], CHEN Zhi-yu[2#], LI Wen-hui[3], GAO Hui[2], GAO Zhan[2], LI Fu-ning[2], DENG Wei[2], XU Bing-ying[1△]

(1 School of Forensic Medicine, Kunming Medical University, Kunming, Yunnan, 650500, China;

2 Affiliated Yan'an Hospital of Kunming Medical University, Kunming, Yunnan, 650030, China;

3 Department of Forensic Medicine, Hainan Medical University, Haikou, Hainan, 570100, China)

ABSTRACT Objective: To investigate the polymorphisms of *VKORC1* in Han population of Yunnan China and compare it with that of other populations home and abroad. **Methods:** Blood samples were collected from 280 Chinese Yunnan Han patients with cardiac valves replacement. *VKORC1* -1639G/A, 1173C/T, 3730A/G polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** For *VKORC1* -1639G/A, 222 subjects (79.3%) were homozygous of AA, 8(2.8%) were GG, and 50 (17.9%) were heterozygous of AG. Two hundred and twenty-four subjects (80.0%) were carriers of TT, while 56 (20.0%) were CT, CC genotype was not found in *VKORC1* 1173C/T. *VKORC1* 3730 A/G showed that 45 (16.1%) were heterozygote of AG, 30 (10.7%) and 205 (73.2%) were homozygote of AA and GG. Both similarities and differences in *VKORC1* polymorphisms were found between Yunnan Han and other populations. **Conclusions:** This study increased the evidence of intrapopulation genotypic variability and highlighted the significant genotypic heterogeneity when different populations are considered, it would be useful to understand clinical pharmacokinetics and drug dosage recommendations for Yunnan Han population.

Key words: *VKORC1*(vitamin K epoxide reductase complex 1); Yunnan Han population; Genotype

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Introduction

Many have speculated that to define an individual's metabolic capacity by genotyping would be an essential tool for safe drug administration, especially for drugs with narrow therapeutic index[1]. Recently, the gene for vitamin K epoxide reductase complex 1 (*VKORC1*), which encodes many drugs' (e.g., warfarin) target proteins, has been identified [2,3]. *VKORC1* is responsible for the metabolism of vitamin K whereby it recycles vitamin K 2, 3-epoxide to activate vitamin K hydroquinone, which is important in activation of clotting factors. *VKORC1*'s polymorphisms were found to be one of the main reasons for the difference in drug dose requirement in different ethnic groups and have a major metabolic effect on certain drugs such as warfarin [4-6]. Many studies about *VKORC1* gene, such as SNPs of 1173, 3730 and -1639, the most common mutations regarding to drug metabolism, have been reported [7-9]. Studies of Chinese Han population about *VKORC1* SNPs have also been established and variations between Han and other populations have been found [4,7,11]. However, the *VKORC1* polymorphism studies in China were performed only in eastern part, little was done about *VKORC1* gene 3730A/G in Han population, not to mention research on population in Yunnan province. To address this imbalance, we explored the genetic polymorphisms of southwest Han in Yunnan China, examining -1639G/A, 1173C/T and 3730A/G SNPs of *VKORC1* genotype profiles. The results were then compared with the data of other populations.

1 Materials and methods

1.1 Patients

Patients, ranging from 13 to 74 years of age, with cardiac valves replacement, from the cardiology department of affiliated Yan'An Hospital, Kunming Medical University, were selected. Subjects with renal insufficiency, liver disease, cancer or any other chronic illnesses were excluded. Prior to the experiment, all procedures were approved by an Ethics Committee, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

1.2 Procedures

First, the genomic DNA was extracted from peripheral blood with phenol-chloroform. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, by Sconce et al[10], was used to detect *VKORC1*. The primers were as follows:

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Author introduction: LIU Jian-xing (1971-), male, PhD, mainly engaged in research of molecular genetics and mechanism of drug abuse and dependence, E-mail: ljxnh@sina.com;

CHEN Zhi-yu(1964-), male, master, mainly engaged in molecular genetics research, E-mail: 444319658@qq.com

#LIU Jian-xing and CHEN Zhi-yu contributed equally to this work

△Corresponding Author: XU Bing-ying, E-mail: bingying_xu@126.com.cn

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VKORC1-1639G/A: forward primer 5'-GAAGGGTAG-GTTCAACAGTAAGG-3', reverse primer 5'-TCAAGTGGTTCT CGTGTCTCA -3'; VKORC1 1173C/T: forward primer 5'-GGTC-TAAGATGAA AAGGAGGG-3', reverse primer 5'-GCTGTTG-GATTGATTGAGGA T-3'; VKORC1 3730A/G: forward primer 5'-GTATCACCACCTATGCTATCAACG-3', reverse primer 5' -TGCTCAGAACATTCCCTCCCT-3'.

1.3 Statistical analysis

Allele frequency was counted by PowerStats(version 12). The data were then analyzed with chi-square analysis. Hardy-Weinberg equilibrium was tested by Arlequin software (version 3.1). SPSS (version 11.0) was used for the analysis of the statistical data. p value of <0.05 was considered statistically significant.

2 Results

A total of 280 patients consented to participate in the study. Genotype and allele frequencies of *VKORC1* are summarized in Table 1. For *VKORC1* -1639G/A, 222 subjects (79.3%) were homozygous of AA, 8 (2.8%) were GG, and 50 (17.9%) were heterozygous of AG. With regard to *VKORC1* 1173C/T, 224 subjects (80.0%) were carriers of TT, 56 (20.0%) were CT, CC genotype was not found. Analysis of *VKORC1* 3730 A/G showed that 45 (16.1%) were heterozygote of AG, 30 (10.7%) and 205 (73.2%) were homozygote of AA and GG, respectively (See table 1).

To compare the genotype and allele frequencies of *VKORC1* between Yunnan Han and other populations, exact test was performed. The results, as well as the reported genotypes and allele frequencies of other populations, are summarized in Tables 2. It shows that AA, GG and TT are the most common genotypes of *VKORC1*-1639G/A, 3730A/G and 1173C/T genes in Yunnan Han population, significantly different from that in populations of southeast Asia, such as Indonesia, Philippines, Thailand and Vietnam; furhter different from data of Caucasian, Italian, American, French, Turkish, Iran and Indian(P<0.05). Compared with Yunnan Han, Iran and American are the populations with the most significant differences. Table 2 also shows that although the three genotypes are most common in Yunnan Han population, the present study found a relatively lower frequencies (79.3%, 73.2% and 80%) compared with other mainland Han and Taiwan Han populations(83.7%, 81% and 86.5%).

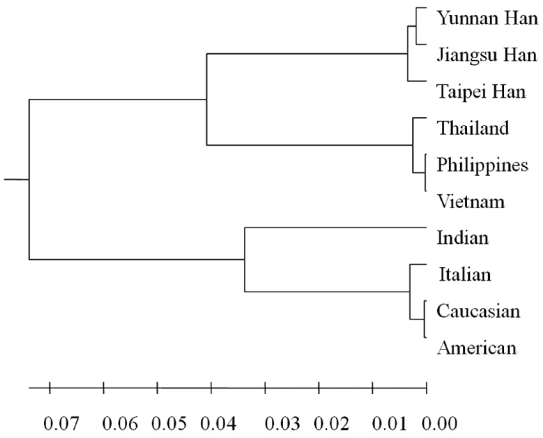

Fig. 1 Evolutionary tree based on genotype frequency of 1173C/T

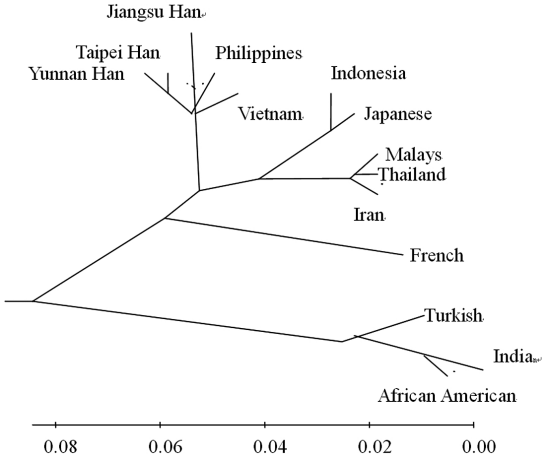

Fig. 2 Evolutionary tree based on genotype frequency of -1639G/A

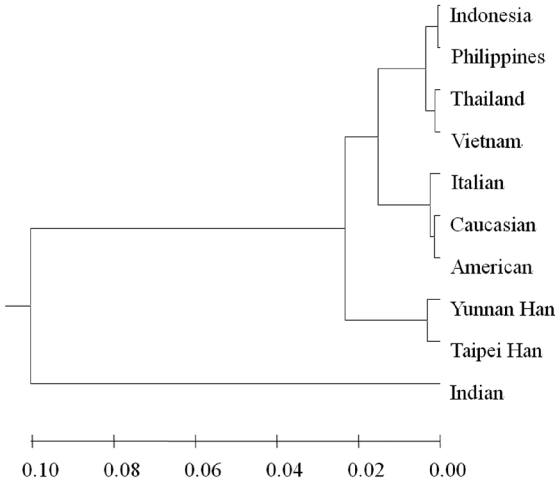

Fig. 3 Evolutionary tree based on genotype frequency of 3730A/G

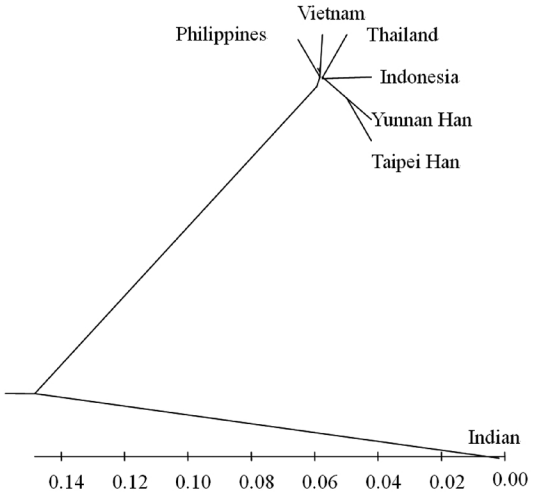

Fig. 4 Evolutionary tree based on genotype frequency of 1173C/T, -1639G/A, 3730A/G together

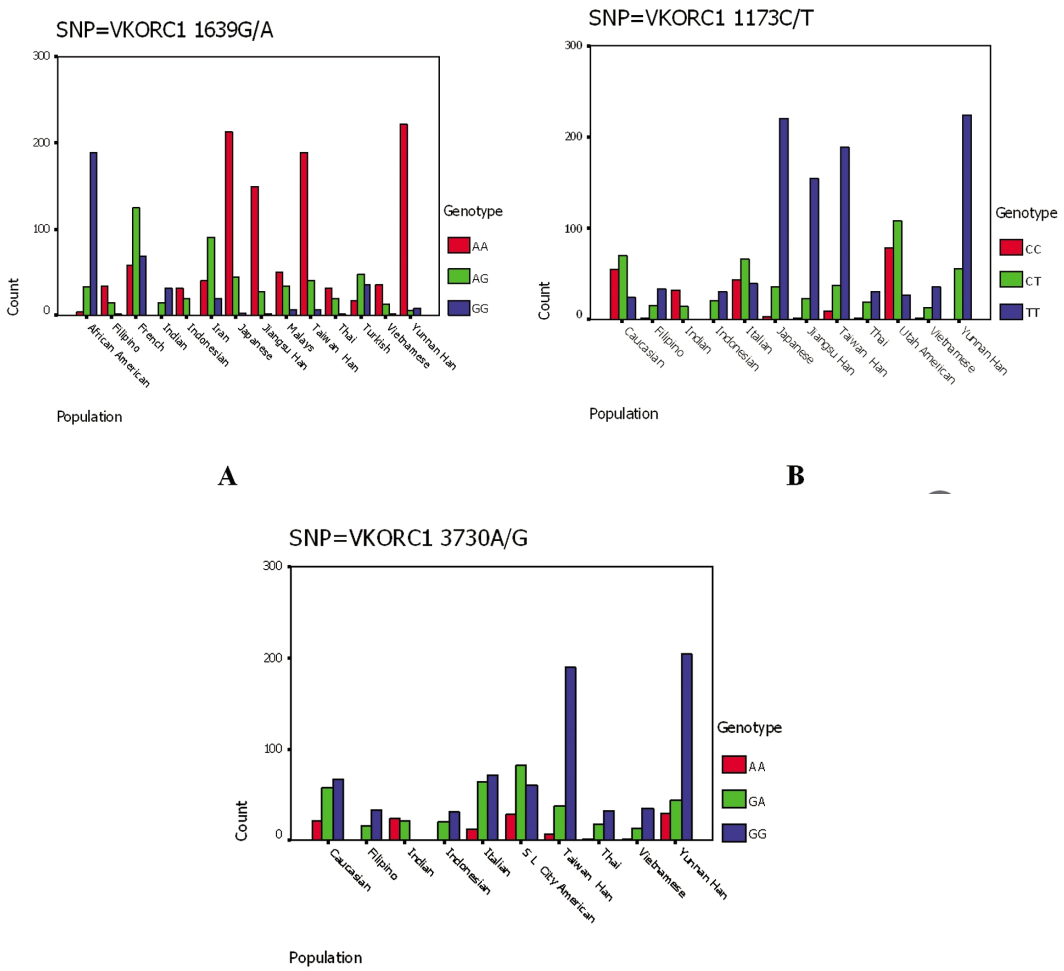

Fig. 5 A, B and C are Genotype contrasts between Yunnan Han and other populations based on *VKORC1* 1639G/A, *VKORC1* 1173C/T and *VKORC1*3730A/G, respectively

Fig. 1-4 are population clusters by UPGMA (unweighted pair-group method with arithmetic means) method based on genotype frequency of 1173C/T, -1639G/A, 3730A/G and the 3 genes together, respectively. There were not so many published data for us to use, so we constructed four clusters of relatively different populations, and the figures were also a little different, but the clustering trends were consistent with each other, Yunnan Han, Jiangsu Han, and Taipei Han clustered first, then they went closely with populations from southeast Asia, and finally, clustered with populations from western countries.

Table 1 Genotype and allele frequency of *VKORC1* genes in Yunnan Han population

Gene		Genotype	Number (%)	Allele	Number (%)
VKORC1	-1639G/A	AA	222(79.3)	A	494(88.2)
		AG	50(17.9)	G	66(11.8)
		GG	8(2.8)		
	1173C/T	TT	224(80.0)	T	504(90.0)
		CT	56(20.0)	C	56(10.0)
		CC	0		
	3730A/G	AA	30(10.7)	A	105(18.7)
		AG	45(16.1)	G	455(81.3)
		GG	205(73.2)		

Table 2 Distrbution of *VKORC1* genes (-1369, 1173 and 3730) genotypes and genes between different ethnic groups

Studies	Study location	Populations	Sample Number (M/F)	Age	*VKORC1* Gene														
					-1639 G>A(%)					3730A>G(%)					1173C>T(%)				
					AA	AG	GG	A	G	GG	GA	AA	A	G	CC	CT	TT	C	T
Jianxing Liu	Yunan China	Yunnan Han	280 (96/184)	59.3±11.6	79.3	18	2.8	88.2	11.8	73.2	16.1	10.7	18.7	81.3	0	20	80	10	90
Miao LY [7,11]	Jiang Su China	Jiangsu Han	178 (74/104)	54.7	83.7	16	0.6	91.6	8.4						0.6	12.9	86.5	7	93
D'Andrea, G[8]	Foggia, Italy	Caucasian	147	40.3 (15-84)						45.4*	39.6	15	34.7*	65.3	37*	46.9	16.3*	60.2*	39.8*
Paola B[12]	Rome, Italy	Italian	148 (78/70)	68.2						48*	43.9*	8.1	30.1	69.9	29*	44.7*	26.4*	50.7*	49.3*
Carlquist JF[13]	S L City, USA	American	213 (104/109)	72±12											37*	50.7*	12.7*	62.2*	37.8*
Carlquist JF[14]	S L City, USA	American	170 (80/90)	71±13						35.3*	48.2*	16.5	40.6*	59.4*					
Cavallari LH[15]	Chicago, USA	African American	226	57±15	1.8**	14.6	83.6**	9.1**	90.9**										
Misa Y[16]	Shizuoka, Japan	Japanese	341 (173/86)	70 (22-89)	83.9	16	0.3	91.8	8.2										
Kyoko O [17]	Tokyo, Japan	Japanese	114 (63/51)	25.2 (20-52)											1.3	13.7	85	8.1	91.9
Ozer N [19]	Istanbul, Turkey	Turkish	100 (39/61)	49.19 ± 11.19	17*	47*	36*	40.5*	59.5*										
Negar A[18]	Shiraz, Iran	Iran	150 (66/84)	20-50	27*	57*	15.9	55.6	44.4										
Gan GG [20]	Kuala Lumpur, Malaysia	Malays	91(46/45)	55	55	37	8	73.6	26.4										
Lee MT [21]	Taiwan, China	Taiwan Han	235		80	17	3	89	11	81	16	3	11	89	3.7	16	80.3	11.7	88.3
		Indian	46		0**	30	70**	15**	85**	0**	48*	52*	76**	24*	70**	30	0**	85**	15**
		Indonesian	51		62	38	0	81	19	61	39	0	19.5	80.5	0	39.5	60.5	19.8	80.2
		Filipino	49		69	28	2.6	83.2	16.8	68	32	0	6	84	2.1	29.9	68	17.1	82.9
		Thai	51		61	36	2.8	79.1	20.9	63	35	2	19.5	80.5	1.9	37.1	61	20.4	79.6
		Vietnamese	49		72	26	2	85	15	72	25.8	2.2	15.1	84.9	2	26	72	15	85

*P<0.05; **P<0.01

3 Discussion

Much of the interindividual and interethnic differences in drug effect is attributed to genetic variations. So it is assumed that in the future, a pharmacogenetic approach can improve public health by determining for each patient which kind of drug will work best, how much to give, and how many times a day to administer [18]. *VKORC1* is a gene that encodes many drugs' (e.g., warfarin) target proteins, it is necessary for the post-translational-carboxylation of vitamin K-dependent clotting factors such as Ⅱ, Ⅶ, Ⅸ and Ⅹ. As described above, *VKORC1* gene is one of the main factors for difference of drug dose requirement, and it significantly attributes to drug metablism variation interindividually and interethnically. *VKORC1* of -1639 G/A, 1173 C/T and 3730A/G, which totally account for much genetic variability of drug dose requirement in Chinese population, have been found to be the most common mutations regarding to drug metablism, especially for warfarin dose requirement [7]. Recently, *VKORC1* gene investigations on Chinese Han populations have been carried out, but few were done together with the three SNPs of -1639 G/A, 1173 C/T and 3730A/G, in addition, these populations are almost all in eastern or northern China. Yunan is a multinational province, adjacent to Burma, Laos, Thailand and Vietnam. Among 55 minorities, there are 26 with population more than 5000 in Yunnan, with 15 of them living only in this province. The mixed-up living model have inevitably resulted in gene exchanges between Han and ethnic minorities. The present study found a relatively lower frequencies of genotypes of *VKORC1* -1639 G/A, 3730 A/G and 1173 C/T compared with other mainland Han and Taiwan Han populations (79.3%, 73.2% and 80% vs 83.7%, 81% and 86.5%; but the p value is not statistically significant), the gene exchange among Yunnan Han and other minorities may be the main reasons, and this should be our future research focus. As indicated by the findings of the present study and by researches done in other countries, each population has a relatively unique allele frequency of *VKORC1* that defines the proper dosage of drugs (particularly warfarin).

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中国云南汉族群体 *VKORC1* 基因多态性研究 *

刘建兴[1#] 陈智豫[2#] 李文慧[3] 高 辉[2] 高 瞻[2] 李福宁[2] 邓 伟[2] 许冰莹[1△]

(1 昆明医科大学法医学院 云南 昆明 650500;
2 昆明医科大学附属延安医院 云南 昆明 650030;3 海南医学院法医学院 海南 海口 570100)

摘要 目的:探讨云南汉族人群维生素 K 环氧化物还原酶亚单位 1(*VKORC1*)基因多态性,并与国内外群体进行比较。**方法:**采集 280 云南汉族心瓣膜置换术病人外周血,获得基因组 DNA,用 PCR-RFLP 方法分析 *VKORC1* -1639G/A,1173C/T,3730A/G 的基因多态性。**结果:**对于 *VKORC1* -1639G/A,共检出 222 (79.3%)名 AA 纯合子,8(2.8%)名 GG 纯合子,50(17.9%)名 AG 杂合子;对于 *VKORC1* 1173C/T,检出 224 (80.0%) 名纯合子 TT,56 (20.0%)名杂合子 CT,未检出 CC 基因型;对于 *VKORC1* 3730 A/G,共检出 30(10.7%)名 AA 基因型,205 (73.2%)名 GG 基因型,45 (16.1%)名 AG 基因型。与不同群体相比,各位点差别不一。**结论:**与其他群体相比,云南汉族人群 *VKORC1* -1639G/A,1173C/T,3730A/G 基因位点具有自己的遗传多态性,其基因多态性在临床药物应用(如华法林)治疗中具有非常重要的意义。

关键词:维生素 K 环氧化物还原酶亚单位 1 (*VKORC1*,vitamin K epoxide reductase complex 1);云南汉族人群;基因型

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作者简介:刘建兴(1971-),男,博士,主要从事法医分子遗传学和毒品中毒及依赖机制研究,E-mail:ljxnh@sina.com;
陈智豫(1964-),男,硕士,主要从事分子遗传学研究,E-mail:444319658@qq.com

为共同为第一作者

△ 通讯作者:许冰莹,E-mail:bingying_xu@126.com.cn

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