Genetic influence on circulating vitamin D among Saudi Arabians

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ABSTRACT

الأهداف: تقييم تأثير النوكليوتيدات الوحيدة والمتعددة الاشكال (SNP) والتي دُرست على مستوى 25 هيدروكسي فيتامين (د) لدى المجتمع السعودي.

الطريقة: هذه دراسة مقطعية أجريت على 283 من الأفراد البالغين والذين لم يستخدموا فيتامين (د) من قبل. جُمعت عينات الدم من المرشحين بعد موافقة واعية لقياس مستويات 25 هيدروكسي فيتامين (د) بالاضافة الى التحليل الجيني ل SNPs في مستقبلات فيتامين (د) VDR rs2228570 and rs1544410، السيتوكروم، و450 CYP2R1 [rs10741657 and rs1993116]، ومكونات المجموعة الخاصة GC [rs2282679 و rs4588].

النتائج: كان متوسط العمر لجميع المرضى 16.7±48 سنة (المدى 20 - 78). وكان هناك فرق ذا دلالة إحصائية هامة بين الآليلات AG، AA وGG لمستقبلات فيتامين (د) rs2228570 (VDR). حيث أن الحاملين للاليل GG ارتبط بمخاطر متزايدة بعدم كفاية فيتامين (د) ($p \leq 0.002$) ونقصه ($p \leq 0.005$). كما أن تحليلات الجين CYP2R1 - rs10741657 أظهرت أن الحاملين للاليل AG والاليل GG لديهم عرضه اكبر لنقص فيتامين (د). الاليل AG (المستوى الطبيعي مقابل عدم الكفاية $P=0.02$ والمستوى الطبيعي مقابل (النقص$p \geq 0.08$) والاليل GG المستوى الطبيعي مقابل النقص $p \leq 0.002$ وعدم الكفايه مقابل النقص ($p=0.001$). أما بالنسبه لمكونات المجموعه الخاصه (GC - rs4588) تبين أن هناك فرق ذا دلالة إحصائية هامة بين المستوى الطبيعي ونقص فيتامين (د) للاليل AC فقط ($p<0.0001$).

الخاتمة: بينت هذه الدراسة أن وجود SNP في لاليل GG لمستقبلات فيتامين (د) rs2228570 وSNP في الجين rs4588 - GC والجين CYP2R1 - rs10741657 كانت مصاحبه لوجود نقص في مستوى فيتامين (د).

Objectives: To examine the effect of most common studied single nucleotide polymorphisms (SNP) on serum 25-hydroxyvitamin D (25OHD) levels in Saudi Arabian population.

Method: A cross-sectional observational study was carried out between July 2014 and October 2015, at King Fahd Hospital of the University (KFHU), Al-Khobar, Kingdom of Saudi Arabia. After informed consent, blood samples from 283 subjects living in the Eastern province were collected for 25-OHD measurement and genetic analysis of SNPs in vitamin D receptor (VDR) [rs2228570 and rs1544410], Cytochrome, P450 family 2 (CYP2R1) [rs10741657 and rs1993116], and Group-specific components (GC) [rs2282679 and rs4588].

Results: Vitamin D deficiency was found in 87.6% and insufficiency in 7.7%. The percentages of the different alleles of the 6 SNPs tested ranged between 0-62.5%. There was significant difference between the AA, AG, and GG alleles of VDR rs2228570. The carries of GG allele was associated with increased risks of vitamin D insufficiency ($p<0.002$) and deficiency ($p \leq 0.005$). The CYP2R1 rs10741657 gene showed that AG and GG allele carriers had significant risk of vitamin D deficiency. AG allele (normal versus Insufficiency $p<0.02$ and normal versus deficiency $p<0.08$) and GG allele normal versus deficiency ($p<0.002$) and insufficiency versus deficiency ($p<0.001$). For group-specific components (GC rs4588), there was only significant difference between the normal and deficiency for the AC allele ($p<0.0001$).

Conclusion: The presence of GG allele of the SNP rs2228570 of VDR gene, SNPs rs4588 of GC gene and CYP2R1 rs10741657 gene was associated with vitamin D deficiency.

Saudi Med J 2016; Vol. 37 (9): 996-1001
doi: 10.15537/smj.2016.9.14700

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Received 7th February 2016. Accepted 15th June 2016.

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In the last 2 decades, vitamin D has occupied the forefront in research worldwide. Hypovitaminosis D has been implicated in many chronic diseases and was found to play a significant role in public health, but consistent trial evidence of amelioration of these conditions with vitamin D therapy is lacking.[1-2] On the other hand, the methodology of assessment of 25-hydroxyvitamin D (25OHD) in the blood improved from radio immunoassay assays (RIA) to high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), which resulted in a more precise diagnosis.[3] Low levels of serum 25OHD has been blamed to be mainly due to inadequate exposure to the sunlight and or low nutritional intake of vitamin D and was reported previously to be influenced by age, gender, race, skin pigmentation, obesity, and seasons of the year.[4] It has been suggested that 10-15 minutes at mid-day sun exposure is enough to get adequate vitamin D. Recent studies have shown that genetic factors can also significantly influence the levels and bioavailability of 25OHD with heritability effect ranging from 53-68.9%.[5] The presence or absence of single-nucleotide polymorphisms (SNPs) in vitamin D related genes can predict the circulating levels of 25OHD. Engelman et al[6] and Wang et al[7] and after a Genome-wide association (GWA) study of 25OHD and 1,25-dihydroxyvitamin D (1,25OH2 D) concluded that there are several SNPs in vitamin D related genes that were associated with vitamin D levels. The 4 vitamin D related genes being most common studied for genetic polymorphism through GWA and were found to alter the vitamin D concentration in the blood are vitamin D binding protein (DBP) also known as group- specific component (GC), vitamin D receptor (VDR), vitamin D metabolism pathway enzymes (cytochrome P450 related enzymes, CYP2R1 and CYP24A1) and the 7-dehydrochlestrol reductase (DHCR7).[8-10] Vitamin D deficiency is common among Saudi Arabian population and studies report deficiency reaching up to 100% even though individuals could be taking adequate vitamin D in their diets and had adequate exposure to sunlight.[11,12] The effect of genetic polymorphism in vitamin D related genes on 25OHD level was mainly studied in patients of European decent, Hispanics, African American, and Chinese. However, such an effect was not well studied among Arabs living in a region of the world where vitamin D is highly prevalent in spite of abundant sun light around the year. We conducted this cross-sectional study with the aim to study the potential contribution of the SNPs in vitamin D related genes on the levels of 25OHD among apparently healthy Saudi Arabians living in the Eastern Province of the country.

Disclosure. Authors have no conflict of interest, and the work was not supported or funded by any drug company. This work was funded by the Deanship of Scientific Research, University of Dammam, AlKhobar, Kingdom of Saudi Arabia (Grant No. 2014220).

Methods. A cross-sectional observational study was carried out in the period between July 2014 and October 2015, at KFHU, Al-Khobar, Saudi Arabia. Informed consent was taken from each subject enrolled in this study, which was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects and it was approved by the Institution Review Board of the Deanship of Scientific Research - University of Dammam, Dammam, Kingdom of Saudi Arabia. Two hundred eighty-three Saudi adult (39 males and 244 females) consecutive individuals between the age of 18-80 years who attended the orthopaedic outpatient clinics of KFHU and who were not receiving any form of vitamin D therapy have been studied. Available convenient sampling was used and patients were excluded if they were diagnosed to have chronic medical illness, such as malignancy or were documented to have organ dysfunction that affect vitamin D status, such as malabsorption, previous major gastrointestinal surgery, chronic liver disease, renal impairment, or nephritic syndrome, in case of use of vitamin D supplement, or use of drugs that can affect vitamin D metabolism, and in case of positive family history of hypocalcemia or vitamin D disorders. Patients with endocrine disorders such as hyperparathyroidism or hyperthyroidism and females who were pregnant, lactating or postpartum were also excluded. After an informed consent, blood samples were collected for measurement of 25-OHD levels and genetic analysis for 6 single nucleotide polymorphisms (SNPs) in 3 vitamin D related genes. Vitamin D3 measurement was made using using DiaSorin (Liaison®, Saluggia, Italy) analyzer. Normal 25OHD levels was defined as ≥30 ng/ml, insufficiency 21-29 ng/ml and deficiency ≤20 ng/ml. We selected 3 candidate genes containing 6 SNPs (2 SNPs from each gene), these are VDR (rs2228570, rs1544410), CYP2R1 (rs10741657, rs1993116), and GC (rs2282679, rs4588). The above SNPs were chosen based on the fact that they were most commonly studied in the Asian population and demonstrated evidence of significant association in previous Genome-wide associations (GWASs) with

vitamin D level variability. The DNA was extracted from 700 µl aliquot of the 283 samples. The average DNA yield was 43 µg (<1-328 µg) by PicoGreen measurement. Samples were normalized after extraction by Nanodrop to 150 ng/µl. During genotyping analysis, an aliquot of each sample was diluted to approximately 4 ng/µl using Taqman® chemistry. Call rates for all markers investigated were 100% and for quality control and to ensure accuracy of the genotypes, all samples were genotyped in duplicate and all genotypes came to be concordant across the duplicates. Serum calcium, phosphorous, alkaline phosphatase, and parathormone levels were performed at the time of 25OHD analysis. Data were collected and entered in the data base and analyzed using social science statistics (http://www.socscistatistics.com). Means and standard deviation were calculated. We compared the baseline characteristics of patients at different categories for vitamin D level using the t-test for continuous variables. The chi-squared test was used to test percentages, odds ratio (OR), confidence intervals (95% CIs) were estimated. All tests were 2-sided and a $p<0.05$ was considered significant. Concentrations of 25OHD across each SNP genotype were tested for statistical significance as a 3 level variable (homozygous wild type, homozygous variant, and heterozygous).

Results. The mean age of all the patients was 48 ± 16.7 (range 18-80) years. Thirty-nine of the patients were males and 244 females. Thirteen (4.6%) subjects have vitamin D level within the normal range, 22 subjects (7.7%) were vitamin D insufficient and 248 (87.6%) were deficient. The mean 25OHD level of patients with normal vitamin D came to be 35.7 ± 3.1 ng/ml as compared with 11.53 ± 6.12 ng/ml in patients with vitamin D insufficiency and deficiency ($p<0.001$). Baseline characteristic of the study population is given in **Table 1**. Patients with vitamin D insufficiency and deficiency are significantly older, have significantly lower calcium level ($p<0.003$), and higher alkaline phosphatase ($p<0.001$) and parathormone levels ($p<0.001$) than patients with normal vitamin D levels. The percentages of the different alleles of the 6 SNPs tested ranged between 0-62.5%. **Table 2** shows the distribution of vitamin D related polymorphisms. Of the 6 SNPs tested, 3 SNPs were associated with 25OHD concentration. There was significant difference between the AA, AG, and GG alleles of VDR rs2228570. **Table 3** shows that the carries of GG allele of rs2228570 SNP was associated with increased risks of vitamin D insufficiency ($p<0.002$, OR: 0.045 and 95% CI: 0.002 to 0.809) and deficiency ($p<0.005$, OR: 0.062, 95% CI: 0.003 to 1.057). The GG allele of CYP2R1 rs10741657 SNP was also significantly over expressed in vitamin D insufficiency ($p<004$, OR: 0.047, 95% CI: 0.002 to 0.857) and deficiency ($p<0.01$, OR: 0.081 and 95% CI: 0.004 to 1.388). With regard to SNPs in vitamin D binding protein, we found that the GG allele of the GC rs4588 was associated only with significant difference between the normal and deficiency of 25OHD ($p<0.0001$ OR: 0.058, 95% CI: 0.003 to 0.006). The other 3 studied SNPs including VDR rs1544410, CYP2R1 rs1993116, and GC rs2282679 did not show any association with vitamin D level. In this study only single SNP association with 25OHD was evaluated and no evaluation for combined SNPs effect had been performed.

Discussion. In the present study, we selected SNPs in vitamin D related genes based upon previous studies from other parts of the world and particularly among Asian population.[13] Our main finding was that genetic

Table 1 - Demographic data of all patients.

Characteristics	All patients	Normal	I and D	*P*-value
Number of patients	283	13	270	
Age, years	48 ± 16.7	36 ± 6.7	50.14 ± 9.8	0.002
BMI, Kg/M^2	25.28 ± 3.91	25.75 ± 4.6	25.59 ± 3.2	0.1
Calcium, mg/dl	9.09 ± 0.39	9.6 ± 0.3	8.7 ± 0.9	0.003
Phosphorus, mg/dl	3.59 ± 0.42	3.71 ± 0.2	3.01 ± 1.2	0.2
Alkaline phosphatase, IU	113.17 ± 26.67	93.62 ± 2.5	135 ± 15.4	0.001
Vitamin D, ng/ml	12.61 ± 7.7	35.7 ± 3.1	11.53 ± 6.12	0.001
Parathormone, pg/mL and pc/ml	9.16 ± 0.32	4.7 ± 0.23	9.76 ± 4.7	0.001

BMI - body max index, N - Normal levels of vitamin D3 (≥30 ng/ml),
I and D - levels of insufficiency and deficiency of vitamin D3 (≤29 ng/ml)

variations in 3 of the 6 SNPs studied were significantly associated with lower serum levels of 25OHD in apparently healthy Saudi Arabian population. The deficiency of vitamin D in this study was over 80% and is related to age. Individuals who had GG allele of the 3 SNPs VDR rs2228570, CYP2R1 rs10741657, and GC rs4588 had significantly lower levels of 25OHD compared with normal population. Till now reports in the literature suggest strong heritable influence on the circulating vitamin D levels in different populations.[14-15] Engelman et al,[16] studied the GC SNPs rs7041 and rs4588 in African Americans and Hispanics involving

Table 2 - Distribution of vitamin D related polymorphisms in apparently healthy Saudi Arabian men and women.

Gene	SNP	Genotype	%	Vitamin D Status (n)		
				N	I	D
VDR	rs2228570	AA	9.9	0	0	28
		AG	31.8	13	4	73
		GG	58.3	0	18	147
	rs1544410	CC	41.3	4	13	100
		CT	58.6	9	9	148
		TT	0.0	0	0	0
CYP2R1 rs10741657		AA	7.7	0	0	22
		AG	46.6	13	5	114
		GG	46.6	0	17	112
	rs1993116	AA	9.2	0	5	21
		AG	44.5	6	8	112
		GG	46.3	7	9	115
GC	rs2282679	GT	34.6	6	8	84
		GG	2.9	0	0	8
		TT	62.5	7	14	156
	rs4588	GT	34.6	10	8	80
		GG	55.2	0	0	156
		TT	10.3	3	14	12

SNP - single-nucleotide polymorphism, n - number of patients, N - normal vitamin D level, I - insufficiency, D - deficiency, VDR - vitamin D receptor, GC - group-specific component

sTable 3 - Influence of high risk genotypes and vitamin D levels.

Gene	SNP	%	*P*-value (OR, 95% CI)
VDR	rs2228570		
GG Allele			
N/I		0/81.9	≤0.002 (0.045, 0.002 to 0.809)
N/D		0/59.3	≤0.005 (0.062, 0.003 to 1.057)
CYP2R1	rs10741657		
GG Allele			
N/I		0/77.3	≤0.004 (0.047, 0.002 to 0.857)
N/D		0/45.3	≤0.01 (0.081, 0.004 to 1.388)
GC	rs4588		
GG Allele			
N/D		0/62.9	≤0.0001 (0.058, 0.003 to 0.006)

VDR - vitamin D receptor, SNP - single-nucleotide polymorphism, N - normal vitamin D level, I - insufficiency, D - deficiency, GC - group-specific component, OR - odds ratio, CI - confidence interval

3 centers and over 1500 patients, and found significant relationship with the lower levels of 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D. In our study, we found similar results for SNP rs4588, but we did not study the SNP rs7041. Instead, we evaluated the effect of SNP GC rs2282679, which showed no relationship with the vitamin D levels, a finding which is different from what was reported recently by Elkum et al,[17] who found positive effect of GC rs2282679 on vitamin D levels among Arabs living in Kuwait. In general, GC SNPs rs4588 and rs2282679 showed consistent and robust association with serum 25OHD in several studies.[5,7,13] Recently both GC SNPs rs4588 and rs2282679 were found to be associated with vitamin D level among Chinese patients[13] and Danish children and adults.[18]

In this study, we also found that SNP CYP2R1 rs10741657 is associated with both insufficiency and deficiency of 25OHD, which is consistent with other studies.[7,19,20] On the other hand, Li et al,[13] did not find any association between SNP CYP2R1 rs10741657 and vitamin D level, while Elkum et al,[17] found that it was positively associated with vitamin D level among South Asian population, but not among Arabs population. We also studied the effect of SNP CYP2R1 rs1993116, and we found that it was not associated with variability in vitamin D, consistent with the finding reported by Li et al,[13] and different from the finding reported by Robien et al,[19] although both studies were carried out on Chinese population. Regarding the SNPs on VDR genes, we found SNP rs2228570 to be associated with low 25OHD level, while SNP rs1544410 was not. Both SNPs were not found to be related to vitamin D level in the previous studies.[13,19] Also, Engelman et al,[16] did not find any effect of SNP rs2228570 on vitamin D level in the African American or Hispanic population.

Low levels of vitamin D are now implicated in many serious diseases including cancer with serious implications.[21,22] Nabi et al,[23] recently hypothesized that there is a link between increased cancer risk and vitamin D deficiency among Saudi Arabs. Alghamdi et al,[24] reported that the highest incidence of breast cancer occurs in young Saudi females. Such a link needs to be further explored in a country with 100% vitamin D deficiency among young medical college students.[12] It is possible that a population in such a sunny country are taking adequate amount of dietary vitamin D and having adequate exposure to sunlight, but genetic polymorphism in vitamin D related genes influencing their vitamin D levels, thus leaving them unprotected with risk of developing serious illnesses. Our study has some limitations, including the small sample size and the small number of SNPs studied. In addition, we did not control for the confounding factors, which can alter the vitamin D levels. This study demonstrated that genetic polymorphism can influence vitamin D in such a population.

In conclusion, we believe that more SNPs should be studied in order to get some clear answers to the problem of high prevalence of low vitamin D levels, which could help in changing our clinical practice with regard to vitamin D supplementation.

Acknowledgment. *The authors would like to thank the Deanship of Scientific Research, University of Dammam, AlKhobar, Kingdom of Saudi Arabia, for funding this project.*

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Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.