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Review Article

Association between Genetic Variants of GC Gene at 4q13.3 and Vitamin D Concentrations in Adult Females

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ABSTRACT

Background: Vitamin D binding protein, encoded by the GC gene (on 4q13.3), plays an important role in transporting vitamin D. Several Genome-Wide Association Studies (GWASs) have established a significant association between variants of GC gene and circulating vitamin D.

Objective: This study aims to determine the association of GC gene polymorphisms with vitamin D concentrations in young healthy Arab females.

Methodology: 214 female subjects from Qatar University were enrolled in this cross-sectional study. The cut-off value for optimal vitamin D levels was set at 30 ng/mL. The serum vitamin D was measured using ELISA, the genotyping of SNPs (rs2298850, rs3755967, rs2282679, rs7041, rs1155563, and rs17467825) of GC gene was performed by TaqMan assay, and the data was analyzed using SPSS software.

Results: The mean age of 214 participants was found to be 21.97 years. Of these, only 182 subjects were included in this study. The data showed that 14.8% were found to have optimal vitamin D levels and 85.2% with sub-optimal levels. All studied SNPs were in HWE except SNPs rs7041 and rs1155563. Using the dominant model for rs2298850, the odds ratio to have low vitamin D is 1.48 (p=0.419). Similarly, rs3755967 has a risk of 1.62 (p=0.294); rs2282679 has an odds ratio of 1.32 (p=0.549); and rs17467825 with a risk of 1.48 (p=0.40). The genotypes for vitamin D levels had no significant difference (p>0.05) for all study subjects.

Conclusion: The current data showed no significant association between risk alleles of SNPs (rs2298850, rs3755967, rs2282679, rs7041, rs1155563, and rs17467825) with vitamin D levels.

Keywords: Vitamin D deficiency; 25-hydroxyvitamin D; GC gene; Vitamin D binding protein; SNPs

Abbreviations: 25-hydroxyvitamin D/calcifediol (25-(OH)D); 1, 25-dihydroxyvitamin D/calcitriol (1,25-(OH)2D); Vitamin D Binding Protein (DBP); Group-specific Component (GC); Ultraviolet radiation B(UVB); Vitamin D Receptor (VDR); Retinoid X Receptor (RXR); Parathyroid Hormone (PTH); DNase Hypersensitivity Site (HSIV); Single Nucleotide Polymorphisms (SNP); Chemiluminescent Immunoassay (CLIA); Chemiluminescent-Microparticle Immunoassay (CMIA); Enzyme Linked Immunosorbent Assay (ELISA); High Performance Liquid Chromatography (HPLC); Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS); Body Mass Index (BMI); Overweight and Obese (OWOB); Waist Circumference (WC); Low Density Lipoprotein (LDL); High Density Lipoprotein (HDL); Triglycerides (TG); Interleukin-6 (IL-6); Minor Allele Frequency (MAF); Hardy-Weinberg Equilibrium (HWE); Confidence Intervals (CI); Analysis of Variance (ANOVA)

INTRODUCTION

Vitamin D, a hormone belongs to a class of steroid derivatives called the secosteroids which are soluble in fat and available through certain foods and dietary supplements. The two most significant forms of Vitamin D are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol), which are obtained from generic supplements, UV-irradiated mushrooms, and fatty fish sources. Unlike other vitamins, it is synthesized in the human skin from exposure to UVB rays from the sun, on its precursor 7-dehydrocholesterol. After synthesis, it is acted upon by the enzymes in the liver hepatocytes to produce 25-(OH)D(calcifediol). This product undergoes further

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hydroxylation in the kidney to form the final product called 1,25-(OH)2D(calcitriol), which circulates the blood vessels in its active form [1].

With the help of parathyroid hormone (PTH), calcitriol is essential for regulating calcium and phosphorus levels in the body and assisting with bone development. High PTH correlates with decreased 1,25-(OH)2D and consequently, low calcium secretion and absorption, leading to vitamin D deficiency. The human body then stimulates PTH to trigger the maturation of bone cells, or osteoclasts, which then secrete calcium from the bone (bone resorption) into the blood to alleviate hypercalcemia. Consequently, the bones become softer and porous, eventually giving rise to osteoporosis [2]. Vitamin D also regulates the immune system, cardiovascular system, and glucose homeostasis [3].

Melanin, a dark pigment in the skin produced as a result of melanocytes exposed to UV radiation, contributes to reduced sun exposure. Those with darker skin, such as the African American population, are more prone to low levels of vitamin D due to high melanin than their Caucasian counterparts [4]. Certain conditions that affect intestinal functions, such as Crohn's disease, inflammatory bowel diseases, ulcerative colitis, cystic fibrosis, etc. causes malabsorption of fats, thus inducing vitamin D deficiency [1]. Additionally, liver dysfunction dramatically impacts vitamin D hydroxylation. For example, hepatic cirrhosis because of viral infection or abusive alcohol consumption causes severe vitamin D deficiency in patients due to extensive damage to their liver cells [5]. Like the liver, diseases of the kidneys considerably affect the hydroxylation process by altering the functions of renal enzymes. As the chronic renal diseases progress, creatinine clearance falls with diminishing glomerular filtration rates, and consequently, decreasing vitamin D levels [4].

The vitamin D binding protein (DBP) is a primary carrier of the metabolites of vitamin D (25-(OH)D and 1,25-(OH)2D) to target organs. DBP is encoded by 35 kb-long GC gene on chromosome 4q13.3 [6]. Structural changes in the DBP due to mutations in GC gene results in suboptimal levels of vitamin D. Single nucleotide polymorphisms (SNPs) can affect gene expression by modification of mRNA splicing and disturb the structure and stability of proteins. Consequently, the protein functions and cellular responses by target cells will be significantly affected [7]. rs7041 and rs4588 are the two most common SNPs known to affect vitamin D concentrations. Studies are still being conducted on the other SNPs scattered throughout the GC gene that are thought to affect serum 25(OH) D levels [6].

According to the values set by the Endocrinology Society, all age groups in Europe, Canada, and the United States have approximately the same increased risk of developing vitamin D insufficiency and deficiency. Despite having sufficient sun exposure, low levels of vitamin D is commonly known to be widespread in places closer to the equator such as South America, Africa, the Middle East, India, and Australia [8]. Qatar, in the Middle East, reports 90 % of its population with suboptimal serum 25-(OH) D levels, due to inadequate dietary choices, genetics, lifestyle, religious and cultural factors. Furthermore, males in this region are found to be less deficient than females, due to the choice of clothing limiting the sun exposure and in turn, correlating with hypovitaminosis D [9].

Based on previous publications, it is hypothesized that the genetic polymorphisms in the GC gene will significantly affect the vitamin D levels in the studied cohort. The aim of this study was to determine the association between genetic variants of GC gene at 4q13.3 and vitamin D concentration in healthy, adult females.

MATERIALS AND METHODS

Study Participants

This research study is both prospective and retrospective. The ethics approval for participation of human subjects was granted by Qatar University Institutional Review Board (QU-IRB 531-A/15). This study involved 214 healthy, female college students of Qatar University, who were enlisted through the university's broadcast emails, social media platforms, and health awareness events like the World Diabetes Day and Vitamin D Awareness Day held on campus. Participating students were encouraged to fill out a Doodle survey to plan their sample donations. Amidst the analysis of results, quite a few data were excluded from the study based on the eligibility criteria for inclusion like ethnicity and liver functions. Since this study's objective was to investigate the association of SNPs in the GC gene with vitamin D levels in the healthy female Arab population, subjects with non-Arab descents and those who are pregnant, lactating, taking vitamin D or calcium supplements within the last three months, and those with renal, hepatic, and autoimmune disorders were excluded.

Methods

The flowchart (supplementary figure S1) presents a concise outline of the methods employed in this study. The concentration of 25-(OH)D was measured by enzyme-linked immunosorbent assay (ELISA) kit, supplied by GENATAUR (Kampenhout, Belgium). The extraction of DNA from blood was performed using QIAamp DNA blood mini kit, provided by QIAGEN (Hilden, Germany). Lastly, the SNP genotyping was carried out by TaqMan SNP Genotyping kit, provided by Life Technologies and Applied Biosystems (Foster City, California).

Blood Collection and Sample Separation

Before their participation and blood sample donations, the volunteers were informed about the study objectives and benefits and risks involved during the procedure. They were, then, requested to fill out a questionnaire and sign a consent form. Blood samples were collected by using the purple EDTA tubes. A certified phlebotomist collected 10 mL of venous blood from students fasting overnight. Then, the samples were centrifuged at 3010 RPM for 10 minutes by ALC PK110 centrifuge. The plasma was subsequently separated and stored at -20 °C for vitamin D measurements that followed. Biochemical assays for glucose, lipid profile, triglycerides, insulin and interleukin (IL-6) were performed in biomedical labs as per the provided instructions of manufacturers.

Vitamin D Concentration Measurements

To increase the sensitivity and specificity of vitamin D detection, ELISA was utilized to measure both vitamin D2 and vitamin D3 (supplementary figure S2). The vitamin D ELISA microtiter plate provided by GENTAUR was covered with monoclonal antibodies: anti-25-(OH)D2 and anti-25-(OH)D3. These antibodies then reacted with vitamin D2 and vitamin D3 in the samples, calibrator, and control. The microplate was washed to remove excess unbound vitamin D. It was followed by treating vitamin D labeled with biotin

and horseradish peroxidase (HRP). This created a competition between vitamin D (in samples, control, and calibrator) and biotin-labeled vitamin D to bind with monoclonal antibodies. The microplate was incubated and then washed again. A chromogenic solution was added and the microplate incubated once more. In the end, a stop solution was added to cease the reaction and the absorbance of microplate read at 450 nm by a spectrophotometer. The absorbance remained inversely proportional to the total 25-(OH)D concentration. The concentration of total vitamin D samples was decided by plotting a calibration curve. The results interpreted were in accordance with clinical chemistry laboratories of Hamad Medical Corporation, which followed the vitamin D status reference values of Endocrinology Society. They are as follows: sufficiency: >30 ng/mL, insufficiency: 21-29 ng/mL, and deficiency: ≤ 20 ng/mL [8].

DNA Extraction

The lysis buffer was added to the blood samples to degrade the cell membrane of the white blood cells and centrifuged to separate and discard the cell lysates, or pellet. The proteins that remained in the supernatant were lysed by the addition of proteases. Lastly, ethanol was used to purify the DNA from the samples.

QIAamp DNA blood mini kit, catalog number 151048063, was used for DNA extraction. To each Eppendorf tube, 200 µL of blood and 20 µL of protease (ratio of 10:1 sample to protease) was added and mixed by micropipette. Then, lysis buffer (200 µL) was added to each tube and mixed by vortex for 15 seconds; the tubes then incubated for 10 minutes at 56 °C. After that, the tubes were centrifuged at 12000 RPM, followed up by the addition of ethanol and vortexing for 15 seconds.

SNP Genotyping

To identify the polymorphisms in GC gene (supplementary table S1), the TaqMan genotyping kit was used. The selected SNPs were known for their associations with DBP expression and vitamin D serum levels, as reported by previous studies [10-14].

Manual calculations (supplementary table S2) were performed to determine the amount of each component to be used from the genotyping kit. Before placing samples in the 96-well reaction plates, the TaqMan Master Mix, nuclease-free water, and 40x TaqMan SNPs (rs2298850, rs3755967, rs2282679, rs7041, rs1155563, and rs17467825) were sufficiently mixed by the vortex. To ensure no DNA contamination, one of the wells in each plate served as a negative control. Thereafter, 23 µL from the reaction solution and 2 µL of sample DNA was added to each well separately. The solution was then mixed by micropipette, the microplates covered, and PCR amplification performed by automated real-time PCR equipment. The thermal cycles were adjusted according to the protocol set by TaqMan SNP genotyping assay.

Statistical Analysis

Statistical analyses were carried out using IBM SPSS Statistics version 24 for Windows [15]. The phenotypic distribution of the cohort was represented by the mean and standard deviation (for continuous data), and frequency count and percentage (for categorical data) to assess the normal distribution curve. Independent samples t-test was employed to build an association between three groups (total subjects, optimal vitamin D subjects, and sub-optimal vitamin D subjects) and phenotype characteristics. The Pearson's Chi-square test was used to evaluate the Hardy-Weinberg equilibrium (HWE), calculate genotypic frequencies (or distribution), likelihood ratio, and the call rate to determine the quality of the selected SNPs. MedCalc statistical software was used to compute the odds ratio (OR) and the 95% confidence intervals (CI) [16]. The data in all tests were interpreted using two-tailed P-values, and the statistical significance value was set at $P<0.05$. Dominant and recessive genetic models were constructed to check for any significance. These models were used to perform a linear regression to account for confounding factors and establish a relationship between vitamin D levels (as the dependent variable) and associated phenotypes (as independent variables). Microsoft Excel 2016 aided in plotting bar graphs, and GraphPad Prism 7 in creating odds ratio distribution [17,18].

RESULTS

General Study Characteristics

The prevalence of vitamin D levels was assessed using SPSS and composed of two groups; subjects with optimal vitamin D levels (>30 ng/mL) and suboptimal vitamin D levels (insufficient/deficient with <30 ng/mL). As shown in **Figure 1**, 182 female subjects were enrolled in this study, with 27 (14.8%) subjects having optimal levels of Vitamin D, and 155 (85.2%) subjects having insufficient (n=17; 9.4%) or deficient levels (n=138; 75.8%).

Phenotypic Characteristics

This cohort was composed of subjects with an average age of 22 years old having a mean BMI of 26.35 kg/m^2 and 85.66 cm for waist circumference. A comparison was made between the two vitamin D groups using statistical methods. As shown in **Table 1**, the data demonstrated that the group with sub-optimal levels has significantly lower mean values for Vitamin D levels (p=0.000); HDL (p=0.021); and triglycerides (p=0.009) than the subjects with the optimal group. On the contrary, subjects with sub-optimal vitamin D levels have significantly higher mean values for BMI (p=0.003) and waist circumference (p=0.010), than the subjects with optimal level. No significance difference (p>0.05) was found for other variables such as OWOB (p=0.091), IL-6 concentration (p=0.283), glucose (p=0.599), insulin (p=0.526), cholesterol (p=0.073), and LDL (p=0.289).

Genotype Distribution of Studied SNPs in Vitamin D Status Groups (Optimal and Sub-optimal)

There are six SNPs that were analyzed in this study: rs2298850, rs3755967, rs2282679, rs7041, rs1155563, and rs17467825 of GC gene on chromosome 4q13.3. The Hardy–Weinberg equilibrium (HWE-P) was evaluated for the studied SNPs. As shown in supplementary table S3, HWE P-values for rs7041 and rs1155563 were less than 0.05, so they were not used in further analyses and interpretation of the findings. Pearson's Chi-Square test prompted the evaluation of frequencies of observed genotypes, and likelihood/risk ratio (χ^2) was used to determine the probability of data. Call rate (93.46%) indicated a successful analysis of SNP genotyping assay. The current data showed no significant difference (p>0.05) in the distribution of the genotypes in the following SNPs with respect to Vitamin D level status: rs2298850 (p=0.655; χ^2=1.154), rs3755967 (p=0.505; χ^2=1.825), rs2282679 (p=0.739; χ^2=0.903), rs7041 (p=0.576; χ^2=0.327), and rs17467825 (p=0.627; χ^2=1.242).

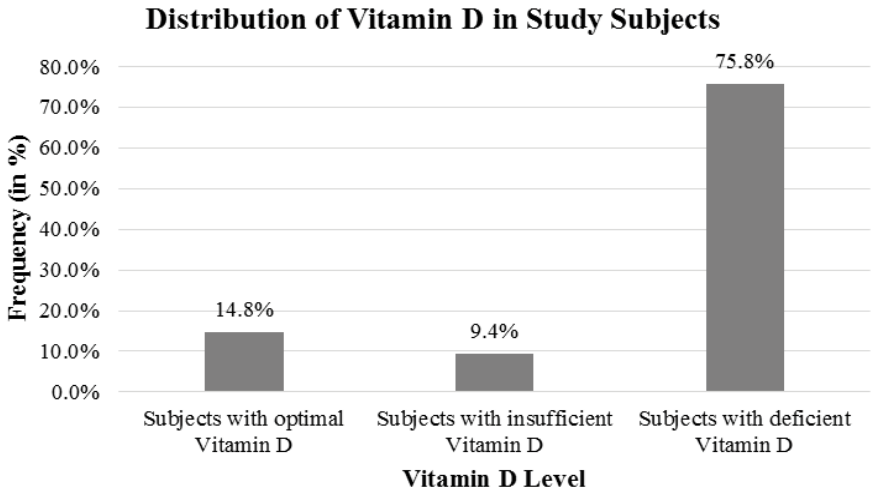

Figure 1: Illustration of the distribution of Vitamin D status among the study subjects, for three groups (optimal, insufficient, and deficient).

Table 1: Phenotype characterization of contributing factors of Vitamin D levels in this cohort.

Variables	Total number of subjects n=182	Optimal Vitamin D Levels (>30 ng/mL) n=27	Sub-optimal Vitamin D Levels (<30 ng/mL; insufficient or deficient) n= 155	P-value (2-tailed)
Vitamin D level(ng/mL)	14.88(14.67)	45.46(10.19)	9.66(6.33)	0.000*
Age(yrs.)	21.97(3.29)	21.93(3.46)	21.90(3.19)	0.967
BMI(kg/m^2)	26.35 (6.89)	23.07(3.71)	27.20(7.15)	0.003*
OWOB	88 (48.4%)	9(33.3%)	79(51.0%)	0.091
Waist Circumference(cm)	85.66(13.29)	80.07(10.20)	87.05(13.41)	0.010*
Glucose(mmol/L)	4.99(0.47)	5.03(0.38)	4.97(0.49)	0.599
Cholesterol(mmol/L)	3.99(1.16)	4.36(0.81)	3.93(1.21)	0.073
LDL(mmol/L)	2.31(1.05)	2.52(0.67)	2.29(1.10)	0.289
HDL(mmol/L)	1.30(0.41)	1.46(0.33)	1.26(0.42)	0.021*
Triglycerides(mmol/L)	0.82(0.46)	0.85(0.28)	0.82(0.49)	0.009*
Insulin(µmol/L)	8.93(5.82)	12.35(5.29)	8.46(5.65)	0.526
IL-6 Concentration(pg/mL)	3.08(3.25)	2.47(0.96)	3.21(3.49)	0.283

Note: * represents statistical significance (p<0.05). The data shown here represent the mean and standard deviation for numerical variables. The number and percentage were used for categorical values, such as OWOB (overweight and obese).

As seen in **Table 2** and **Figures 2a-f**, for all subjects in optimal and sub-optimal groups, the predominant genotype for rs2298850 is CC homozygous with G as the minor allele (14.84%). The SNPs rs3755967 and rs17467825 have predominant AA homozygous and G for the minor allele (18.13% and 17.03%, respectively). rs2282679 has CC homozygous as the most prevalent genotype and minor allele A (15.93%). rs7041 has GT heterozygous as the predominant genotype and minor allele T (40.66%). Lastly, rs1155563 has the expression of CT heterozygous alone with no major or minor allele (C:50%, T:50%). Similarly, the genotypic distribution of the SNPs was obtained for optimal, insufficient, and deficient groups of vitamin D (see supplement 4).

Association of Genetic Models with Vitamin D Status (Optimal and Sub-optimal)

Using the genotype frequencies for each SNP (Table 2), the MAF obtained was employed to construct dominant and recessive models. For rs1155563, dominant and recessive models could not be constructed since all samples have CT as the genotype, with no major or minor allele. Table 3 shows no significant association with vitamin D status among the study subjects (p>0.05) for both genetic models for the following five SNPs: rs2298850 (Dominant, p=0.43; Recessive, p=0.94), rs3755967 (Dominant, p=0.31; Recessive, p=0.88), rs2282679 (Dominant, p=0.56; Recessive, p=0.94), rs7041 (Dominant, p=0.58; Recessive, p=0.39), and rs17467825 (Dominant, p=0.40; Recessive, p=0.94). **Table 3** and supplementary figures S3 & S4 shows the odds of getting low vitamin D level below the optimal level expressed as odds ratio and 95% CI.

Vitamin D Levels Based on the Genotype

The data (supplementary table S4) were analyzed by ANOVA, with the post-hoc test for multiple Dunnett's comparisons between different genotypes. This post-hoc test revealed no significant differences (p>0.05) between the pair-wise genotypic groups of the studied SNPs, and the intergroup difference was not significant as follows: rs2298850 (p=0.739), rs3755967 (p=0.403), rs2282679 (p=0.707), rs7041 (p=0.307), and rs17467825 (p=0.719).

Correlation Coefficient between the Vitamin D Level and the Phenotypic Characteristics of the Study Subjects

The vitamin D level of this study, which is a dependent factor, was evaluated for its correlations with other independent factors or variables (supplementary tables S5 & S6). It was found that the independent variables such as age, BMI, OWOB, waist

Table 2: Genotypic distribution and minor allele frequency (MAF), for each studied SNP for optimal and sub-optimal groups.

SNP	Vitamin D Status	A/a	MAF (%)	P-value	Subject's n (%)			P-value		Likelihood Ratio
					CC	CR	RR	Dominant	Recessive	
rs2298850	Total subjects (n=182)	G/C	14.84%	0.655	130 (71.4%)	50 (27.5%)	2 (1.1%)	0.43	0.94	1.154
	Optimal Vitamin D subjects (n=27)		11.11%		21 (77.8%)	6 (22.2%)	0 (0.0%)			
	Sub-optimal Vitamin D subjects (n=155)		15.48%		109 (70.3%)	44 (28.4%)	2 (1.3%)			
rs3755967	Total subjects (n=182)	G/A	18.13%	0.505	119 (65.4%)	60 (33.0%)	3 (1.6%)	0.31	0.88	1.825
	Optimal Vitamin D subjects (n=27)		12.96%		20 (74.1%)	7 (25.9%)	0 (0.0%)			
	Sub-optimal Vitamin D subjects (n=155)		19.03%		99 (63.9%)	55 (34.2%)	3 (1.9%)			
rs2282679	Total subjects (n=182)	A/C	15.93%	0.739	126 (69.2%)	54 (29.7%)	2 (1.1%)	0.56	0.94	0.903
	Optimal Vitamin D subjects (n=27)		12.96%		20 (74.1%)	7 (25.9%)	0 (0.0%)			
	Sub-optimal Vitamin D subjects (n=155)		16.45%		106 (68.4%)	47 (30.3%)	2 (1.3%)			
rs7041	Total subjects (n=182)	T/G	40.66%	0.576	34 (18.7%)	148 (81.3%)	0 (0.0%)	0.58	0.39	0.327
	Optimal Vitamin D subjects (n=27)		42.59%		4 (14.8%)	23 (85.2%)	0 (0.0%)			
	Sub-optimal Vitamin D subjects (n=155)		40.32%		30 (19.4%)	125 (80.6%)	0 (0.0%)			
rs1155563	Total subjects (n=182)	C/T	50.00%	-	0 (0.0%)	182 (100.0%)	0 (0.0%)	-	-	-
	Optimal Vitamin D subjects (n=27)		50.00%		0 (0.0%)	27 (100.0%)	0 (0.0%)			
	Sub-optimal Vitamin D subjects (n=155)		50.00%		0 (0.0%)	155 (100.0%)	0 (0.0%)			
rs17467825	Total subjects (n=182)	G/A	17.03%	0.627	122 (67.0%)	58 (31.9%)	2 (1.1%)	0.4	0.94	1.242
	Optimal Vitamin D subjects (n=27)		12.96%		20 (74.1%)	7 (25.9%)	0 (0.0%)			
	Sub-optimal Vitamin D subjects (n=155)		17.74%		102 (65.8%)	51 (32.9%)	2 (1.3%)			

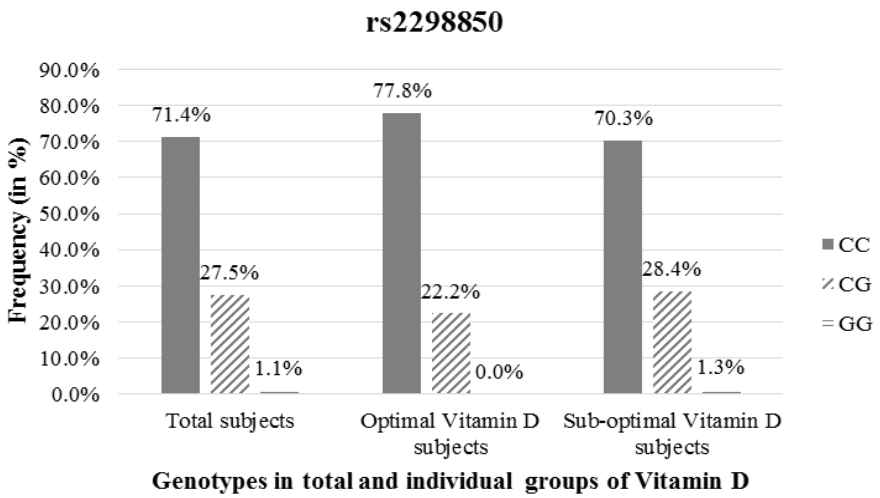

Figure 2a: Graphic representation of rs2298850 among optimal and sub-optimal Vitamin D groups.

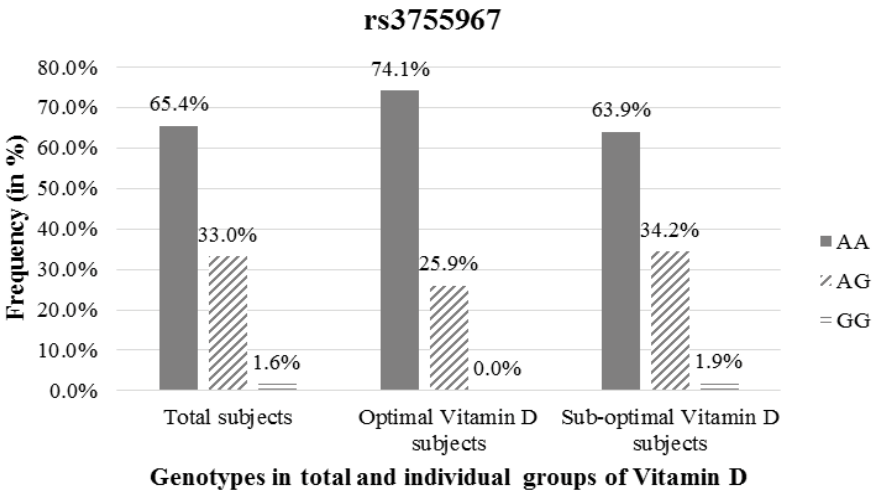

Figure 2b: Graphic representation of rs3755967 among optimal and sub-optimal Vitamin D groups.

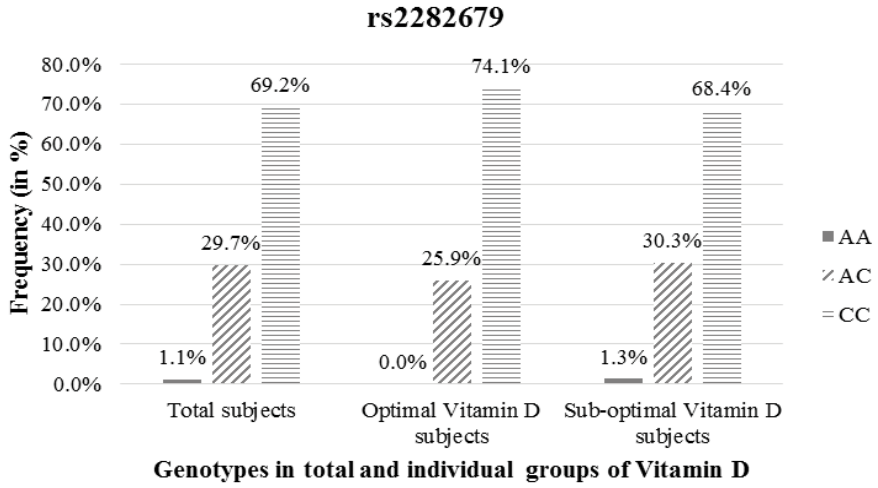

Figure 2c: Graphic representation of rs2282679 among optimal and sub-optimal Vitamin D groups.

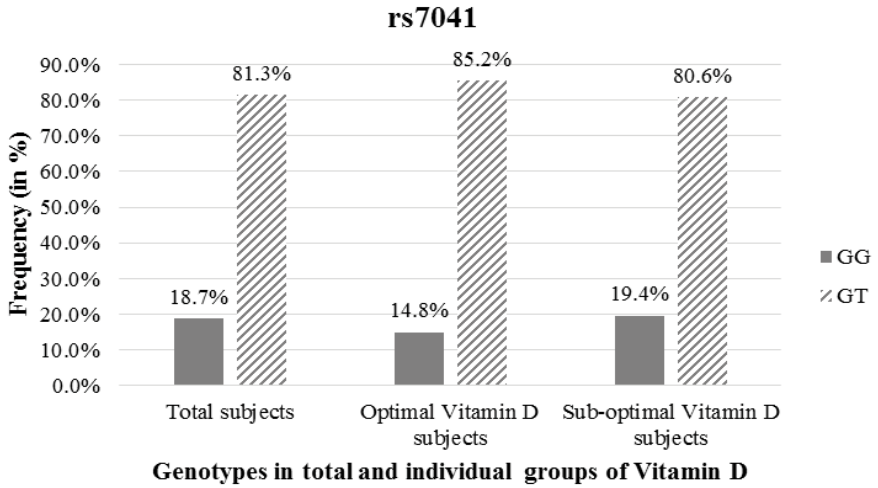

Figure 2d: Graphic representation of rs7041 among optimal and sub-optimal Vitamin D groups.

circumference, IL-6, and triglycerides showed insignificant negative correlation. In other words, the inverse linear relationship was seen with vitamin D; age (R=-0.042, p=0.574); BMI (R=-0.120, p=0.108); OWOB (R=-0.067, p=0.372); waist circumference (R=-0.153, p=0.051); IL-6 (R=-0.090, p=0.310); and triglycerides (R=-0.022, p=0.782). However, the correlation between vitamin D level with independent variables like glucose, cholesterol, LDL, HDL, and insulin showed an insignificant proportional linear relationship. Glucose (R=0.054, p=0.493); cholesterol (R=0.107, p=0.177); LDL (R=0.067, p=0.400); HDL (R=0.138, p=0.080); and insulin (R=0.182, p=0.051). The Pearson correlation values were calculated using bivariate correlation analysis and two-tailed P-value was significant at <0.05.

Association between Vitamin D Level (ng/mL) as the Dependent Variable and the Phenotype and Genotypes as Independent Variables

Linear regression analysis (**Table 4**) was performed to find out the associations between vitamin D levels (dependent variable)

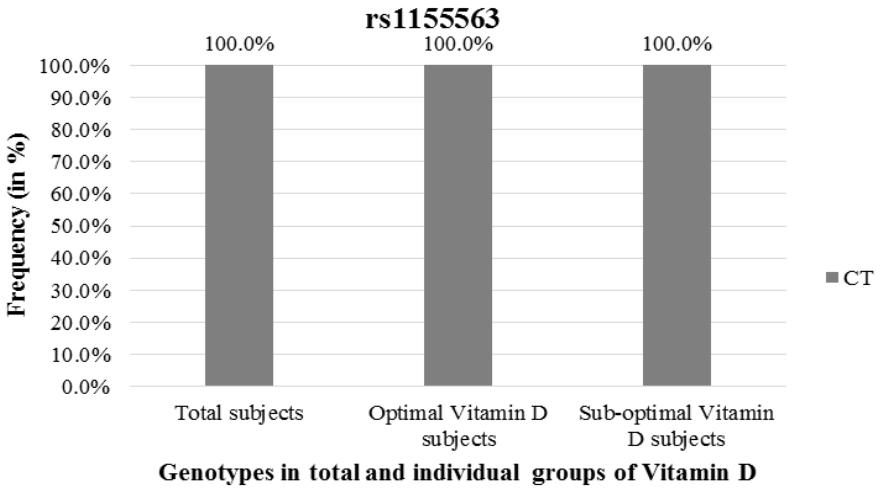

Figure 2e: Graphic representation of rs1155563 among optimal and sub-optimal Vitamin D groups.

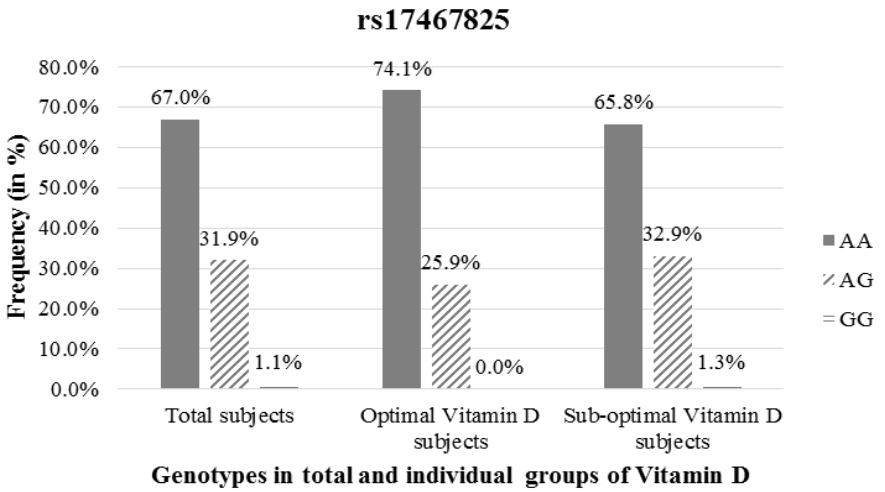

Figure 2f: Graphic representation of rs17467825 among optimal and sub-optimal Vitamin D groups.

Table 3: Genetic models constructed for each SNP and determination of the risk allele/genotype by odds ratio.

Category	Genetic Model	Genotype	Odds Ratio	95% CI	P-value
rs2298850	Dominant	GG+CG	1.48	0.56–3.90	0.419
		CC			
	Recessive	CC+CG	1.12	0.05–23.89	0.94
		GG			
rs3755967	Dominant	GG+AG	1.62	0.64–4.06	0.294
		AA			
	Recessive	AA+AG	0.79	0.04–15.77	0.88
		GG			
rs2282679	Dominant	AA+AC	1.32	0.52–3.33	0.549
		CC			
	Recessive	CC+AC	1.12	0.05–23.89	0.94
		AA			
rs7041	Dominant	TT+GT	0.72	0.23–2.25	0.58
		GG			
	Recessive	GG+GT	5.65	0.10–291.01	0.39
		TT			
rs17467825	Dominant	GG+AG	1.48	0.59–3.73	0.4
		AA			
	Recessive	AA+AG	1.12	0.05–23.89	0.94
		GG			
Note: The statistical significance was set at $p<0.05$.					

Table 4: Association of vitamin D levels and other independent factors.

Independent Variable	β-Unstandardized Coefficient	Standard Error	t value	P-value
BMI	-0.255	0.158	-1.615	0.108
WC	-0.177	0.09	-1.964	0.051
HDL	5.194	2.944	1.764	0.08
TG	-0.731	2.63	-0.278	0.782
rs2298850	-1.813	2.411	-0.752	0.453
rs3755967	-3.085	2.281	-1.352	0.178
rs2282679	-1.992	2.383	-0.836	0.404
rs17467825	-1.83	2.316	-0.79	0.43
Note: t-value = Unstandardized coefficient/Standard error. The statistical significance was set at p<0.05.				

and various selected independent variables; BMI, WC, HDL, TG, rs2298850, rs3755967, rs2282679, and rs17467825 in their dominant models. They all showed P-values >0.05 which is insignificant. The data indicate that all independent variables are not significantly associated or have no effect on vitamin D level.

DISCUSSION

The deficiency in vitamin D levels has become a main global issue in the world, with children and adults of all ages equally at elevated risk. Vitamin D is a steroid hormone and is essential for immunity, bone development, reduction of cancer risk and other vital functions. It is transported in the human body by the DBP which is encoded by GC gene. The genetic variations (SNPs) affect the expression of DBP and hence, decrease vitamin D level in blood. This study was conducted to look for the association between SNPs and the vitamin D concentrations, in healthy young adult Arab females.

The vitamin D level was measured in 182 female subjects, out of which 14.8% of them showed an optimal level of vitamin D (>30 ng/mL), while 85.2% of subjects showed a low level of vitamin D (<30 ng/mL). Such a large percentage is indicative that most participants suffer from vitamin D deficiency. These findings agree with a study done in Saudi Arabia, which showed that vitamin D deficiency was present in 87.6 % and insufficiency in 7.7% of the participants [13].

The phenotypic characteristics were analyzed for all subjects in this study. The present data demonstrated subjects with low vitamin D levels are associated with obesity phenotypes such as BMI and central obesity by waist circumference and with dyslipidemia (decreased HDL and high TG). A study that was done in Kuwait by Elkum et al. showed Arabs with high mean significant BMI and age (p<0.0001) with sub-optimal vitamin D levels. Likewise, the results were similar for BMI in this study, but the age was found to be an insignificant contributor to low-level of vitamin D [10].

In this study, the association between the genetic variants (SNPs) in GC gene and vitamin D level was investigated in adult female participants, who were healthy with no diseases or conditions impairing vitamin D metabolism. The most studied genetic variants or SNPs (rs2298850, rs3755967, rs2282679, rs7041, rs1155563, and rs17467825) were included in this study. Since rs7041 and rs1155563 deviated from the HWE, they were not included in the interpretation of the correlation and regression analyses. The major finding of the present study is that all the above four SNPs (rs2298850, rs3755967, rs2282679, and rs17467825) in the GC gene were found to be insignificantly contributing to the low-level of vitamin D in adult females.

The data presented for rs2298850 in this cohort did not show any significant association between vitamin D levels, with G as the risk allele. On the other hand, the findings of Elkum et al.'s study showed statistical significance with vitamin D, among Arabs (p=0.0374) and South Asians (p=0.0103) living in Kuwait; however, with C as the risk allele. The dissimilarity between the present research and Kuwait's study could be because of inclusion of large sample size consisting of both genders and a broad age group in the latter study, in contrast with the current study [10].

For SNP rs3755967, the current data showed no statistical significance with G as the risk allele among young Arab females. However, Suaini et al. found that rs3755967 was significantly associated with insufficient vitamin D level (risk allele A) in Caucasian infants in Australia. The obvious difference between their data and the present data is the ethnicity and age group [14].

In this study, rs2282679 has no significant association with serum 25(OH)D (minor allele A). This finding is different from the study by Elkum et al. who showed that rs2282679 (minor allele T) is significant in affecting vitamin D level in Arabs in Kuwait [10]. In their study, vitamin D was measured in Arabs and South Asian, and included both genders male and female. Five SNPs (rs17467825, rs3755967, rs2282679, rs7041 and rs2298850) in the GC gene were investigated. All of them were found to be significantly associated with vitamin D level. On the other hand, rs7041 and rs2282679 were the only two that were significantly associated with affecting vitamin D level in both Arabs and South Asians. Here, the difference in ethnicity, age and gender influenced the action of SNP in the GC gene. However, another study done in King Fahd Hospital of the University in Saudi Arabia agrees with the present results. It was found that there was no significant association between rs2282679 (but with minor allele G) and vitamin D level [13].

The current data for rs7041 shows no significant association for this cohort, although several studies have proved its significance about vitamin D levels. For example, a study conducted by Wood et al. demonstrated statistical significance (p=0.01) with serum vitamin D, risk allele T, in the United Kingdom's Caucasian subjects with α-1 antitrypsin deficiency. The discrepancy arises due to the inclusion of a different ethnic group and unhealthy subjects [12].

For rs1155563, the current data did not present with any significant association with vitamin D, and the only genotype found was CT. However, the results of Elkum et al. contradict with the results of this cohort. In their study, rs1155563 demonstrated a significant association with vitamin D level (p=0.0289) among the Arab

population, and CC, CT, and TT genotypes with C as the risk allele. The difference in results could be due to the inclusion of both genders and large age group [10].

There was no significant association found in SNP rs17467825 with vitamin D levels (risk allele G) in this cohort. Similar studies done in healthy Danish children and adults revealed that rs17467825 (risk allele G) is significantly associated (p=0.0230) to serum vitamin D concentrations. The disparity occurs because of the involvement of a large age group, gender, and ethnicity [11].

Further studies should involve a larger sample size with bigger age group, to observe a significant association between the GC gene and vitamin D serum concentrations. Other SNPs in the GC gene should also be analyzed to find out if there is any significant association with vitamin D in this population. The countries in the Middle East are majorly dependent on international vitamin D definitions as set by the Endocrinology Society. Therefore, the cut-off values for vitamin D status for this region can be re-evaluated to fit the Arab population better.

CONCLUSION

For the cohort studied in this research, the key finding revealed an insignificant association between vitamin D status and SNPs rs2298850, rs3755967, rs2282679, rs7041, rs1155563, and rs17467825. The current results of this study suggest that for healthy Arab young females, there are no genetic factors (in GC gene) associated with sub-optimal levels. On the contrary, it can be said that the physiological and environmental factors like an individual's lifestyle and clothing choices and other genetic factors like mutations in renal and hepatic enzymes play an equally important role in defining the vitamin D status in this study.

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COMPLIANCE WITH ETHICAL STANDARDS

The experimental design including participation of human subjects was approved by the Qatar University Institutional Review Board (QU-IRB 531-A/15), and all participants provided written informed consent.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Elham Sharif (corresponding author) designed and supervised the project. Nasser Rizk, Hajera Thakur, Tasnim Kurdi and Mariam Alwakeel equally contributed to data collection, data analysis and manuscript draft. All authors equally contributed to the final editing and revision of the manuscript.

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