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Original Article

Frequency and association of the genetic polymorphisms rs10741657 in *CYP2R1*, rs6013897 in *CYP24A1* and rs2282679 in *GC* with vitamin D profile and thyroid function in Saudi Arabian women with hypothyroidism

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Abstract: Objectives: To investigate whether variations in genes linked with vitamin D (VitD) metabolism [including a single nucleotide polymorphism (SNP) in *CYP2R1* (rs10741657), *CYP24A1* (rs6013897) and *GC* (rs2282679)] are associated with vitD and thyroid function in subjects with hypothyroidism compared to subjects without hypothyroidism. Methods: This study included 84 women aged ≥50 years with hypothyroidism and 91 healthy women in the same age group. The levels of total serum 25(OH)D, free 25(OH)D and vitamin D binding protein (VDBP) and thyroid function parameters, including TSH, free T3 and free T4, were quantified. Sanger DNA sequencing was used to detect rs10741657, rs6013897 and rs2282679. Results: No associations were detected between rs10741657, rs6013897 or rs2282679 and the vitD profile, which included VDBP, total vitD, and free vitD. The SNP rs2282679 in *GC* was associated with thyroid function, which was measured by TSH levels, but not with 25(OH)D. Conclusion: This research supports the association of genetic variation in *GC* with thyroid function. Although our findings did not reveal an association of selected SNPs in *CYP2R1* and *CYP24A1* with hypothyroidism, further studies with an expanded number of genetic variants in genes regulating vitD metabolism are needed to confirm the link between genetic variations in vitD genes and hypothyroidism.

Keywords: Vitamin D genetic polymorphism, hypothyroidism, CYP2R1, CYP24A1, GC

Introduction

Vitamin D (vitD) is primarily present in the circulation as 25-hydroxyvitamin D (25(OH)D), which is considered the representative marker of vitamin D status. 25(OH)D is first activated in the liver by 25-hydroxylase (encoded by *CYP27A1* and *CYP2R1*) and then in the kidney by 1α-hydroxylase (encoded by *CYP27B1*) to ultimately form the bioactive form of vitD, which is 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) [1]. $1,25(OH)_2D$ exerts its effect by binding to vitD receptors (VDR) present in target tissues. In addition, its inactivation is succeeded by 24-hydroxylase (*CYP24A1*) [2-4]. VitD is stored in adipose tissue and is primarily carried by vitamin D binding protein (VDBP), which is genetically encoded by *GC*. Approximately 85-90% of 25(OH)D is attached to VDBP, and a lower percentage (approximately 10-15%) is attached to albumin, while less than 1% is free [5]. Free vitD is most likely the active form of vitD that travels into target tissues [6, 7].

In earlier studies, low concentrations of 25(OH)D were related to autoimmune diseases, including Hashimoto thyroiditis, which is considered the main cause of hypothyroidism [8, 9]. In addition, several studies have confirmed the association between inadequate 25(OH)D levels and immune disease risk and severity [10-12]. Previous studies have suggested that low 25(OH)D levels limit the production of 1,25(OH)D by immune cells. This in turn debilitates innate immunity, overstimulates the inflammatory immune response, and disrupts the func-

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tion of T-cells, macrophages, and dendritic cells - all of which leads to the development of autoimmune diseases [11].

Genetic variations in genes related to the vitD metabolic pathway (including *CYP2R1, CYP-24A1*, *VDR, GC and CYP27B1)* have been frequently correlated with 25(OH)D levels [13-15]. VitD single-nucleotide polymorphisms (SNPs) are associated with thyroid autoimmune diseases [16]. However, this association has been most extensively studied with SNPs in *VDR* (mainly four SNPs including rs731236, rs7975232, rs2228570 and rs1544410) [3, 17, 18]. Therefore, the goal of the present study was to explore the correlation of vitD-related genetic polymorphisms in genes other than *VDR,* specifically SNPs in *CYP2R1 (*rs10741657), *CYP24A1* (rs6013897) and *GC* (rs2282679), with vitD and thyroid function test (TFT) results in subjects with hypothyroidism compared with subjects without hypothyroidism.

Subjects and methods

Study structure and recruitment

This study included 84 women (aged ≥50 years) diagnosed clinically with hypothyroidism according to thyroid stimulating hormone levels (TSH) above 4.68 mIU/L. The participating patients were compared with controls (n=91) of the same age group who were free of illness and had sufficient vitamin D levels [25(OH)D level ≥20 ng/ml]. All the participants were recruited from Jeddah, Saudi Arabia. The study took place at King Fahad Medical Research Centre (KFMRC), King Abdulaziz University (KAU), Jeddah, Saudi Arabia. All participants signed an informed consent form to participate in the study. Females with malignancy, malabsorption syndrome, hepatic or renal disease, rheumatoid arthritis, or intake of medication that may influence vitD levels, including vitD nutritional supplementation, anticonvulsant and glucocorticoids, were excluded from the study. In addition, females with levels of creatinine and liver enzymes higher than normal were excluded (the normal clinical level of serum creatinine in females <105 μmol/L; aspartate aminotransferase <45 U/L; alanine aminotransferase <50 U/L and alkaline phosphatase 80-280 U/L). The results of hypothyroidism patients were validated by comparing them with the results of non-hypothyroidism healthy controls. These controls were matched with hypothyroidism samples for age, sun exposure, oral vitD intake and BMI but with adequate vitD level. To investigate the association of vitD genetic polymorphisms with vitD and thyroid function in subjects with hypothyroidism subjects compared to controls, a number of SNPs in genes linked with the vitD metabolic pathway were investigated. These SNPs included rs10741657 in CYP2R1, rs6013897 in CYP24A1 and rs2282679 in GC. The associations of these SNPs with the vitD profile [total 25(OH)D, free 25(OH)D and VDBP] and TFTs [thyroid stimulating hormone (TSH), free triiodothyronine (T3) and free thyroxin (T4)] were assessed, as were other biochemical parameters [intact PTH, calcium (Ca), phosphate (PO_4), magnesium (Mg), albumin, blood glucose, lipid profile, creatinine and liver enzymes].

The ethics of the Declaration of Helsinki were considered in the study. Ethical approval for this study was obtained from the Research Ethics Committee in the Unit of Biomedical Ethics, Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University (KAU) (ref no.013-CEGMR-02-ETH).

Blood biochemical analysis

Blood samples were collected from all participants in plain and EDTA tubes. The concentrations of 25(OH)D and intact PTH in serum were quantified via a chemiluminescence immunoassay (CLIA) using a LIAISON autoanalyzer (DiaSorin Inc., Stillwater, MN, USA). The intra-assay and interassay coefficients of variation (CVs) for the samples were <5.5%. TFT parameters in serum were measured with immunoassays utilizing VITROS ECiQ (Ortho-Clinical Diagnostics Inc., Rochester, NY, USA).

Serum concentrations of lipids, glucose, calcium (Ca), magnesium (Mg), phosphate (PO_4), liver enzymes, albumin, and creatinine were assessed by the colorimetric method with a VITROS 250 Clinical Chemistry autoanalyzer (Ortho-Clinical Diagnostics Inc., Rochester, NY, USA). The intra-assay CV of the samples was 3.7%, and the interassay CV was 4%. vitD levels were categorized based on the Institute of Medicine (IOM) recommendations [19] (vitD deficiency: 25(OH)D level <12 ng/ml, vitD insufficiency: 25(OH)D concentration 12-19 ng/ml,

Table 1. Design of the PCR primers used for the selected SNPs in the *CYP2R1*, *CYP24A1* and *GC* genes

GENE	Primer Design	PCR size (bp)	*PCR (°C)
CYP2R1	F: 5'-TGTTCCCATGTCCTAAGCAA-3'	374	60
	R: 5'-CTGTCAGCCCTGGAAGACTC-3'		
CYP24A1	F: 5'-TCTCCTGAGGCAGGAACTGT-3'	477	
	R: 5'-TGATCCAAATGTCCGCACTA-3'		
GC	F: 5'-AGGATGCAATAAGGGACACG-3'	338	
	R: 5'-TGTTAGCCAGGATGGTCTCC-3'		

*Annealing PCR temperature. F is forward, and R is reverse.

and vitD sufficiency 25(OH)D level 20-50 ng/ml).

Single-nucleotide polymorphism analysis

Genomic DNA extraction was performed first with a DNA isolation kit (53104, Qiagen, Hilden, Germany), and then the concentration and purity of the DNA were evaluated with a NanoDrop spectrophotometer (ND-1000 UV-VIS). The selected genetic variants linked to vitD (rs10741657 in *CYP2R1*, rs6013897 in *CYP24A1* and rs2282679 in *GC*) were screened in the DNA samples by Sanger sequencing. The primers were first designed via the web-based Primer3 (v. 0.4.1) program (**Table 1**) and then synthesized (Macrogen Inc., Seoul, Korea) and prepared at a 3.2 pmol/μl concentration by adding nuclease-free water. The DNA samples were subsequently amplified using touch-down PCR, in which 1 μl each of forward and reverse primer were mixed and vortexed with 10 μl of GoTaq® Green Master mix (M7123, Promega, WI, USA), 11 μl of nuclease-free water and 2 μl of DNA sample in PCR tubes. The PCR tubes were subsequently spun down and placed in a thermal cycler (VERITI 96, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). To assess the quality and average molecular weight of the genomic DNA, the PCR products were visualized using agarose gel electrophoresis.

Sanger sequencing was performed first through purification of PCR products (using ethanol, sodium acetate and EDTA) and then by mixing 2 μl of purified PCR product and 5× sequencing buffer with 1 μl of primer, 1 μl of BigDye Terminator V3.1 Cycle Sequencing Kit (cat#4337455, Applied Biosystems, ThermoFisher Scientific, MA, USA) and 4 μl of nuclease-free water. The cycle sequencing reaction was then completed by placing the samples in a thermal cycler. Afterward, the cycle sequencing products were purified, denatured, and finally loaded into a sequencer (3500 genetic analyzer, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA), which produced sequencing data in the form of ABI sequence traces. Each sequence trace was paired with the corresponding reference sequence via BioEdit software (version 7.2.5).

Statistical analysis

The SPSS program (v.20 SPSS Chicago Inc., 2011) was used to statistically analyze the data in this study. Data normality was assessed by the Kolmogorov-Smirnov test. Parametric data are shown as the means ± SDs, whereas nonparametric data are depicted as the medians (IQRs). Comparisons of the hypothyroidism group with the control group were performed via independent t tests for parametric data, whereas comparisons between two groups of nonparametric data were performed via the Mann-Whitney test. The Kruskal-Wallis H test was used to compare the results of three genotype groups of each SNP. Statistical tests showing P values ≤0.05 were considered statistically significant.

Results

General characteristics of the participating women

The overall features of the patients in the two groups, those with and without hypothyroidism, are presented in **Table 2**. There was a significant difference (P<0.0001) in the level of total 25(OH)D between the two groups, with higher median total 25(OH)D in the control subjects than in the hypothyroidism subjects (30 g/ml in the controls compared with 21 ng/ml in the hypothyroidism subjects). On the other hand, the free 25(OH)D level did not significantly differ between the hypothyroidism group and the healthy group. According to the IOM classification of vitD status [14], 28% of the participants with hypothyroidism had vitD deficiency, 17%

Table 2. Characteristics of the patients with hypothyroidism and control subjects in the study

Variable	Hypothyroidism subjects (n=84)	Control subjects (n=91)	*P* value
Age (years)	58 (51-60)	56 (50-62)	0.93
BMI (kg/m^2)	32.4±7.5	29.7 (24.3-34.2)	0.28
Serum total 25(OH)D (ng/ml)	21 (11-26)	30±7	<0.0001*
Serum free 25(OH)D (pg/ml)	5.22 (3.7-7.7)	4.9 (4.09-6.3)	0.70
Serum VDBP (μg/ml)	342±151	374 (164-837)	0.31
Serum Intact PTH (pg/ml)	11.9 (7.8-16.1)	13±7	<0.0001*
Serum Albumin (g/L)	43±6	47±11	0.27
Serum Ca (mmol/L)	2.5 (2.4-2.7)	2.4±0.27	0.093
Serum PO_4 (mmol/L)	1.32 (1.25-1.51)	1.4±0.17	0.37
Serum Mg (mmol/L)	0.8 (0.8-0.9)	0.8 (0.7-0.9)	0.15
Serum total cholesterol (mmol/L)	5±1.07	5.5±1.5	0.061
Serum triglyceride (mmol/L)	1.2 (0.92-1.77)	1.4±0.55	0.59
Serum HDL-C (mmol/L)	1.5±0.44	1.25 (1.1-1.4)	0.071
Serum LDL-C (mmol/L)	4.4±2.8	3.6±1.1	0.002*
Serum VLDL-C (mmol/L)	0.55 (0.42-0.81)	0.64±0.25	0.67
Serum AST (U/L)	25 (22-27)	23 (19-27)	0.24
Serum ALT (U/L)	24 (20-31)	16 (11-20)	<0.0001*
Serum ALP (U/L)	96±30.5	91 (71-106)	0.60
Serum creatinine (μmol/L)	56 (49-70)	70±20	0.35
Serum TSH (mIU/L)	2.8 (1.4-5.5)	2.7 (1.9-3.6)	0.83
Serum Free T_4 (ng/dL)	1.25 (1.08-1.42)	1.28±0.31	0.72
Serum Free T_3 (pg/ml)	3.21 (2.72-3.74)	4 (3.75-4.35)	<0.0001*
Fasting glucose (mmol/L)	5.3±1.5	5.2±0.6	0.27

*Significant correlation (P<0.05). Normally distributed data are presented as the means ± SDs. Nonnormally distributed data are presented as medians (IQRs). Comparisons between the hypothyroidism group and the control group were performed via independent t tests for parametric data, whereas the Mann-Whitney test was used to compare nonparametric data between the two groups. BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; Ca, calcium; PO_4, phosphate; Mg, magnesium; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TSH, thyroid stimulating hormone; free T4, free thyroxin; T3, free triiodothyronine.

had vitD insufficiency, and the remaining 50% had a sufficient vitD level.

Intact PTH, free T3 and LDL-C levels were greater in the controls than in the patients with hypothyroidism (P<0.0001 for intact PTH and free T3; P=0.002 for LDL-C). On the other hand, the hypothyroidism group had significantly greater ALT levels than did the control group (P=0.007).

Variations and frequency of the SNPs rs10741657 in CYP2R1, rs6013897 in CYP24A1 and rs2282679 in GC among the participants

When screening for the three SNPs (rs10741657 in *CYP2R1,* rs6013897 in *CYP24A1* and rs2282679 in *GC*) using Sanger DNA sequencing, the obtained chromatograms (**Figure 1**) revealed that the homozygous AA genotype was the reference genotype for rs10741657, whereas the homozygous TT genotype was the reference genotype for rs6013897 and rs2282679. It was also shown that homozygous GG replaced AA in rs10741657 and TT in rs2282679, whereas homozygous AA replaced TT in rs6013897. In addition, heterozygous AG, TA and TG genotypes were observed for rs10741657*,* rs6013897, and rs2282679, respectively.

The frequencies of the genotypes and alleles for the three SNPs found in the study subjects are shown in **Table 3** and **Figure 2**. The minor allele frequencies (MAFs) of rs10741657A*,* rs6013897A and rs2282679G in the hypothyroidism subjects were 0.29, 0.31 and 0.20,

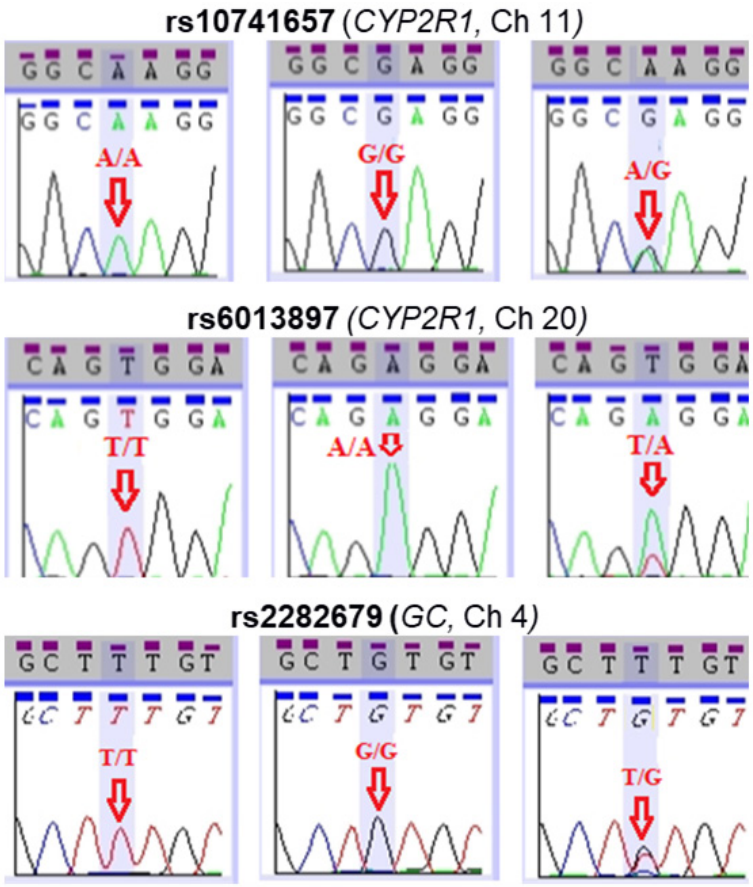

Figure 1. Sanger sequencing chromatograms showing the polymorphisms rs10741657, rs6013897 and rs2282679 among the participants.

respectively, whereas they were 0.21 for rs10741657A, 0.30 for rs6013897A and 0.10 for rs2282679G in the control subjects (**Figure 2**).

Associations of the SNPs rs10741657 in CYP2R1, rs6013897 in CYP24A1 and rs2282679 in GC with vitD and the thyroid function test

Total 25(OH)D, free 25(OH)D and VDBP serum levels were not significantly different between the three genotypes of each SNP in either the hypothyroidism group or the control group (P>0.05) (**Table 3**). Moreover, there was no statistically significant difference in the levels of the other biochemical parameters measured between the genotypes of the three SNPs in women with hypothyroidism, except for the serum TSH level. The serum TSH level was significantly different between the genotypes of rs2282679 (P=0.048), with the median TSH level being greater in the TT genotype group (3.41 mIU/L) than in the heterozygous TG genotype group (2.61 mIU/L) (**Table 4**). In addition, the biochemical results in the control group were not significantly different between the genotypes of each genetic variant (P>0.05) (data not shown).

Discussion

In this study, vitD status was lower in the hypothyroidism group than in the control group. This was anticipated, as several prior studies have revealed a robust connection between vitD deficiency and hypothyroidism and a higher prevalence of vitD deficiency among subjects with hypothyroidism [20, 21]. However, this study revealed no associations between the selected vitD SNPs (rs10741657, rs6013897 and rs2282679) in genes encoding vital proteins involved in the vitD metabolic pathway (*CYP2R1*, *CYP24A1* and *GC*) and the vitD profile [including VDBP, total 25(OH)D, and free 25(OH)D]. This contradicts previous findings that revealed a positive relationship between these genetic polymorphisms (in *CYP2R1*, *CYP24A1* and *GC*) and 25(OH)D [13, 14]. A recent systematic review of genetic polymorphisms in vital genes in the vitD metabolic pathway revealed that vitD is linked with rs2282679 in *GC*, rs10741657 in *CYP2R1* and rs6013897 in *CYP24A1* in 77%, 66%, and 17% of the studies, respectively [16]. This contrast might be due to the different ethnic populations studied and methods used to measure 25(OH)D in these studies compared with those used in this study.

VitD is essential for the normal functioning of numerous organs, including the thyroid gland. Consequently, vitD deficiency is not unanticipated as a risk factor for the development of several thyroid diseases, including hypothyroidism. Nevertheless, the interconnection between vitD and thyroid function is unclear [22]. In this study, the MAFs of the investigated vitD SNPs (rs10741657 in *CYP2R1* and

Table 3. Frequency of the genotypes of the studied SNPs and their associations with vitD parameters among patients with hypothyroidism and control subjects

Group Type	SNP (Gene)	Genotype	Frequency	Total 25(OH)D *P* value	*P*-value	Free 25(OH)D *P* value	*P*-value	VDBP *P* value	*P*-value
Hypothyroidism Group (n=84)	rs10741657 *(CYP2R1)*	AA	0.07	0.37	>0.05	0.85	>0.05	0.83	>0.05
		GG	0.48						
		AG	0.45						
	rs6013897 *(CYP24A1)*	TT	0.53	0.57		0.91		0.99	
		AA	0.15						
		TA	0.32						
	rs2282679 *(GC)*	TT	0.62	0.63♦		0.67♦		0.36♦	
		GG	0.02						
		TG	0.36						
Control Group (n=91)	rs10741657 *(CYP2R1)*	AA	0.01	0.39♦		0.93♦		0.068♦	
		GG	0.59						
		AG	0.40						
	rs6013897 *(CYP24A1)*	TT	0.50	0.30		0.52		0.76	
		AA	0.10						
		TA	0.40						
	rs2282679 *(GC)*	TT	0.90	0.88♦		0.87♦		0.19♦	
		GG	0.10						
		TG	0	-		-		-	

Nonsignificant correlation (P>0.05). The median (IQR) differences in 25(OH)D and VDBP among the three SNP categories were determined via the Kruskal-Wallis test. ♦The Mann-Whitney test was used to compare the two most common groups of genotypes. VDBP, vitamin D-binding protein; 25(OH)D, 25-hydroxyvitamin D; SNP, single-nucleotide polymorphism.

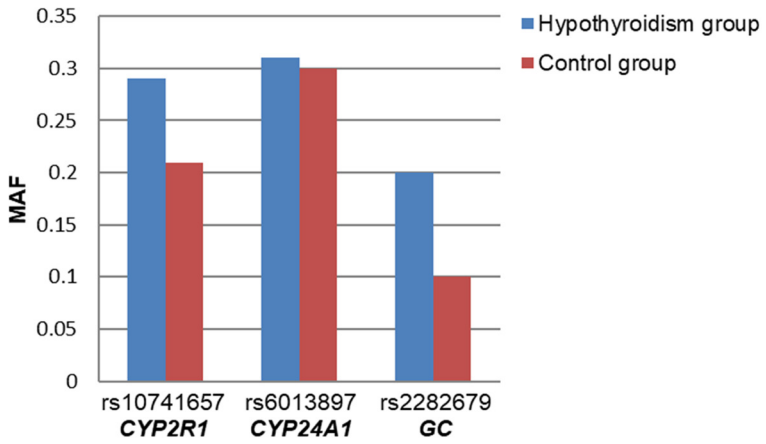

Figure 2. MAFs of the studied SNPs in the subjects of the study.

rs6013897 in *CYP24A1*) in the hypothyroidism group were similar to those in the control group with one exception: rs2282679 in *GC* had a twice as high MAF in the hypothyroidism females compared with the controls (0.20 compared with 0.1). The SNP rs2282679 in *GC* was associated with thyroid function represented by measured TSH levels but not with 25(OH)D levels. This finding could not be explained, as it was expected that this SNP would show an association with vitD, as observed previously in several studies [16, 23-25]. The level of 25(OH)D was found in a previous study to be related to the TSH level in patients with hypothyroidism after adjustment for age, sex and BMI [26]. A meta-analysis that included 20 case-control studies reported lower 25(OH)D levels in patients with autoimmune thyroid disease than in controls [27]. Research linking vitD-related SNPs with thyroid disease, with the exception of SNPs in *VDR*, is limited. The findings of studies investigating the relationship between vitD status and thyroid function are inconsistent. Therefore, challenges remain to make a strong judgment on how vitD status influences thyroid function. In healthy individuals, the 25(OH)D level is either negatively associated with or not associated with TSH [22].

Conclusion

The present study supports the association of genetic variation in *GC*, specifically rs2282679, with thyroid function. These findings support

Table 4. Biochemical parameter levels of the different genotypes of the studied SNPs in the hypothyroidism group

Variable (N=84)	rs10741657 *(CYP2R1)*			*P* value	rs6013897 *(CYP24A1)*			*P* value	rs2282679 *(GC)*			*P* value
	AA	GG	AG		TT	AA	TA		TT	GG	TG	
Serum Intact PTH (pg/ml)	50 (28-61)	28.9 (17.7-47.4)	28.4 (24.5-49.8)	>0.05	28.1 (21.8-49.9)	28.6 (18.7-57.9)	30.2 (21.8-56.8)	>0.05	29.3 (20.2-49.9)	-	28.5 (22.6-58.3)	>0.05
Serum Albumin (g/L)	38 (31-39)	45 (39-48)	43 (40-47)		43 (38-47)	44 (38-49)	45 (41-47)		43 (39-47)	-	43 (39-48)	
Serum Ca (mmol/L)	2.38 (2.12-2.43)	2.6 (2.3-2.8)	2.5 (2.4-2.7)		2.43 (2.37-2.64)	2.56 (2.34-2.66)	2.58 (2.46-2.77)		2.51 (2.35-2.64)	-	2.51 (2.38-2.72)	
Serum PO_4 (mmol/L)	1.29 (1.20-1.45)	1.33 (1.24-1.52)	1.34 (1.28-1.56)		1.34 (1.25-1.61)	1.38 (1.95-1.52)	1.31 (1.26-1.46)		1.33 (1.24-1.54)	-	1.32 (1.27-1.49)	
Serum Mg (mmol/L)	0.70 (0.65-1)	0.8 (0.7-0.9)	0.8 (0.8-0.9)		0.81 (0.80-0.90)	0.80 (0.70-0.93)	0.80 (0.81-0.90)		0.80 (0.73-0.90)	-	0.80 (0.82-0.91)	
Serum total cholesterol (mmol/L)	4.4 (3.8-5.3)	5.1 (4.5-5.6)	5 (4.5-5.5)		5 (4.3-5.9)	5.4 (4.6-6.2)	5 (4.4.3-5.4)		5.05 (4.5-5.7)	-	4.7 (4.2-5.5)	
Serum triglyceride (mmol/L)	0.97 (0.59-1.27)	1.18 (0.88-2.09)	1.2 (0.95-1.8)		1.27 (0.93-1.86)	1.55 (1.12-2.23)	1.03 (0.82-1.29)		1.22 (0.94-1.89)	-	1 (0.77-1.30)	
Serum HDL-C (mmol/L)	1.4 (1.2-1.7)	1.5 (1.08-1,80	1.4 (1.2-1.7)		1.5 (1.2-1.7)	1.6 (1.8-1.9)	1.35 (1.07-1.6)		1.35 (1.1-1.7)	-	1.6 (1.3-1.7)	
Serum LDL-C (mmol/L)	2.9 (1.99-2.96)	3.09 (2.4-3.4)	2.8 (2.5-3.2)		2.8 (2.36-3.5)	3.18 (2.6-3.45)	2.91 (2.14-3.17)		2.91 (2.51-3.52)	-	2.78 (1.99-3.14)	
Serum VLDL-C (mmol/L)	0.58 (0.27-0.63)	0.54 (0.41-0.96)	0.55 (0.43-0.82)		0.62 (0.43-0.85)	0.72 (0.51-1.02)	0.47 (0.38-0.59)		0.56 (0.43-0.87)	-	0.47 (0.35-0.63)	
Serum AST (U/L)	23 (17-26)	25 (21.8-28.8)	25 (22-27)		24 (18-25)	26 (24-33)	27 (22-29)		26 (22-28)	-	24 (21-26)	
Serum ALT (U/L)	23 (18-24)	27.5 (23.8-32.5)	22 (16-33)		24 (21-29)	25 (20-34)	25 (18-33)		25 (20-32)	-	24 (22-32)	
Serum ALP (U/L)	118 (96-122)	90 (70-107)	90 (69-110)		88 (63-106)	96 (78-111)	101 (86-129)		95 (83-110)	-	88 (69-118)	
Serum creatinine (µmol/L)	51 (44-102)	55 (50-73)	59 (49-66)		59 (50-70)	53 (47-68)	55 (49-79)		56 (50-65)	-	53 (49-78)	
Serum TSH (mIU/L)	3.5 (1.79-3.70)	2.97 (1.05-7.20)	2.66 (1.36-5.46)		2.77 (1.78-5.87)	4.48 (3.07-18.76)	1.99 (0.48-3.85)		3.41 (1.81-7.22)	-	2.61 (0.24-3.5)	0.048*
Serum Free T_4 (ng/dL)	1.37 (1.32-1.43)	1.35 (0.99-1.71)	1.20 (1.14-1.33)		1.26 (1.3-1.4)	1.07 (0.89-1.8)	1.32 (1.17-1.51)		1.21 (1.05-1.41)	-	1.35 (1.07-1.70)	>0.05
Serum Free T_3 (pg/ml)	2.73 (1.40-3.72)	3.5 (2.61-3.73)	3.15 (2.83-3.78)		3.44 (2.81-3.77)	3.13 (2.77-3.68)	2.85 (2.64-3.85)		3.19 (2.81-3.75)	-	3.65 (2.59-3.72)	
Fasting glucose (mmol/L)	5.5 (4.6-6.5)	5.3 (4.8-5.8	5.4 (4.7-6.4)		5.3 (4.7-6.2)	5.7 (5-6.4)	5.3 (4.9-6.3)		5.3 (4.8-6.4)	-	5.4 (4.7-5.8)	

*Significant correlation (P<0.05). Median (IQR) differences in the results of biochemical tests between three SNPs categories were determined using Kruskal-Wallis test. Mann-Whitney test was used to compare between two groups of genotypes. PTH, parathyroid hormone; Ca, calcium; PO_4, phosphate; and Mg, magnesium. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TSH, thyroid stimulating hormone; free T4, free thyroxin; T3, free triiodothyronine; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; and VLDL-C, very low density lipoprotein cholesterol.

the need for further research on SNPs in *GC*, the gene encoding VDBP, and influences on the bioavailability of vitD in circulation. Although our findings did not reveal an association of the other two SNPs related to vitD (rs10741657 in *CYP2R1* and rs6013897 in *CYP24A1*) with hypothyroidism, these findings cannot negate the association of vitD SNPs with hypothyroidism. The small number of SNPs investigated in the current study is considered one of its limitations. Further studies with expanded numbers of SNPs in genes interrelated with vitD metabolism are needed to ascertain the existence of a link between genetic polymorphisms in vitD-associated genes and hypothyroidism.

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Disclosure of conflict of interest

None.

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