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# Association of VDR-gene variants with factors related to the metabolic syndrome, type 2 diabetes and vitamin D deficiency

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ABSTRACT

The prevalence of metabolic syndrome (MetS) is rising alarmingly in the Saudi Arabian population. This study was conducted to assess the association between vitamin D receptor (VDR) polymorphisms and genetic susceptibility to components of the metabolic syndrome, type 2 diabetes mellitus (T2DM), and vitamin D deficiency in the Saudi Arabian population. Five-hundred-seventy Saudi individuals (285 MetS and 285 controls) were enrolled in this cross-sectional study. TaqI, BsmI, ApaI and FokI single nucleotide polymorphisms (SNPs) of the VDR gene were genotyped. The CT genotype and allele T of BsmI were associated with lower HDL-C levels [OR 0.60 (0.37, 0.96), p = 0.03] and obesity [OR 1.4 (1.0, 1.90), p = 0.04], respectively. The CT genotype and the dominant model CT + TT of BsmI were associated with increased risk of diabetes [OR 1.7 (1.2, 2.4), p = 0.007], and [OR 1.5 (1.1, 2.2), p = 0.01], respectively. On the contrary, the CT and CT + CC genotypes of FokI exhibited an association with a reduced risk of diabetes [OR 0.70 (0.49, 0.99), p = 0.05] and [OR 0.67 (0.48, 0.94), p = 0.02], respectively. The allele C of FokI was associated with lower risk of developing T2DM [OR 0.73 (0.56, 0.95), p = 0.02]. The prevalence of vitamin D deficiency was lower in subjects with the AC genotype of ApaI [OR, 0.34 (0.14, 0.80), p = 0.01]. Components of the MetS such as obesity, low HDL and T2DM were associated with the *VDR* gene. FokI and BsmI have protective and facilitative effects on the risk for T2DM, while the ApaI genotype was associated with reduced vitamin D deficiency.



## 1. Introduction

The components of metabolic syndrome (MetS) (dyslipidemia, hyperglycemia, hypertension and obesity), individually and cumulatively, increase the risk of developing T2DM and cardiovascular diseases (CVD) (Alkharfy et al., 2012). Moreover, the involvement of vitamin D in the prevalence of metabolic syndrome has also been suggested (Ford et al., 2005). The mechanism of action regarding the effect of vitamin D either includes binding of the active metabolite 1,25 $(OH)_2D_3$ with the cytosolic/nuclear VDR or via non-genomic pathways (Lips, 2006). Cytosolic/Nuclear VDR is a member of the steroid/thyroid hormone receptor family that functions as a transcriptional activator of many genes (Uitterlinden et al., 2004b). Polymorphisms in the VDR gene that produce variation in the activity of the VDR have been described in various populations (Valdivielso and Fernandez, 2006). Polymorphisms in the VDR gene have also been shown to be associated with the components of MetS, obesity and T2DM in different populations (Bid et al., 2009; Filus et al., 2008). In addition, increased susceptibility to type 1 diabetes has also been associated with allelic variations of the VDR gene (McDermott et al., 1997; Pani et al., 2000). Most VDR gene polymorphisms, including the BsmI, ApaI and TaqI restriction fragment length polymorphisms, are located at the 3′ untranslated region (3′ UTR) of the gene (Panierakis et al., 2009), while the FokI polymorphism, is localized within the 5′ end of the gene, near the promoter region (Uitterlinden et al., 2004b). Recently, we demonstrated co-segregation between VDR and HLA alleles in T2DM patients in the Saudi Arabian population (Al-Daghri et al., 2012a). Numerous cross-sectional studies have noted significant negative associations among

*Abbreviations:* MetS, metabolic syndrome; VDR, vitamin D receptor; T2DM, type 2 diabetes mellitus; single SNPs, nucleotide polymorphisms; IDF, International Diabetes Federation; HDL, high density lipoprotein; PHCC, Primary Health Care Centers; OR, odds ratio.

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circulating levels of 25-hydroxyvitamin D and cardiometabolic risk factors, highlighting potential extra skeletal functions of this sterol hormone (Al-Daghri et al., 2012b). However, there are still very limited studies that combine VDR gene polymorphisms with the components of the MetS, T2DM, and vitamin D deficiency in this part of the world.

Therefore, the aim of this study was to examine the association of four single nucleotide polymorphisms (SNPs) in intron 8 (BsmI, ApaI) exon 9 (TaqI) and exon 2 (FokI) of the VDR gene with components of MetS, T2DM, and vitamin D deficiency in the Saudi Arabian population.

## 2. Materials and methods

### 2.1. Study design

Five-hundred-seventy Saudi individuals (285 MetS patients and 285 healthy controls) were enrolled in the study. These individuals are part of the Biomarker Screening in Riyadh Project (RIYADH COHORT), a capital-wide epidemiologic study taken from over 17,000 consenting Saudis coming from different Primary Health Care Centers (PHCCs). The MetS includes waist circumference ≥102 cm for men and ≥88 cm for women, triglycerides ≥1.7 mmol/l and HDL-Cholesterol <1.03 mmol/l for men and 1.29 mmol/l for women, blood pressure ≥130/85 and fasting plasma glucose levels ≥5.6 mmol/l. Diagnosis was based on the International Diabetes Federation (IDF), which defines MetS as central obesity plus 2 other factors. Healthy control subjects were those who did not match the criteria employed for the selection of MetS subjects. A generalized questionnaire aimed to seek demographic information, past medical history, current medication and family history was given to all participating subjects. Those with co-morbidities that needed medical attention were excluded from the study. Written and informed consents were taken before inclusion. Ethics approval was granted by the Ethics Committee of the College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia (KSA).

### 2.2. Anthropometry and blood collection

Participating subjects were requested to return to their respective PHCCs after an overnight fast (>10 h) for anthropometry and blood withdrawal. Anthropometry included height (to the nearest 0.5 cm), weight (to the nearest 0.1 kg), waist and hip circumference utilizing a standardized measuring tape in cm, systolic and diastolic blood pressure measurements, and BMI calculated as weight in kg divided by height in square meters. Overweight was defined as having a BMI of 25–29.9 kg/m$^2$, obesity ≥30 < 34.9 and morbid obesity >35. Vitamin D deficiency was defined as 25-(OH)D level <50 nmol/L (Bischoff-Ferrari et al., 2006). Blood was transferred immediately to a non-heparinized tube for centrifugation. Serum was then transferred to a pre-labeled plain tube, stored in ice, and delivered to the Biomarker Research Center in King Saud University on the same day.

### 2.3. Biochemical analysis

Fasting serum samples were stored in a −20 °C freezer prior to analysis. Fasting glucose (FG), lipid profile, albumin, phosphorus and calcium were measured using a chemical analyzer (Konelab, Vantaa, Finland). Serum 25-hydroxyvitamin D [25-(OH)D] was measured by enzyme linked immunosorbent assays (ELISA) (IDS Ltd, Boldon Colliery, Tyne & Wear, UK). The intra-assay variation was 1.4–7.9% and inter-assay variation was <21%.

### 2.4. VDR gene analysis

Whole blood was collected in EDTA-containing tubes and genomic DNA was isolated from whole blood by using the blood genomic prep minispin kit (GE healthcare, Piscataway, NJ, USA), stored at −20 °C until analyzed. The four VDR SNPs (rs731236, rs1544410, rs7975232, rs10735810) were evaluated by allelic discrimination Real-time PCR using pre-designed TaqMan probes (Applied Biosystems, Foster City, CA, USA). The PCR consisted of a hot start at 95 °C for 10 min followed by 40 cycles of 94 °C for 15 s and 60 °C for 1 min. Fluorescence detection takes place at a temperature of 60 °C. All assays were performed in 10 μl reactions, using TaqMan Genotyping Master Mix on 96-well plates using an ABI 7000 instrument (Applied Biosystems, Foster City, CA, USA). Control samples representing all possible genotypes and a negative control were included in each reaction.

### 2.5. Statistical analyses

Data were analyzed using the Statistical Package for the Social Sciences for Windows (SPSS version 16.0, Chicago, IL, USA) and are expressed by mean ± standard deviation (SD). Data was checked for normality using Kolmogorov–Smirnov test. All non-Gaussian variables were either log or square root transformed. Independent sample t-test was used to test control and metabolic syndrome groups. Analysis of variance (ANOVA) was performed between genotypes for each parameters followed by Bonferroni post-hoc test. A chi-square test was used to show the allele and genotype frequency for each SNP in patients and controls. Odds ratios (ORs) and 95% confidence intervals are calculated by binomial logistic regression for the allele, genotype with metabolic diseases/complications after adjustment for covariates including gender, age and body mass index (BMI). Haplotype frequencies were estimated by the Expectation–Maximization algorithm (EM algorithm) implemented in PROC Haplotype in SAS Genetics statistical software package (SAS institute, Cary, NC, USA). The most common haplotype was used as the reference and rare haplotypes were dropped from the analysis. Tests of departures from LD were performed by using the likelihood ratio test (LR test) of linkage disequilibrium as used in PROC Allele of SAS Genetics. Pairwise LD estimations were performed using Haploview 4.2. A power estimation, based on previous studies (Filus et al., 2008; Velayoudom-Cephise et al., 2011; Ye et al., 2001), showed that our study design for specific aims (BMI, Vit D, HDL-C) had 80% power to detect a similar sized-effect ($\alpha = 0.05$). Significance was set at $p < 0.05$.

## 3. Results

The anthropometric, epidemiologic and metabolic characteristics of MetS and controls are depicted in Table 1. Anthropometric and

**Table 1**
Anthropometric, epidemiologic, and metabolic characterization of the MetS and control individuals.

| | Control | MetS | p-Value |
|---|---|---|---|
| N | 285 | 285 | – |
| Age (years) | 42.2 ± 16.2 | 49.5 ± 12.7 | <0.001 |
| BMI (kg/m$^2$) | 28.7 ± 7.2 | 32.6 ± 6.2 | <0.001 |
| Hips (cm) | 92.6 ± 25.6 | 112.7 ± 12.4 | <0.001 |
| Waist (cm) | 79.4 ± 21.7 | 105.3 ± 11.7 | <0.001 |
| Systolic BP (mm Hg) | 123.2 ± 16.4 | 127.9 ± 13.5 | 0.002 |
| Diastolic BP (mm Hg) | 76.8 ± 10.1 | 79.7 ± 7.8 | 0.001 |
| Cholesterol (mmol/l) | 5.2 ± 1.7 | 5.5 ± 1.1 | 0.02 |
| Glucose (mmol/l) | 7.4 ± 0.73 | 9.0 ± 0.65 | <0.001 |
| Triglycerides (mmol/l) | 1.5 ± 0.35 | 2.0 ± 0.34 | <0.001 |
| HDL (mmol/l) | 0.91 ± 0.32 | 0.89 ± 0.32 | 0.54 |
| LDL (mmol/l) | 3.9 ± 1.0 | 4.3 ± 1.0 | <0.001 |
| Pi (mmol/l) | 1.1 ± 0.31 | 1.2 ± 0.23 | 0.39 |
| Ca (mmol/l) | 2.5 ± 0.34 | 2.5 ± 0.24 | 0.56 |
| Corr. Ca (mmol/l) | 2.4 ± 0.35 | 2.5 ± 0.21 | 0.01 |
| Albumin (g/l) | 45.5 ± 7.7 | 44.4 ± 5.2 | 0.18 |
| Vitamin D (nmol/l) | 32.5 ± 14.2 | 26.8 ± 13.2 | <0.001 |

Data are presented as mean ± standard deviation; statistical significance is shown. Individuals with metabolic syndrome as defined by IDF; controls: Individuals free from metabolic syndrome; BP: blood pressure; HDL: high density lipoproteins; LDL: low density lipoproteins; Pi: phosphate ion; Corr. Ca: corrected calcium.