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Association of vitamin D receptor gene polymorphisms with insulin resistance and response to vitamin D

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ABSTRACT

The objectives of the study were to determine associations between single nucleotide polymorphisms (SNPs) of the vitamin D receptor (*VDR*) gene and insulin resistance and the effects of these SNPs on changes in insulin sensitivity in response to vitamin D supplementation. The research described here was an extension of the Surya study. Genotyping of the Cdx-2, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I SNPs was carried out on 239 South Asian women in New Zealand using polymerase chain reaction–based techniques. Associations of these genotypes and 3′ end haplotypes with insulin resistance were determined using multiple regression analysis. Associations between SNP genotypes and responses in insulin sensitivity to vitamin D supplementation (4000 IU vitamin D_3 per day) were also determined for a subset (81) of these women. *Bsm*I BB, *Apa*I AA, and *Taq*I tt genotypes were significantly associated with lower insulin resistance compared with *Bsm*I bb, *Apa*I aa, and *Taq*I TT, respectively, in the cohort of 239 women. Furthermore, homozygosity of the haplotypes baT and BAt was associated with higher and lower insulin resistance, respectively, compared with no copies of their respective alleles. Of the 81 subjects who were supplemented with vitamin D, women with the *Fok*I Ff genotype showed a significantly greater improvement in insulin sensitivity (increase of 29.4 [2.9, 38.1]) compared with women with the *Fok*I FF genotype (increase of 2.3 [−11.5, 10.1]). This study has highlighted the association of vitamin D responsiveness and insulin resistance with *VDR* gene polymorphisms. This is the first study to determine associations between all three. Genotyping of the *VDR* gene may provide a predictive measure for insulin resistance in response to vitamin D intervention.

Author contributions: Assoc Prof. Jane Coad and Dr. Pamela von Hurst conceived and gained funding for the Surya study. Assoc Prof. Coad, Dr. von Hurst and Assoc Prof. Welma Stonehouse carried out the original Surya study and provided the buffy coat samples. Reema Jain carried out all the experimental work under the supervision of Dr. Colleen Higgins and Assoc Prof. Donald Love. Assoc Prof. Welma Stonehouse and Dr. von Hurst provided Ms. Jain with guidance for the statistical analysis. Ms. Jain wrote the manuscript with significant input from all authors.

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1. Introduction

Vitamin D is a fat-soluble vitamin produced endogenously when the skin is exposed to ultraviolet-β radiation: a small amount may also be ingested from the diet. The actions of vitamin D are mediated by the vitamin D receptor (VDR), a nuclear receptor with a DNA-binding domain that acts through vitamin D response elements located near the start site of target genes [1]. The presence of the VDR in pancreatic β-cells [2] supports the findings of earlier studies that suggested that vitamin D affected insulin secretion [3], and the discovery of a vitamin D response element on the insulin receptor gene suggests a mechanism by which vitamin D deficiency could affect insulin sensitivity [4]. Epidemiological and prospective studies have proposed an inverse relationship between vitamin D status and risk of developing diabetes, insulin resistance (IR), and metabolic syndrome [5-10].

Recent studies have suggested that single nucleotide polymorphisms (SNPs) within the *VDR* gene may influence the stability, quantity, and activity of VDR protein and the rate of *VDR* gene transcription [11]. Genetic association studies have reported a link between *VDR* gene polymorphisms and IR, type 2 diabetes mellitus, obesity, and responsiveness to calcium and vitamin D supplementation [12-14]. Five polymorphisms of the *VDR* gene (Cdx-2, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I) have been most commonly studied; and allelic variations have been shown to increase risk of poor bone health [15,16], IR [17], and diabetes [18] in a range of populations.

Genotype may also potentially affect the response to treatment. Manchanda et al [19] demonstrated a significantly better response to drug therapy for benign prostate hyperplasia patients with a particular variant of the *Taq*I genotype. However, to our knowledge, there have been no studies that have investigated the effects of *VDR* genotypes on the response in IR to vitamin D supplementation.

In New Zealand, the prevalence of type 2 diabetes mellitus in South Asians has been reported as being 3 times the national average [20]. In addition, South Asian migrants around the world demonstrate a high incidence of vitamin D deficiency [21-24]. The Surya study investigated a group of 239 South Asian women who were screened for vitamin D deficiency and IR to determine entry suitability for a vitamin D intervention trial investigating the effect of improved vitamin D status on IR. The present study is part of the Surya study, and the aims were 2-fold:

1. To ascertain if there was an association between SNP alleles at 5 loci (Cdx-2, *Fok*I, *Bsm*I, *Apa*I, and *Taq*I) and haplotypes at 3 loci (*Bsm*I, *Apa*I and *Taq*I) in the *VDR* gene and IR levels of 239 South Asian women in an observation study (phase 1).
2. To determine if allelic variants and haplotypes of the *VDR* gene affected the response of insulin sensitivity to vitamin D supplementation in 81 women from the observation study who took part in a subsequent intervention/prospective study (phase 2).

2. Methods and materials

2.1. Ethics

The study was approved by the Massey University Human Ethics Committee (Southern A), reference no. 06/67; and the subjects gave written consent before participating in the study. The registered trial number for phase 2 was ACTRN12607000642482.

2.2. Participants and study design

Genetic analysis was carried out on 239 South Asian women living in Auckland, New Zealand, who participated in the Surya study. The methodology for the Surya study was reported in detail in von Hurst et al [25]. In brief, in the first phase of the study, the health and lifestyle of 239 South Asian women (>20 years old) living in Auckland, New Zealand, were investigated. The serum 25(OH)D concentration of all 239 women screened was less than 80 nmol/L. Phase 2 was a randomized placebo-controlled intervention trial investigating the effects of vitamin D supplementation on IR in those subjects who were vitamin D deficient and insulin resistant [26]. Inclusion criteria for the intervention phase of the study were serum 25(OH)D less than 50 nmol/L and IR (homeostasis model assessment 1 for IR [HOMA-IR] >1.93). Women with overt diabetes (fasting serum glucose [FSG] >7.2 mmol/L) were referred and excluded. Eighty-one women who met the inclusion criteria completed the 6-month randomized controlled trial, receiving either 4000 IU vitamin D_3 daily (n = 42) or a placebo (n = 39). Responsiveness to the intervention was assessed by end point change in IR (homeostasis model assessment 2 for IR) and insulin sensitivity (homeostasis model assessment 2 for insulin sensitivity [HOMA2%S]) [27]. The women in phase 2 were matched for age and body mass index (BMI) and then randomly assigned to either the vitamin D or placebo interventions. Serum 25(OH)D concentrations, IR, and insulin sensitivity were measured; and buffy coat samples were collected for genetic analysis as described below. The HOMA-IR and the HOMA2%S were calculated using FSG and fasting serum insulin). The HOMA-IR [28] was used for assessing IR in 239 women in phase 1, and HOMA2%S [27] was used for assessing insulin sensitivity in phase 2 participants (see later).

2.3. Genomic DNA extraction

Genomic DNA was extracted from 50 μL buffy coat samples (adjusted with 150 μL of phosphate buffered saline) using the Invitrogen Purelink Genomic DNA kit (Carlsbad, CA, USA) according to the manufacturer's instructions, with the following alterations to increase the yield. First, the incubation time for protein digestion at 55°C was increased from 10 to 15 minutes. Second, the incubation time for the elution step was increased from 1 to 2 minutes. The purified DNA was eluted in 50 μL of elution buffer and stored at –20°C.

2.4. RFLP-PCR genotyping/haplotyping of *BsmI*, *ApaI*, and *TaqI* SNPs

The restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) method was adapted from Uitterlinden et al [29] and modified for the analysis of the *Bsm*I (rs 1544410), *Apa*I (rs 7975232), and *Taq*I (rs 731236) SNPs. The *VDR* gene region containing all 3 SNPs was amplified using the primers VDR1 (5′-CAACCAAGACTACAAGTACCGCGT-CAGTGA-3′) and VDR4 (5′-GCAACTCCTCATGGCT-GAGGTCTC-3′) [29]. Polymerase chain reaction (PCR) amplification was carried out in a final volume of 75 μL containing approximately 150 ng of DNA, 35.5 μL of 2× GoTaq Green Master Mix GreenMasterMix (Promega, Fitchburg, WI, USA) and 3 μL each of 10 μmol/L of the primers. Thermal cycling was carried out in a Techne TC-512 machine (Stone, Staffordshire, UK) as follows: 95°C for 2 minutes; followed by 30 cycles of 94°C, 60°C, and 72°C for 1 minute each; and a final extension of 72°C for 10 minutes. The PCR products were analyzed in a 1% agarose gel in 1× Tris acetate EDTA buffer at 75 V for 1.5 hours.

Ten microliters of each PCR product was incubated with restriction enzymes separately, and in combination, to provide a haplotype (*Bsm*I, *Apa*I ,and *Taq*I SNPs) for each individual. For each digest, 1.25 U of *Bsm*I (New England Biolabs Ipswich, MA, USA) or *Taq*I (Roche Applied Science, Penzberg, Germany) or 10 U of *Apa*I (Roche Applied Science) was used with 1.25 μL of 10× restriction buffer (150 mmol/L Tris-HCl [pH 7.5], 250 mmol/L NaCl, and 35 mmol/L $MgCl_2$). The reactions were incubated at 65°C for 45 minutes for *Bsm*I and *Taq*I and at 31°C for 45 minutes for *Apa*I [29]. In the case of digestions using all 3 enzymes, 2.5 U of each restriction enzyme was added to 20 μL of PCR together with 2.5 μL of 10× restriction enzyme buffer. The reactions were incubated for 45 minutes at 31°C (for digestion with *Apa*I) and then for 45 minutes at 65°C (for digestion with both *Bsm*I and *Taq*I). Digestion products were analyzed in a 1.4% agarose gel electrophoresed in 1× Tris acetate EDTA for 2.5 hours at 70 V. The presence of *Bsm*I, *Apa*I, and *Taq*I restriction enzyme sites was designated as lowercase *b*, *a*, and *t*, respectively, whereas absence was designated as uppercase *B*, *A*, and *T*, respectively.

The *Bsm*I, *Apa*I, and *Taq*I genotypes of 3 individuals (homozygotes and heterozygotes) were confirmed by sequencing the PCR products using an ABI 3100-Avant Genetic Analyzer (Applied Biosystems California, USA) and BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems); sequencing was undertaken by Ecogene DNA-based Diagnostics, Auckland, New Zealand. Sequence analysis was performed using Geneious Pro v.4.7 software [30].

2.5. RFLP-PCR genotyping of *FokI* SNP

The *Fok*I SNP (rs 10735810) was analyzed by RFLP-PCR using a modification of the method described by Rezende et al [31]. The PCR amplification was carried out in a 15-μL reaction volume containing 0.3 μL of each 10-μmol/L forward primer 5′-GATGCCAGCTGGCCCTGGCACTG-3′ and reverse primer 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′, approximately 50 ng of DNA, and 7.5 μL of GoTaq Green Master Mix (Promega). The PCR was carried out as above with an initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 63°C for 30 seconds, and extension at 72°C for 30 seconds. The final extension was conducted at 72°C for 3 minutes. Ten microliters of each PCR product was incubated with 10 U of *Fok*I (New England Biolabs) enzyme at 37°C overnight with 2 μL of the supplied restriction enzyme buffer in a total volume of 20 μL. The digested fragments were separated by electrophoresis in a 2.5% agarose gel in 0.5× Tris borate EDTA buffer at 120 V for 45 minutes.

2.6. Allele-specific multiplex PCR genotyping of Cdx-2 SNP

The method of Fang et al [32] was modified for the amplification of the Cdx-2 SNP (rs11568820). The PCR amplification was carried out in a 10-μL total volume with 1 μL of 0.8 to 1.2 μmol/L of each of the primers G-For (5′-AGGATAGA-GAAAATAATAGAAAACATT-3′) and A-Rev (5′-ACGTTAAGTT-CAGAAAGATTAATTC-3′) and 1 μL of 1.2 μmol/L of each of the primers G-Rev (5′-AACCCATAATAAGAAATAAGTTTTTAC-3′) and A-For (5′-TCCTGAGTAAACTAGGTCACAA-3′), 5 μL of GoTaq Green Master Mix (Promega), and approximately 50 ng of DNA. Thermocycling was carried out as above with an initial denaturation at 96°C for 5 minutes; followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 45 seconds; and a final extension at 72°C for 5 minutes. The PCR products were separated by electrophoresis in a 2.5% agarose gel in 0.5× Tris borate EDTA at 120 V for 1 hour.

2.7. Data analysis

Statistical analyses were performed using the SPSS package for Windows version 17 (SPSS, Chicago, IL). Genotype frequencies were examined for compliance with Hardy-Weinberg equilibrium using χ^2 analysis for the whole group (239 subjects) and for 81 subjects who entered the intervention phase of the study.

2.8. Statistics for phase 1

Categorical data are reported as frequencies, nonnormally distributed data as median (25, 75 percentiles), and normally distributed data as mean ± SD. Normality was tested using Kolmogorov-Smirnov and Shapiro-Wilk tests. The HOMA-IR was normalized by logarithmic transformations and is described as geometric mean (95% confidence interval [CI]). Associations between HOMA-IR (log data) and genotypes/haplotypes for each SNP for all 239 participants were assessed separately by multiple regression analyses. Body mass index was included in the model as the confounding factor. The genotype and haplotypes were polychotomous categorical variables, so they were handled by creating binary dummy variables. The *reference group* was defined as the homozygous group that had the higher frequency, that is, more commonly occurring in 239 participants. For example, the *Bsm*I bb genotype (n = 74) was selected as the control group compared with the BB genotype (n = 46). The homozygous group was selected as the control group, as a relationship could be better observed between 2 extreme

groups (BB vs bb) than between heterozygous and homozygous groups (Bb vs bb).

Haplotype alleles refer to the 5 possible allelic combinations for the 3 SNPs that exist in this population, whereas *haplotype genotypes* refer to the 11 combinations of these haplotype alleles that were shown to occur in these individuals. The *allele dose* was defined as the number of copies of a particular haplotype allele within an individual [29]. The associations between allele dose and HOMA-IR and between the 3 most frequent haplotype genotypes (BAt-baT, baT-baT, and baT-BAT) and HOMA-IR were also analyzed by multiple regression, while controlling for BMI; the least frequent alleles were not analyzed. All assumptions for multiple regression analysis were met; namely, residuals were normally distributed and independent (Durbin-Watson test), and variance was constant (as assessed by plotting standardized residuals against the predicted values). The level of significance was $P < .05$.

2.9. Statistics for phase 2

Differences in the genotype frequencies between placebo and vitamin D–supplemented groups were assessed using χ^2 analysis. The response to vitamin D treatment was assessed as changes in insulin sensitivity as measured by HOMA2%S. The change in HOMA2%S over the 6-month intervention period was calculated by subtracting the baseline value from the end point value. Because HOMA2%S is a result of an algorithm and was highly skewed, a nonparametric test (Kruskal-Wallis test) was used to compare the changes in HOMA2%S between different genotypes within placebo and vitamin D–treated groups separately. Post hoc analysis was done using the Mann-Whitney *U* test with Bonferroni adjustments (α/number of comparisons); the level of significance after Bonferroni adjustment was set at .016 (.05/3). Analyses that addressed haplotype allele dose and haplotype genotype could not be carried out because the frequencies of both were too small.

3. Results

3.1. Participants

Table 1 summarizes the characteristics and biochemical data for the 239 women in phase 1 of this study. The majority of participants were Indians (91%) followed by Sri Lankans (6%) and Pakistanis (3%). The women were more than 20 years of age, with a mean age of 40.6 ± 10.3 years for the whole cohort. They were well educated, with a mean of 16.2 ± 3.5 years of education, and had lived in New Zealand for 6 (4, 10) years. The mean BMI was 25.7 ± 4.3 kg/m^2, which is greater than the specific cutoff values for healthy BMI for Asian Indians of 23 kg/m^2 [33], indicating that a high proportion of the women were overweight/obese. The average blood pressure levels and lipid profiles of the women were within reference ranges. It was observed that they had low average serum 25(OH)D concentrations of 28 (18, 44.3) nmol/L. More than half (53%) of the women were insulin resistant as defined by the reference value of 1.93 [34].

Table 1 – Characteristics of 239 participants in the Surya study (phase 1)

Characteristic	Mean ± SD [a]
Age (y)	40.6 ± 10.3
Education (y)	16.2 ± 3.5
Years in New Zealand	6 (4, 10)
BMI (kg/m^2)	25.7 ± 4.3
Serum 25(OH)D (nmol/L)	28 (18, 44.3)
Systolic blood pressure (mm Hg)	117 (106, 127)
Diastolic blood pressure (mm Hg)	77.8 ± 10.3
TC (mmol/L)	4.8 ± 0.8
LDL-C (mmol/L)	2.9 ± 0.7
HDL-C (mmol/L)	1.3 (1.1, 1.5)
TG (mmol/L)	1.1 (0.8, 1.6)
FSI (mU/L)	9.3 (5.98, 12.9)
FSG (mU/L)	4.7 (4.4, 5.0)
HOMA-IR	2 (1.2, 2.8)
Insulin resistant (%) (HOMA-IR ≥1.93)	53%

LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FSI, fasting serum insulin; TG, triglyceride; TC, total cholesterol.
[a] Normally distributed data presented as mean ± SD and data not normally distributed as median (25th, 75th percentile).

3.2. Frequencies of genotypes of 5 SNPs

The most common genotypes for the *Bsm*I, *Apa*I, and *Taq*I SNPS were Bb, Aa, and TT, respectively, whereas for *Fok*I and Cdx-2, they were FF and AG, respectively (Table 2). The genotype frequencies at each SNP locus were in Hardy-Weinberg equilibrium for the cohorts of 239 and 81 participants.

The haplotypes, or combinations of SNP genotypes carried on each chromosome within an individual, was determined for the *Bsm*I, *Apa*I, and *Taq*I SNPs in all 239 participants. Five different haplotype alleles were identified for the 3′ end of the *VDR* gene, with the most common being baT (44.8%) and BAt (30.9%) (Table 3). Eleven haplotype genotypes were identified in this study (Table 3). The most common genotypes were BAt-baT (26.8%) and baT-baT (21.8%).

Table 2 – Frequencies of genotypes of all 5 SNPs of 239 participants in the Surya study

SNP	Genotype	Frequency (%)
*Bsm*I	BB	46 (19.3%)
	Bb	**119 (49.8%)**
	bb	74 (30.9%)
*Apa*I	AA	77 (32.2%)
	Aa	**110 (46%)**
	aa	52 (21.8%)
*Taq*I	TT	**113 (47.3%)**
	Tt	103 (43.1%)
	tt	23 (9.6%)
*Fok*I	FF	**139 (58.2%)**
	Ff	82 (34.3%)
	ff	18 (7.5%)
Cdx-2	AA	43 (18%)
	AG	**132 (55.2%)**
	GG	64 (26.8%)
Total		239

Figures in bold show the most common genotype.

Table 3 – Frequencies of haplotype on 1 chromosome (haplotype allele) and on 2 chromosomes (haplotype genotype) of 239 participants in the Surya study

	Frequency (%)
Haplotype alleles [a]	
baT	214 (44.8%)
BAt	148 (30.9%)
bAT	52 (10.9%)
BAT	63 (13.2%)
bAt	1 (0.2%)
Total	478 (100%)
Haplotype genotypes [b]	
BAt-baT	64 (26.8%)
baT-baT	52 (21.8%)
baT-BAT	26 (10.9%)
BAt-BAt	22 (9.1%)
bAT-baT	20 (8.4%)
BAt-bAT	20 (8.4%)
BAt-BAT	19 (7.9%)
BAT-bAT	8 (3.4%)
BAT-BAT	5 (2.1%)
bAT-bAT	2 (0.9%)
BAt-bAt	1 (0.3%)
baT-bAt	–
Total	239 (100%)

[a] Number of chromosomes.
[b] Number of individuals.

3.3. Association between genotypes and IR (phase 1)

The genotype and haplotype data were used to determine if there were any associations with IR as calculated by HOMA-IR (Table 4). There was no association between the Cdx-2 and *Fok*I genotypes and IR in this population. In contrast, the genotypes *Bsm*I BB ($P = .026$), *Apa*I AA ($P = .035$), and *Taq*I tt ($P = .022$) were significantly associated with lower IR compared with bb, aa, and TT genotypes, respectively. The unstandardized regression coefficients (*B*) presented in Table 4 can be interpreted as predicting the change in the dependent variable (HOMA-IR) when the value of the independent variable (genotype) changes with 1 unit. This analysis suggests that the presence of the b, a, and T alleles and B, A, and t alleles, each in the homozygous state, is associated with high and low IR, respectively.

Table 5 shows an evaluation of the association between allele dose effect for the 2 haplotype alleles with the highest frequency and IR. Because the frequencies of other haplotype alleles were too low, only the 2 with the highest frequencies were selected for further analyses. Homozygosity of the haplotype alleles baT and BAt appears to be associated with IR ($P = .032$ and $P = .03$, respectively) compared with no copies of their respective alleles. Therefore, baT-baT and BAt-BAt are predicted to be associated with high and low IR, respectively, compared with no copies of their respective alleles. Thus, for these haplotypes to play a role in IR, it appears that they must be present in the homozygous form; heterozygosity of these alleles did not show a significant association. When association between highly frequent haplotype genotypes and IR was evaluated, BAt-baT and baT-BAT did not show significant association with IR ($P = .305$ and .289 relative to baT-baT, respectively; data not shown).

Table 4 – Prediction of IR (HOMA-IR) by *VDR* genotypes of 239 participants in the Surya study

Genotype (n)	Unstandardized regression coefficients (95% CI) (B) [a]	HOMA-IR [b]	P [c]
Cdx-2 [d]			
GG (64) [e]	–	2.1 (1.8-2.4)	–
AG (132)	0.92 (0.79-1.07)	1.9 (1.7-2.1)	.267
AA (43)	0.95 (0.78-1.15)	1.9 (1.6-2.33)	.579
*Fok*I [d]			
FF (139) [e]	–	1.9 (1.7-2.1)	–
Ff (82)	1.00 (0.87-1.15)	1.98 (1.7-2.3)	.97
ff (18)	0.92 (0.71-1.18)	2.0 (1.5-2.7)	.5
*Bsm*I [d]			
bb (74) [e]	–	2.1 (1.8-2.4)	–
Bb (119)	0.91 (0.79-1.06)	2.0 (1.8-2.2)	.226
BB (46)	0.81 (0.67-0.97)	1.7 (1.5-2.0)	**.026**
*Apa*I [d]			
AA (77) [e]	–	1.8 (1.5-2.0)	–
Aa (110)	1.12 (0.97-1.3	2.0 (1.8-2.3)	.131
aa (52)	1.21 (1.01-1.45)	2.1 (1.8-2.4)	**.035**
*Taq*I [d]			
TT (113) [e]	–	2.0 (1.8-2.2)	–
Tt (103)	0.95 (0.83-1.08)	2.0 (1.8-2.3)	.42
tt (23)	0.77 (0.61-0.96)	1.5 (1.2-1.9)	**.022**

Multiple regression analysis using log-transformed HOMA-IR as the dependant variable and genotypes as the predictor variables, while controlling for BMI. Figures in bold show $P < .05$. Prediction equation for Cdx-2: log (HOMA-IR) = −1.232 + 0.076 BMI − 0.086AG − 0.054AA. *Fok*I: log(HOMA-IR) = −1.292 + 0.076 BMI + 0.003Ff − 0.085ff. *Bsm*I: log(HOMA-IR) = −1.217 + 0.076 BMI − 0.09Bb − 0.215BB. *Apa*I: log(HOMA-IR) = −1.386 + 0.076 BMI + 0.115Aa + 0.193aa. *Taq*I: log(HOMA-IR) = −1.243 + 0.076 BMI − 0.056Tt − 0.268tt.
[a] Presented as the antilog of the unstandardized regression coefficient: the increment or decrement in HOMA-IR compared with the control group (in brackets is the 95% CI).
[b] Presented are geometric means (95% CI in brackets).
[c] Significance of differences vs the control group.
[d] R^2 for models (including genotype and BMI) Cdx-2 = 0.313; *Fok*I = 0.308; *Bsm*I = 0.324; *Apa*I = 0.323; *Taq*I = 0.323.
[e] Control group.

3.4. Effect of genotype on response in insulin sensitivity to vitamin D supplementation

The genotype distributions for the 5 SNPs were not significantly different between the group treated with vitamin D and the placebo group. The Surya study showed that increasing serum vitamin D concentrations led to enhanced insulin sensitivity (HOMA2%S) compared with the placebo group [26]. The second aim of the study presented here was to determine whether *VDR* gene polymorphisms play a role in vitamin D–related improvements in insulin sensitivity. Table 6 shows that the change in insulin sensitivity among *Fok*I ($P = .005$) and *Taq*I ($P = .024$) genotypes was significantly different in the vitamin D–treated group; however, no association was observed between *VDR* genotypes and insulin sensitivity in response to placebo treatment.

Post hoc analysis showed that insulin sensitivity in women with the *Fok*I Ff genotype increased significantly more compared with women with the *Fok*I FF genotype ($P < .016$) (Table 6). Only 8 women (4 in the vitamin D and 4 in the placebo groups) carried the *Fok*I ff genotype. Women carrying

Table 5 – Prediction of IR (HOMA-IR) by *VDR* haplotype allele dose in 239 participants

Haplotype allele (n) [a]	Copy number (n)	Unstandardized regression coefficients (*B*) (95% CI) [b]	HOMA-IR [c]	P [d]
baT (214)	0 [e] (77)	–	1.8 (1.5-2)	–
	1 (110)	1.12 (0.97-1.3)	2 (1.8-2.3)	.128
	2 (52)	1.22 (1.02-1.45)	2.1 (1.8-2.4)	**.032**
BAt (148)	0 [e] (113)	–	1.98 (1.8-2.2)	–
	1 (104)	0.94 (0.82-1.08)	2 (1.76-2.2)	.369
	2 (22)	0.77 (0.61-0.97)	1.6 (1.2-2)	**.03**

Multiple regression analysis using log-transformed HOMA-IR as the dependant variable and genotypes as the predictor variables, while controlling for BMI. Figures in bold show $P < .05$.

[a] Other haplotype alleles were not considered because of low numbers.

[b] Presented is the antilog of unstandardized regression coefficient: the increment or decrement in HOMA-IR as compared with the control group (in brackets is the 95% CI).

[c] Presented is the geometric mean (95% CI).

[d] Significance of differences vs the control group.

[e] Control group.

the *FokI* Ff genotype would be expected to exhibit a greater improvement in insulin sensitivity following vitamin D supplements compared with the placebo group.

When further post hoc analyses were carried out for alleles at the *TaqI* SNP, significant differences were observed between women with Tt and tt genotypes ($P < .016$) but not between TT and tt, and TT and Tt genotypes (Table 6); however, the number of *TaqI* tt (n = 5) participants was small.

Because the participants were matched according to age and BMI before randomization into the vitamin D and placebo groups, these 2 confounding factors were not considered during analyses. Although changes in insulin sensitivity in

Table 6 – Differences in changes in insulin sensitivity (ΔHOMA2%S) over a 6x-month period between genotypes within vitamin D–supplemented and placebo groups (phase 2)

SNP (n)	Placebo		Vitamin D group	
	Frequency (n)	ΔHOMA2%S *	Frequency (n)	ΔHOMA2%S *
Cdx-2				
AA (19)	11	−2.5 (−20.6, 18.8)	8	19.3 (-1.1,37.8)
AG (36)	16	−6.3 (−30.3, 11.6)	20	4.95 (−5.5, 20.3)
GG (26)	12	−7.95 (−26.4, 4.5)	14	6.4 (−4.1, 43.8)
P value †		.821		.441
FokI				
FF (39)	20	−2.6 (−24.5, 18.5)	19	2.3 (−11.5, 10.1) [a]
Ff (34)	15	−7.9 (−32.2, 5.8)	19	29.4 (2.9, 38.1)[b]
ff (8)	4	−5.4 (−29.3, 16.1)	4	−1.8 (−12.3, 7.3)
P value †		.537		**.005**
BsmI				
BB (13)	6	−14.3 (−33.1, 2.4)	7	5.4 (−18.6, 11.4)
Bb (41)	17	−16.6 (−29.6, 3.1)	24	3.4 (−3.6, 32.7)
bb (27)	16	−1.5 (−7.8, 18.8)	11	20 (−2.5, 29.4)
P value †		.263		.395
ApaI				
AA (21)	9	−20.6 (−37.6, 8.1)	12	1.8 (−13.2, 11.1)
Aa (41)	19	−2.6 (−23, 5.8)	22	4.05 (−2.5, 37.1)
aa (19)	11	−2.3 (−25, 18.8)	8	20.2 (−0.2, 28.2)
P value †		.412		.219
TaqI				
TT (40)	23	−5.9 (−32.2, 17.4)	17	11.4 (−3.4, 31.3)
Tt (36)	14	−2.9 (−20.8, 7.8)	22	4.1 (−1.8, 32.7)[a]
tt (5)	2	−28.2 (−35.8, NA)	3	−18.6 (−48.6, NA)[b]
P value †		.366		**.024**

Different superscripts (*a*, *b*) indicate significant differences within genotypes ($P < .016$ after Bonferroni adjustment). However, post hoc analysis for *TaqI* between Tt and tt could not be considered because of extremely low frequency of tt (n = 5). Figures in bold show $P < .05$. NA indicates not applicable.

* Change in HOMA2%S expressed as median (25th, 75th percentile).

† As determined by Kruskal-Wallis test.

women with Cdx-2 AA, *Bsm*I bb, *Apa*I aa, and *Taq*I TT genotypes failed to reach significance in the vitamin D–supplemented group, there seemed to be a trend toward having a greater increase in insulin sensitivity compared with the other genotypes. The effect of haplotype allele dose and haplotype genotype on the response to this intervention in vitamin D and placebo groups could not be assessed because the frequencies were too low.

4. Discussion

To our knowledge, this is the first study to examine the associations between *VDR* gene polymorphisms, IR/sensitivity, and the response to treatment with vitamin D supplementation in a vitamin D–insufficient (25[OH]D <80 nmol/L) subject group.

This study found that certain *VDR* genotypes and haplotype alleles may be of predictive value for increased IR in these vitamin D–insufficient South Asian women and that the response in insulin sensitivity to vitamin D supplementation may be affected by certain *VDR* genotypes. The major strength of this study was to determine the haplotypes of 239 individuals by RFLP-PCR instead of "predicting" the haplotypes in a population by using software that results in potentially missing rare haplotypes.

Similar to a previous study [35], we also found that the *Bsm*I bb genotype was associated with high IR along with the *Apa*I aa and *Taq*I TT genotypes; this has not been shown before. Furthermore, the haplotypes BAt and baT were also associated with lower and higher IR, respectively, but only when in the homozygous form as compared with no copies of the respective alleles. An association of baT-baT and BAt-BAt haplotype genotype with higher and lower IR, respectively, could be due to linkage disequilibrium between the baT haplotype allele and a larger number of adenines (n = 18-24) in the nearby polyA variable number of tandem repeats (VNTR), whereas the BAt haplotype is linked to a smaller number of adenines (n = 13-17) [36]. Previous studies, summarized by Uitterlinden et al [37], suggested that the BAt haplotype allele might confer a better response than baT to treatments affecting bone mineral density. This might mean that BAt together with a smaller number of adenines could confer greater messenger RNA stability, resulting in higher levels of VDR protein being present in the target cell. With more VDR protein, a target cell may respond better to vitamin D or continue to function effectively in a reduced vitamin D environment. The zygosity of the BAt haplotype would be important, as 2 copies would result in the maximum amount of VDR protein compared with 1 or no copy. These studies illustrate the importance of analyzing multiple polymorphisms simultaneously to identify associations that could not have been predicted by analyzing single SNPs.

We also investigated the association between *VDR* genotypes and response to vitamin D supplementation as measured by changes in insulin sensitivity. The improvement/increase in insulin sensitivity (calculated by HOMA2%S) among those individuals with the *Fok*I Ff genotype was significantly greater compared with those with the *Fok*I FF genotype. A trend was also observed for the *Taq*I TT genotype, together with the Cdx-2 AA, *Bsm*I bb, and *Apa*I aa genotypes, being associated with greater improvement in insulin sensitivity following vitamin D supplementation. These findings could be explained from the observations in phase 1 of the study, namely, that individuals with the *Bsm*I bb, *Apa*I aa, and *Taq*I TT genotypes had higher IR (thus, lower insulin sensitivity) than those with the BB, AA, and tt genotypes, respectively, providing more room for improvement when serum 25(OH)D was increased to sufficiently high concentrations. The substantial change in insulin sensitivity in response to vitamin D treatment with the Cdx-2 AA and *Fok*I Ff genotypes could have been due to an overestimation of the magnitude of the association due to low numbers. At the same time, we cannot exclude the possibility that the response to treatment could be significantly different for each of the 5 SNPs. However, the role of these SNPs in increasing IR of an individual is unknown. A study with a larger cohort of individuals with the same study design for phase 2 may increase the number of individuals per genotype group considerably and may help to achieve stable and precise point estimates. The low numbers of certain genotypes (*Bsm*I BB, *Apa*I AA, and *Taq*I tt) and haplotypes (eg, BAt-BAt) in the phase 2 subjects could be explained by the inclusion criteria of IR (HOMA-IR >1.93). Because the BB, AA, and tt genotypes were found to be associated with low IR, fewer individuals with these genotypes and haplotype were included in phase 2.

4.1. *Strengths and weaknesses of this study*

Subjects who received 4000 IU/d of vitamin D over a period of 6 months showed improvement in insulin sensitivity, but this improvement was seen only after 6 months of supplementation and only when the serum 25(OH)D concentration was at least 80 nmol/L [25,26]. There are limitations to the Surya study that could be addressed with a larger study that included participants of both sexes who were treated with vitamin D for longer. This study included women of South Asian ethnicity; and although the genotype associations are not likely to be sex specific, it would be important to ensure that our findings are also true for men. Furthermore, it would be important to determine if the associations can be applied to other ethnicities or are specific to South Asians. The women investigated in this study all had serum 25(OH)D concentrations less than 80 nmol/L at baseline. In the intervention group, insulin sensitivity did not improve until serum concentrations were raised above this level. Therefore, it is possible that the associations observed in this study may not be seen in a vitamin D–replete population.

This study has highlighted the association of clinical phenotypes such as vitamin D responsiveness and IR with *VDR* gene polymorphisms. For instance, it could be predicted that individuals with the baT haplotype, which is the genetic background leading to higher IR, may respond well to vitamin D treatment if they are also carrying the Cdx-2 AA and *Fok*I Ff genotypes. Further exploration of the association between genotypes and response to vitamin D supplementation is required in a larger study. Thus, this study is an example of research into personalized treatment strategies. If we can identify individuals who are at greater risk for IR and those

who are more likely to be responsive to vitamin D supplementation, such treatment could be modified in terms of duration and dosage.

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Conflict of Interest

The authors are unaware of any potential conflicts of interest with regards this work.

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