Vitamin D status and its relation with insulin resistance and VDR-FokI polymorphism in Iranian non-melanoma skin cancer (NMSC) patients: a case-control study

Fatemeh Rezaiian
  Shaheed Beheshti University of Medical Sciences

Sayed Hossein Davoodi
  Shaheed Beheshti University of Medical Sciences

Bahareh Nikooyeh
  Shaheed Beheshti University of Medical Sciences

Amir Houshang Ehsani
  Tehran University of Medical Sciences

Ali Kalayi
  Shaheed Beheshti University of Medical Sciences

Nastaran Shariatzadeh
  Shaheed Beheshti University of Medical Sciences

Maliheh Zahedirad
  Shaheed Beheshti University of Medical Sciences

Tirang R. Neyestani (✉ neytr@yahoo.com)
  https://orcid.org/0000-0002-0953-2594

Research article

Abstract

Background: Sunlight exposure, the main source of endogenous vitamin D synthesis, may increase the risk of non-melanoma skin cancer (NMSC) development. Vitamin D receptor (VDR) polymorphisms are associated with 25(OH)D levels, cancer development and insulin resistance. This study was aimed to examine the associations among vitamin D status, VDR FokI polymorphism, insulin resistance and NMSC. Methods: This case-control study included 73 diagnosed cases of NMSC and 72 healthy controls from dermatology clinics at Razi Hospital, Tehran, Iran. A questionnaire was used to assess sunlight exposure. The extracted DNA from whole blood samples were genotyped. Fasting serum 25-hydroxyvitaminD (25(OH)D)), lipid profile, glucose, and insulin were measured. To evaluate insulin resistance, HOMA-IR formula was used. Results: We found a significant higher duration of cumulative sunlight exposure in cases compared with controls (p<0.001). However, 25(OH)D concentrations were not significantly different between cases and controls (30±15 vs. 29±15 ng/mL, p=0.78). Higher levels of insulin (p = 0.004) and HOMA-IR score (p= 0.019) were observed in Ff and ff genotype of FokI. We did not observe any significant increased risk of NMSC due to f allele, as compared with FF (OR =2.33, 95% CI 0.81-6.75, p=0.12). The components of lipid profile, fasting serum glucose, iPTH and anthropometric measures did not differ significantly across VDR genotypes. Conclusion: In conclusion, sunlight exposure was associated with NMSC risk. VDR FokI polymorphisms appears to influence insulin resistance in the NMSC patients. Keywords: sun exposure; non-melanoma skin cancer; polymorphisms; insulin resistance

Introduction

Suboptimal vitamin D status reported from many countries in different age and sex subpopulations is now considered a serious global health problem (1). The importance of vitamin D deficiency (VDD) is not confined to the deleterious effects on musculoskeletal system. A growing body of evidence shows the predisposing effect of VDD in many human pathologies including cardiovascular disease, diabetes, autoimmune disorders (2) and certain cancers (3-4). Skin cancers are among the most common types of human neoplasms (5). In Iran the incidence of skin cancers is increasing (6) comprising approximately 15% of all cancers (7).

Exploration of vitamin D receptor (VDR) on a vast variety of tissues and cells including basal cell and squamous cell carcinomas indicated new roles for the vitamin (8). Though several lines of evidence indicate a protective role for vitamin D against colorectal, breast, prostate and pancreatic cancers (9) , the association of vitamin D and skin cancers is intriguing. While the main natural source of vitamin D is solar ultra violet beam (UVB), direct exposure to the same wavelength of UVB has been known as the major culprit of skin cancers (10). It is, therefore, speculated that subjects with skin cancers may have longer periods of sun exposure and hence higher vitamin D status than the general population (11).

The possible link between insulin resistance (IR) and human cancers, including skin malignancies, can be a new argument (12). The associations between IR and several malignancies including colon, liver, pancreas (13), endometrium (14), breast (15), lung (16) and thyroid cancers (17) have been reported. The ameliorating

effect of vitamin D on IR has been shown by clinical trials (18). Whether this effect can mediate any anti-cancer property of vitamin D is still unknown.

Despite previous studies on relationship between 25(OH)D3 and IR, the molecular mechanisms of the role of VDR in insulin resistance remains to be determined (19-21). The *limited evidence* available suggests that two common VDR gene polymorphisms (BsmI and FokI) might have an effect on BMI, insulin resistance and serum HDL-cholesterol (21-24). However, little information is available regarding this association in cancer patients.

On the other hand, the nutrigenetic effect of VDR polymorphisms on response to vitamin D intake has been already documented (25). VDR polymorphism is characterized by altered expression levels leading to decrease or increase in vitamin D activity (26-27). FokI (rs2228570) polymorphism is the only known polymorphism that generates an altered protein (28). VDR polymorphisms have been linked to several cancer risks including prostate, breast, bowel (29-30) and skin malignancies (31-32). However, the associations of VDR polymorphisms with skin cancer risk has not been understood clearly yet (33). Data from a meta-analysis suggest that there might be a possible positive link between VDR FokI (rs2228570) and BsmI (rs1544410) polymorphisms and cutaneous malignant melanoma (CMM) and non-melanoma skin cancer (NMSC) risks (34). However, in a systematic review *no* significant *association was observed* between TaqI (rs731236) , BsmI (rs1544410) and FokI (rs2228570) polymorphisms and NMSC risk (33). With regard to the possible role of vitamin D in skin cancers and notably NMSCs, several issues may raise: (1) Do NMSC patients have longer duration of occupational direct sun exposure than unaffected people? And if yes (2) Does it have any influence on their vitamin D status? In other words, do NMSC patients have higher vitamin D status as compared to the unaffected people as reported by some studies (35-36)? (3) Is there any association between IR and NMSC? And finally (4) Is there any association among VDR FokI polymorphisms (rs2228570), vitamin D status and NMSC risk? To answer these questions, a hospital-based case-control study was conducted.

Subjects And Methods

Participants and Clinical Samples

This case-control study included 65 participants with a BCC or SCC within 3 months of diagnosis who were visited at the dermatology clinics at Razi Hospital of Tehran University of Medical Sciences in a period between September 2016 and April 2018. The controls were composed of 65 unrelated healthy volunteers who were matched for age and sex to the patients with NMSC (Table I). All participants completed a questionnaire including information on marital status, education level, medication and supplement use history, disease history, sunscreen use and mean hours of daily sun exposure. Participants who were taking any nutritional supplement including vitamin D, calcium, omega-3 and antioxidant, or medications that modify vitamin D metabolism (corticosteroids, estrogens and calcitonin) for at least 3 months preceding the study, history of any other cancers and renal or liver diseases were excluded from the study. Fasting blood samples were obtained from all participants for DNA genotyping and biochemical analyses. The study procedures were approved by the Research Council and the Ethical Committee of the National Nutrition and

Food Technology Research Institute (NNFTRI), respectively. All participants signed a written informed consent form.

Anthropometry and blood pressure

Weight was measured with light clothing and without shoes using a digital scale (Seca 808; Seca, Hamburg, Germany) to the nearest of 0.1 kg. Height was measured using a stadiometer (Seca 216, Seca, Hamburg, Germany) to the nearest of 0.1 cm. Body mass index (BMI) was calculated using the equation body weight (kg)/height^2 (m). BMI was categorized as follows: underweight (< 18.5), normal (18.5–24.9) and overweight (> 25.0) in accordance with the 2004 World Health Organization (WHO) recommendations.

Hip circumference (HC) and waist circumference (WC) were both measured by a measuring tape to the nearest 0.1 cm. WC was measured at the approximate midpoint between the lowest rib and iliac crest after a normal expiration. Hip circumference (HC) was also measured at the level of the greater trochanters. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a digital sphygmomanometer (BC08; Beurer GmbH, Ulm, Germany).

Laboratory investigations

Glycemic status and lipid profile

Fasting serum glucose, total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined using enzymatic colorimetric methods (all from Pars Azmoon, Tehran, Iran). Serum insulin was measured by means of an enzyme immunoassay (EIA) kit (Demeditec Diagnostics GmbH, Kiel, Germany). Homeostasis model assessment of insulin resistance (HOMA-IR was used as an index of insulin resistance and was calculated by the following formula (37):

HOMA-IR= ((fasting insulin (mU/mL) x fasting glucose (mg/dL))/405)

Serum calcidiol and iPTH measurements

Serum 25(OH)D and iPTH were assayed using EIA kits (both from Euroimmun, Medizinische Labordiagnostika AG, Germany). Subjects were categorized as vitamin D deficient if 25(OH)D concentration was below 20 ng/ml, insufficient with concentrations 20-29 ng/ml and sufficient with concentrations of at least 30 ng/ml (38-41).

2.3. DNA Extraction and Genotyping

Genomic DNA was isolated from whole blood samples using PrimePrep Genomic DNA isolation kit (GeNet Bio, Daejeon, South Korea) according to the manufacturer's protocol. For VDR FokI polymorphism (rs2228570) the forward primer was 5'- GTCAAAGTCTCCAGGGTCAG -3', and the reverse primer used was 5'-GCCTGCTTGCTGTTCTTAC -3'. Genotyping was done by high-resolution melting (HRM) assay using Step one plus (Applied Biosystems, Foster City, USA). The PCR reactions were carried out in a final volume of 20 μL using the 5x Hot FIREPol HRM Mix (HRM PCR buffer, HotStarTaq Plus DNA Polymerase, nucleotides and EvaGreen dye), 0.3 nM of forward and reverse primers each (final concentration) and 30 ng DNA under the

following conditions: initial denaturation-activation step at 95°C for 15 min, followed by a 40-cycle program (denaturation at 95°C for 15 s, annealing at 61°C for 20 seconds, 72°C for 20 seconds) and HRM step from 60 to 95°C rising at 0.1°C per second. Curves for each duplicate were checked on the shape and peak height to meet reproducibility. Normalized and temperature-shifted melting curves from HRM, suggestive of SNP, were distinguished, and direct Sanger sequencing was used to confirm genotyping results from the samples.

Statistical analyses

Data were expressed as mean±SD for continuous variables and frequencies for categorical variables. Normality of data was checked using Kolmogrov-Smirnov test. For between-group comparison of variables, independent sample t test, Mann−Whitney, or χ^2 tests were used when appropriate. Means of variables were compared among the different polymorphism groups using ANOVA or Kruskal-Wallis tests for data with normal or non-normal distribution, respectively. Tukey's HD correction for multiple comparisons was applied, as required. The associations between VDR FokI polymorphism and risk of NMSC were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression analyses. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit chi-squared test to compare the observed genotype frequencies with the expected frequencies among controls. All statistical analyses were done with SPSS software IBM SPSS Statistics version 23.A two-tailed value of less than 0.05 was considered statistically significant.

Results

Characteristics of the study participants

Overall, 145 participants including 73 NMSC patients and 72 healthy controls were enrolled (Table 1). The mean age of the patients and controls was 56±9 and 58±8 years, respectively, men consisted the greater proportion of both study groups. Compared with the healthy controls, NMSC subjects were significantly less educated (p=0.006). Anthropometric measures including waist circumference (p=0.016), hip circumference (p=0.02), percentage of visceral fat (p=0.008) and blood pressure (p=0.001) were all significantly greater in cases than in controls. However, BMI did *not statistically differ between two groups (p=0.8)*.

Despite significantly longer duration of sun exposure (including occupational exposure) in cases than in controls (p<0.001), there was no significant between-group difference in concentrations of circulating 25(OH)D and distribution of vitamin D status (Table 2). Though fasting serum glucose showed no significant between-group difference, serum concentrations of insulin as well as HOMA-IR were both higher in NMSC group than in controls. Other variables did not show any significant between-group difference.

Our analyses revealed that the frequencies of VDR variants in our study population were in HW equilibrium (chi^2 value = 5.094, p=0.07 for control). The genotypes of FF, Ff and ff were 57%, 26%, 16% in NMSC cases and 68.1%, 23%, 8% in healthy controls, respectively. The results of the evaluation of the association between VDR FokI polymorphism (rs2228570) and NMSC risk are demonstrated in Table 3. Logistic regression analysis did not show any significant increased risk of NMSC due to f allele (ACG codon), as compared with FF (ATG codon).

On comparison of different variables among VDR FokI variants, the measures of serum insulin and HOMA-IR were significantly higher in Ff and ff than in the FF genotype (Table 4). WHR values were higher in ff than in Ff and FF variants (p=0.032). There was no significant between-FokI-variant difference in other variables.

Discussion

We found a significant association between cumulative (including occupational) sunlight exposure and increased risk of NMSC. However, no significant difference in circulating concentrations of calcidiol between NMSC subjects and their healthy counterparts was detected. A great body of evidence indicates that chronic sunlight exposure induces most non-melanoma skin cancers (42). It is roughly estimated that only 25% of life span UV exposure occurs before the age of 18 (43). Despite the potential role of sun exposure in skin cancer development and also the potential protective effect of vitamin D against various malignancies, it is still the matter of debate that how much sunlight is needed to provide adequate levels of circulating calcidiol without exerting carcinogenicity. Findings from studies on the association between circulating calcidiol concentrations and NMSC convey conflicting results. Some case-control studies indicated an association between higher prediagnostic concentrations of circulating calcidiol and an increased risk of basal cell carcinoma (BCC) development (35-36). Along the same line of evidence, some prospective cohort studies reported an increased risk of non-melanoma and melanoma skin cancers with increasing concentrations of serum 25(OH)D (44-45). In contrast, a case-control study from Iran reported high prevalence of vitamin D deficiency in both BCC patients and healthy people (46). One of the reasons of these discrepancies may be the very high prevalence of suboptimal serum calcidiol concentrations in Iran (47-50) which may veil any possible effect of vitamin D status on NMSC risk. Longer duration of sun exposure in the NMSC group despite no significant difference in circulating calcidiol between two groups might have contributed in development of the disease.

We found no significant association between VDR FokI polymorphisms (rs2228570) and NMSC risk. Accordingly, in a nested case-control study within Nurses' Health Study, there was no significant association between FokI ff genotype and skin cancers whereas BsmI BB variant was associated with increased risk of squamous cell carcinoma (SCC) (51). In contrast with this report, a meta-analysis suggested a possible role for polymorphisms of VDR FokI and BsmI in relation to skin cancer risks including malignant melanoma and NMSC (34). Altogether, previous studies investigating the association of VDR polymorphisms with NMSC risk have generated controversial results which can be explained by genetic variations in populations and types of data collected.

Several studies have examined the possible interactions between VDR polymorphisms and vitamin D status (25, 52). However, we found no significant difference in circulating calcidiol concentrations among VDR FokI variants. But interestingly, Fok-I Ff and ff SNPs were related to increased insulin resistance in the whole study population. Furthermore, these genotypes were related to higher blood pressure, BMI, WC, total cholesterol and LDL-C in both NMSC and control participants. Our findings are consistent with previous reports (53).

VDR FokI polymorphism has been previously found to be associated with anthropometric and biochemical components of metabolic syndrome (MetS), as in a study VDR BsmI polymorphism was associated with BMI whereas FokI VDR polymorphism was related to insulin sensitivity and serum HDL cholesterol in men (22). In a case-control study, FokI VDR was significantly associated with the components of lipid profile, calcidiol and interleukin (IL)-6 plasma levels in patients without MetS and associated with HOMA-IR, serum insulin, 25(OH)D and IL-6 levels as well as WC and BMI in MetS group (21). These findings are largely in accord with those of our study. Notwithstanding, VDR gene is not the foremost determinant of 25(OH)D serum concentrations, as stated by genome-wide association (GWA) experiments (54-55). However, the evidence is indicative of the potential role of VDR gene in the pleiotropic functions of $1,25(OH)_2D_3$ and in insulin secretion (56). The role of increased IR in development of NMSCs needs to be clarified by further studies.

To the best of our knowledge, this is the first report of a possible association between Fok-I VDR gene polymorphism and IR in a NMSC population. As IR and type 2 diabetes are caused by a set of interplays between genetic and lifestyle, large-scale, population-based studies are essential to further explore the relationship between this SNP and IR and its association with skin cancer development.

Some limitations of this study are acknowledged. Participants in a hospital-based case–control study may not be representative of the whole community. Secondly, comparison of the results from different studies for association between sunlight exposure and skin cancer could be very difficult because of the various methods used to estimate sunlight exposure. In case–control studies recall bias occurs because cases tend to recall past exposures more accurately than controls. Moreover, self-reporting of sunlight exposures in the past may result in measurement error due to difficulty in recalling sun exposure habits. Like some other studies, some important NMSC risk factors, including family history and skin type of white subjects were not evaluated. However, some studies reported that Iranian skin types, based on Fitzpatrick scale, can be classified as "lightly pigmented", i.e. skin types I to IV from north to south, respectively, based on the Fitzpatrick scale (57-58) and there is no relationship between skin type and minimal erythermal dose (59). We did not examine any possible association between NMSC and other VDR polymorphisms, including BsmI.

Our data on vitamin D status were limited to a single measurement of 25(OH)D in the blood sample obtained at the enrollment time and this measurement does not necessarily reflect vitamin D status of the subjects in critical periods of life mainly childhood and young adult. Future well-designed prospective studies are to be performed to overcome the aforementioned limitations.

Conclusion

In conclusion, our results suggest that *VDR FokI* polymorphisms are not significantly associated with the development of NMSC in Iranian subjects. However, this lack of association might be due to our study's small sample size and low genetic power to detect disease susceptibility.

The present study suggests an interaction between VDR FokI polymorphism and measures of insulin resistance and circulating 25(OH)D. FokI VDR polymorphisms may be linked to insulin resistance and might represent a genetic determinant for developing MetS in NMSC patients. These findings have clinical implications. Some studies revealed that components of MetS including raised blood pressure and deranged glucose and lipid metabolism may contribute in development of skin cancers (60) and also treatment of MetS can improve skin conditions (61). Therefore, weight control, having a healthy diet and improvement of vitamin D status may all help NMSC prevention. Further studies on large population are essential, besides the genome wide association studies to determine the direct effect of VDR polymorphisms on insulin resistance.

Abbreviations

BCC: Basal cell carcinoma

BMI: Body Mass Index

DBP: Diastolic blood pressure

EIA: Enzyme immunoassay

HWE: Hardy-Weinberg equilibrium

HC: Hip circumference

HRM: high-resolution melting

HOMA-IR: Homeostasis model assessment of insulin resistance

25(OH)D: 25-hydroxycalciferol

IU: International unit

MetS: Metabolic syndrome

NMSC: Non-melanoma skin cancer

SBP: Systolic blood pressure

SCC: Squamous cell carcinoma

UV: Ultraviolet

VDD: Vitamin D deficiency

VDR: Vitamin D receptor

WC: Waist circumference

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

The study procedures were approved by the Research Council and the Ethical Committee of the National Nutrition and Food Technology Research Institute (NNFTRI), respectively. All participants signed a written informed consent form.

Consent for publication

Not applicable

Availability of data and materials

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

TN and FR designed the study. FR, AK, NS and MZ contributed to sample collection and performed the experiments. BN, TN and FR analysed the data. FR and TN wrote the paper with input from all authors.

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Tables

Table 1. Comparison of age, gender, duration of sun exposure and certain anthropometric data of NMSC subjects and healthy controls

characteristic	NMSC (n=73)	Controls (n=72)	P value
Age (years), Mean ± SD	56±9	58±8	0.36
Sex			
male	50 (68.5%)	43 (59.7%)	0.30
female	23 (31.5%)	29 (40.3%)	
Sun exposure			
Negligible	8 (11.0%)	8 (11.1%)	
10-60 min	13 (17.8%)	38 (52.8%)	<0.001
60 min to 2 h	8 (11.0%)	9 (12.5%)	
> 2 h	44 (60.3%)	17 (23.6%)	
BMI (kg /m^2)	28.04±4.3	27.88±3.8	0.80
Waist Circumference (cm)	101±10	97±9	0.016
Hip Circumference (cm)	105.4±7	102.6±6	0.02
WHR	0.95±0.05	0.94±0.05	0.14
Body fat (%)			
Truncal fat	35.7±10	34.8±8	0.53
Visceral fat	14.3±5	12.2±4	0.008
DBP (mm Hg)	13.1±2.0	12.1±1.6	0.001
SBP (mm Hg)	8.3±2	7.3±1	<0.001

BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; WHR: waist to hip ratio

Table 2. Comparison of vitamin D, glycemic and lipidemic status between NMSC patients and healthy controls

Variable	Cases (n_1=73)	Controls (n_2=72)	p-value
25(OH)D (ng/mL)	30±15	29±15	0.78
Deficient (<20ng/mL), n(%)	20(27.4)	22(30.6)	
Insufficient (20-29.9 ng/mL), n(%)	23(31.5)	21(29.2)	0.9
Sufficient (>30ng/mL), n(%)	30(41.1)	29(40.3)	
iPTH (pg/mL)	46.0±20	40.5±23	0.14
Fasting serum glucose (mg/dL)	97.7±18	94.7±34	0.52
Insulin(µIU/mL)	14.8±9	12.2±6	0.048
HOMA-IR	3.7±2.8	2.8±1.8	0.037
Triglyceride(mg/dL)	129.8±75	133.3±68	0.77
HDL(mg/dL)	49.2±9	48.8±10	0.83
LDL(mg/dL)	106.3±27	105.0±27	0.78
Total cholesterol(mg/dL)	180.6±34	179.8±35	0.9

HOMA-IR: homeostasis model assessment of insulin resistance; iPTH: Intact parathyroid hormone

Table 3. Comparison of distribution of different VDR Fok-I SNPs in NMSC patients and healthy controls

Fok-I (rs2228570) SNP	Cases (n_1=73)	Controls (n_2=72)	OR	95% CI	p value
FF	42(57%)	49(68%)	-		
Ff	19(26%)	17(23%)	2.33	0.806-6.75	0.12
ff	12(16%)	6(8%)	1.30	0.602-2.82	0.50
Ff+ff	31(42%)	23(31%)	1.57		0.19

Table 4. Comparison of anthropometric, blood pressure and biochemical measures among VI FokI (rs2228570) genotypes

ariable	Genotype (n=145)				p valuea	p valueb
	FF (n=90)	Ff (n=35)	Ff (n=16)	Ff+ff (n=51)		
aist(cm)	98.2±10	98.8±9	103.3±9	100.3±9	0.13	0.21
ip (cm)	103.6±7	104.4±6	105.2±6	104.7±6	0.65	0.39
HR	0.94±0.05	0.94±0.05	0.98±0.06	0.95±0.06	0.03	0.21
runcal fat (%)	34.9±9	34.8±8	38.2±8	35.9±8	0.35	0.25
isceral fat (%)	12.9±4	14.0±4	13.7±4	13.9±4	0.50	0.51
MI (kg/m$^{2)}$	27.6±4	28.3±3	28.8±3	28.4±3	0.48	0.26
BP (mmHg)	12.5±1.9	13.0±1.8	12.2±1.8	12.7±1.9	0.32	0.52
BP (mmHg)	7.8±1.90	7.9±1.28	7.3±1.01	7.7±1.2	0.40	0.60
5(OH)D (ng/mL)	30.6±16	28.0±14	30.1±15	28.7±14	0.69	0.46
TH (pg/mL)	41.3±20	48.3±24	42.7±25	46.4±24	0.29	0.18
SG (mg/dL)	95.5±28	99.8±31	92.0±12	97.4±26	0.60	0.70
sulin (μIU/mL)	12.0±6	16.5±9	15.0 ±9	16.0±9.2	0.01	0.004
OMA-IR	2.9±2.1	4.1±2.7	3.5±2.9	3.9±2.8	0.04	0.02
riglyceride(mg/dL)	128.1±70	130.6±69	154.6±84	137.8±74	0.41	0.43
otal holesterol(mg/dL)	179.5±34	177.5±37	189.8±33	181.3±36	0.48	0.77
DL-C (mg/dL)	49.6±10	46.9±9	50.2±9	47.9±9	0.33	0.32
DL-C (mg/dL)	104.9±26	106.1±31	108.8±23	106.9±28	0.87	0.68

a p values stands for difference between VDR FokI genotypes (FF, Ff, ff); P-values are obtained from ANOVA with post hoc Tukey HSD

b p values stands for difference between VDR FokI FF and Ff+ff genotypes.