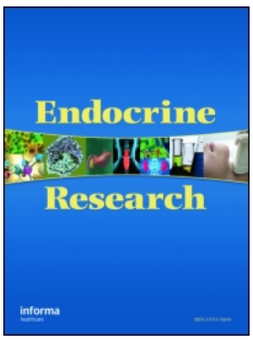

Endocrine Research

ISSN: 0743-5800 (Print) 1532-4206 (Online) Journal homepage: http://www.tandfonline.com/loi/ierc20

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To cite this article: Laura Bertoccini, Federica Sentinelli, Frida Leonetti, Diego Bailetti, Danila Capoccia, Flavia A. Cimini, Ilaria Barchetta, Michela Incani, Andrea Lenzi, Efisio Cossu, M. Gisella Cavallo & Marco G. Baroni (2017): The vitamin D receptor functional variant rs2228570 (C>T) does not associate with type 2 diabetes mellitus, Endocrine Research, DOI: 10.1080/07435800.2017.1305965

To link to this article: http://dx.doi.org/10.1080/07435800.2017.1305965

Published online: 07 Apr 2017.

ENDOCRINE RESEARCH
http://dx.doi.org/10.1080/07435800.2017.1305965

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REVIEW

The vitamin D receptor functional variant rs2228570 (C>T) does not associate with type 2 diabetes mellitus

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ABSTRACT

Aim: Vitamin D acts through the binding to the vitamin D receptor (VDR). Several polymorphisms in VDR gene have been studied. Among these, the rs2228570 C>T (FokI) variant has been demonstrated to be functional, leading to a protein with a different size and activity. So far, genetic studies on the association between VDR gene rs2228570 single nucleotide polymorphism (SNP) and type 2 diabetes mellitus (T2DM) showed contradictory results. Thus, we performed an association study in a large cohort of adult Italian subjects with T2DM and in nondiabetic controls. **Materials and methods**: For this study, 1713 subjects, 883 T2DM patients and 830 controls, were genotyped for the polymorphism. All participants without a diagnosis of diabetes underwent oral glucose tolerance test (OGTT), with measurement of glucose and insulin levels. Indices of insulin resistance (Homeostatic model assessment of insulin resistance, insulin sensitivity index), secretion (homeostatic model assessment for beta-cell, corrected insulin response at 30 minutes) and disposition index were calculated. **Results**: Genotype distributions and allele frequencies did not show difference between T2DM subjects and controls. We did not find significant differences among the three genotypes regarding gender, age, BMI, waist, hip, waist-to-hip ratio, and blood pressure. There were also no significant differences in lipid parameters, aspartate aminotransferase, and alanine aminotransferase levels. We tested for association with OGTT-derived data and surrogate indices of insulin resistance and secretion. We did not find significant differences among the genotypes in any of above-mentioned parameters. Furthermore, vitamin D levels were measured in a subgroup of subjects. We did not find significant differences among the genotypes. **Conclusions**: Our study does not provide evidence for the association of the rs2228570 polymorphism with T2DM in a Caucasian population.

ARTICLE HISTORY
Received 3 October 2016
Revised 16 February 2017
Accepted 28 February 2017

KEYWORDS
SNP; type 2 diabetes; VDR gene; vitamin D

Introduction

The 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], the biologically active form of vitamin D, plays a central role in a large variety of metabolic pathways. Vitamin D acts at the molecular level through the binding to the vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily of transcriptional regulators (1). The VDR regulates positively or negatively gene transcription by binding to vitamin D responsive elements (VDREs and nVDREs, respectively), located in the promoter region of target genes (2). The VDR is expressed in many different cell types such as pancreatic b-cells (3), vascular smooth muscle cells (4), osteoblasts and chondrocytes (5), liver, adipose tissue (6), muscle (7), dendritic cells, and lymphocytes (8).

Several polymorphisms in the gene encoding the VDR, such as rs2228570 (FokI), rs11568820 (Cdx2), rs1544410 (BsmI), rs7975232 (ApaI), and rs731236 (TaqI), have been previously studied. The last 3 polymorphisms are located between the 8 and 9 exons and lay in an area with unknown function.

Recently, we have provided evidence, for the first time, of an association between the functional rs11568820 variant of the VDR gene and type 2 diabetes mellitus (T2DM) in an Italian adult population. This variant was also associated with reduced HOMA-B (homeostatic model assessment for beta-cell function), CIR30 (corrected insulin response at

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30 minutes) and DI (disposition index),suggesting a reduced insulin secretion independent from insulin sensitivity (9).

A different case is the rs2228570 C > T SNP. This polymorphism was detected in the early 1990s, is in exon 2, and consists of a C–T change (10,11). The change is inside a start codon, so when the C variant is present, an alternative start site is used, leading to a protein with a different size. Most of the functional experiments conducted so far, in COS7 and Hela cells, show that the shorter form of the protein (424 aa) is more active than the long form (427 aa) in terms of its transactivation activity as a transcription factor (12).

It is important to note that the rs2228570 polymorphism is not in linkage disequilibrium (LD) with any of the other VDR polymorphisms, and therefore can be considered a putative independent marker in the VDR gene, and also the LD area surrounding this polymorphism seems to be very small (12).

A very recent meta-analysis, which included 12 papers on the rs2228570 polymorphism involving 2218 cases and 1859 controls, demonstrated that the T allele and TT genotype of rs2228570 were significantly associated with T2DM, only among Chinese populations (13). In contrast, no significant association was observed among other ethnic cohorts (from Poland (14), India (15), and Saudi Arabia (16)) involving a total of 772 cases and 658 controls (13).

A relatively recent study has demonstrated the rs2228570 polymorphism of the VDR gene as a possible risk factor for T2DM (17).

Furthermore, previous studies, performed in two small Caucasian cohorts, reported that rs2228570 SNP was associated with indices of insulin resistance and with insulin sensitivity (18,19).

Other association has been found between the rs2228570 SNP, out of 30 SNPs of the VDR gene, with winter 25(OH)D levels in a twin population of 198 MS patients and healthy controls (20).

So far, genetic studies on the association between rs2228570 SNP and T2DM showed contradictory data, probably due to studies performed in different ethnic cohorts or in very small populations, showing overall unreliable data. Thus, we performed an association study in a large cohort of adult Italian subjects with T2DM and in nondiabetic controls.

Materials and methods

Population

For this study, we selected 1713 subjects, 883 affected by T2DM (male = 430, female = 453) and 830 nondiabetic controls (male = 230, female = 600), among subjects attending the Internal Medicine outpatient clinics of Sapienza University of Rome and the Metabolic and Diabetes Unit of the Department of Experimental Medicine, Sapienza University of Rome. All subjects without a diagnosis of diabetes underwent a standard 75 g oral glucose tolerance test (OGTT) with measurements of glucose and insulin at baseline and after 30, 60, 90, and 120 minutes. Subjects were classified according to the ADA 2015 diagnostic criteria in normal glucose tolerant (NGT) and affected by T2DM (21). The 257 subjects presenting impaired fasting glucose, 222 impaired glucose tolerance, and 188 with both conditions after the OGTT were excluded from the study.

All subjects had a complete work-up including clinical examination, anthropometric measurements, and laboratory tests. BMI was calculated as body weight (kg)/height (m^2). The diagnosis of hypertension was based on the presence of elevated systolic (>140 mmHg) and/or diastolic (>90 mmHg) blood pressure, and/or the current use of antihypertensive medications.

Laboratory determinations

The study cohort underwent fasting blood sampling to assess FBG, glycosylated hemoglobin, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) by standard laboratory methods. Insulin was measured by radioimmunoassay (ADVIA Insulin Ready Pack 100, Bayer Diagnostics, Milan, Italy), with intra- and inter-assay coefficients of variation <5%. Low-density lipoprotein (LDL) cholesterol value was obtained using Friedwald formula.

Serum 25(OH) vitamin D concentration (25 (OH)D) was measured by a validated colorimetric method LAISON (DiaSorin SpA, Saluggia, VC).

Surrogate indices of insulin resistance and secretion

HOMA-IR for insulin resistance assessment, HOMA-B for insulin secretion evaluation, and insulin sensitivity index (ISI) indices were calculated as previously shown by Matthews et al. (22) and Matsuda et al. (23). The corrected insulin response at 30 minutes (CIR30) to estimate insulin secretion was calculated as glucose-independent parameter of β-cell function (100 x I30/[G30 x (G30–3.89)] with I30 = insulin at 30 min of OGTT in μUI/ml and G30 = glucose at 30 min of OGTT in mmol/l) (24). The main advantage of this parameter lies in its independence from the initial or 30′ glucose levels. DI, i.e., insulin secretion adjusted for insulin sensitivity, was calculated using the formula CIR30 × ISI (25).

Genotyping assay

The rs2228570 C>T transition of the human VDR gene was assayed using the TaqMan assay ID C_12060045_20 (Applied Biosystems, Foster City, CA), in a total volume of 10 μl on an Eco^{TM} Real-Time PCR System by Illumina (San Diego, CA,). The plate was run at 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 1 minute for 50 cycles. Allele frequencies were in Hardy Weinberg Equilibrium.

Statistical analysis

All statistical analyses were performed with SPSS 17.0 statistical package. Categorical variable distribution was compared by χ^2 test. Differences between continuous variables across the genotype classes were evaluated by ANOVA including gender, age, and BMI as covariates. Skewed variables were logarithmically transformed before the analyses.

Power calculation: Considering minor allele frequencies in controls of 0.46 (probability of exposure) with an odds ratio of 1.21 (13), we will be able to reject the null hypothesis with power 0.80 with our sample size. The type I error probability associated with this test of this null hypothesis was 0.05.

Ethics statement

The adult study was reviewed and approved by the Ethical Committee of Policlinico Umberto I, Sapienza University of Rome and conducted in conformance with the Helsinki Declaration. Written consent was obtained from all subjects before the study.

The study was approved by the Ethical Committee of the University of Rome.

Results

In the study population, the T-allele frequency of rs2228570 was 0.33, similar to those reported in HAPMAP-TSI (0.38 in a population from Tuscany in Italy).

Genotype distributions and allele frequencies in NGT and T2DM subjects did not show difference between the two populations (Table 1). We then analyzed the clinical characteristics of the study subjects, comparing the three genotypes. We did not find significant differences among genotypes regarding gender, age, BMI, waist, hip, waist-to-hip ratio, and blood pressure (data not shown). There were also no significant differences among the three groups in plasma concentration of total cholesterol, HDL and LDL cholesterol, and circulating triglycerides, AST and ALT levels (data not shown).

Given the reported association of the rs2228570 VDR variant and indices of insulin resistance (18,19), we then tested for association with OGTT-derived data, including glucose and insulin at baseline (0⊠), and surrogate indices of insulin resistance (HOMA-IR and Matsuda Insulin Sensitivity Index), and of insulin secretion (HOMA-B and CIR30). Given that both β-cell function and insulin sensitivity may be impaired in T2DM, we excluded from the analyses all T2DM subjects. We did not find significant differences among the three genotypes in any of above-mentioned parameters (data not shown).

The same analyses were performed according to dominant (TT versus CT + CC) and recessive (TT +

Table 1. Association of rs2228570 polymorphism with T2DM.

	T2DM *n* = 883	NGT *n* = 830	*p*
Genotype *n* (%)			
CC	395 (44.7)	378 (45.5)	
CT	379 (43)	359 (43.3)	
TT	109 (12.3)	93 (11.2)	n.s.
Allele frequency (%)			
C-allele	66.2	67.2	
T-allele	33.8	32.8	n.s.

CT versus CC) models, and did not yield any significant difference in all metabolic, clinical, and OGTT-derived parameters.

Serum 25(OH)D levels were measured in a subgroup (n = 637, NGT = 166, and T2DM = 471) of our study subjects. We did not find significant differences among the three genotypes (CC = 22.7 ± 11.1, CT = 22.6 ± 10.6, TT = 23.5 ± 11.2, $p = 0.806$).

When we performed the same analyses according to a dominant and recessive genetic model, again we observed no differences between genotypes.

Furthermore, we compared 25(OH)D levels among males and females; as expected females showed a significantly lower level of Vitamin D compared to males (21.2 ± 11.1 versus 24.3 ± 10.3, $p < 0.001$ respectively). This difference was present only in T2DM subjects (females versus males: 22.8 ± 11.8 versus 24.9 ± 10.4, $p = 0.036$), showing a relation with metabolic status.

Discussion

In the present study we tested the hypothesis that rs2228570 polymorphism of VDR gene might be associated with T2DM. Indeed vitamin D system has been shown to play a significant role in T2DM and in cardiovascular diseases (26,27).

Several reports in the past, although in small cohorts, have shown association of VDR variants, including the rs2228570 variant examined in our study, with diabetes and diabetes-related alterations (13,17–19). At variance, we were unable to find association between rs2228570 SNP and metabolic traits, including diabetes in a large cohort. We also analyzed the rs2228570 variant in males and females separately. However, no significant differences in clinical and metabolic parameters between the genotypes within each sex group were observed. When we performed the same analyses according to a dominant and a recessive genetic model, again we observed no differences between genotypes.

We also tested the association between 25(OH)D levels and rs2228570 genotypes, but we could not find any difference. To the best of our knowledge, this is the first study that analyzed the rs2228570 polymorphism in a large and very well clinically characterized cohort of Caucasian origin. Indeed, there is only one study by Malecki et al. performed in a reasonable sized population of 548 Caucasian subjects. In line with our observations, the authors were unable to detect an association between this VDR polymorphism and T2DM (14). Our study, performed in 1713 subjects, had the power to define the exact role of this variant in diabetes.

Furthermore, a very recent meta-analysis on the rs2228570 polymorphism demonstrated that this VDR variant was significantly associated with T2DM only among Chinese populations and particularly in studies with a small sample size (<200). The rs2228570 polymorphism was not associated in other cohorts of different ethnic origin (13).

One limitation of our study is the fact that 25(OH)D levels were not available for all subjects, since vitamin D levels were obtained from clinical records. However, vitamin D levels were available in almost 40% of our population, giving a sufficient number of subjects to estimate possible differences. Also, our data are related to subjects of Caucasian origin, and therefore not applicable to other populations. On the other hand, VDR variants have been studied mostly in populations of Asian origins, and one strength of our study relies on the selective analysis in a large and very well clinically characterized cohort of Caucasian origin.

From all the studies published so far, it appears evident that the association of VDR rs2228570 polymorphism with T2DM is contradictory and a possible reason for this divergence could be explained by the heterogeneity of the studies performed in various ethnic groups but also, and primarily, by the small samples size analyzed.

In conclusion, our study does not provide evidence for the association of the rs2228570 VDR polymorphism with T2DM in a Caucasian population.

Funding

This work was funded by research grants from the Regione Autonoma della Sardegna (Grant RAS 2007 n. CRP-59453), and from the Foundation Banca d'Italia (projects 2015), all awarded to Marco G. Baroni.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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