Interaction between vitamin D receptor gene polymorphisms and 25-hydroxyvitamin D concentrations on metabolic and cardiovascular disease outcomes

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Received 29 November 2013; received in revised form 24 January 2014; accepted 26 January 2014
Available online 25 February 2014

Abstract

Aim – 25-hydroxyvitamin D (25OHD) concentrations have been shown to be associated with major clinical outcomes, with a suggestion that individual risk may vary according to common genetic differences in the vitamin D receptor (VDR) gene. Hence, we tested for the interactions between two previously studied VDR polymorphisms and 25OHD on metabolic and cardiovascular disease-related outcomes in a large population-based study.

Methods – Interactions between two previously studied VDR polymorphisms (rs7968585 and rs2239179) and 25OHD concentrations on metabolic and cardiovascular disease-related outcomes such as obesity- (body mass index, waist circumference, waist‐hip ratio (WHR)), cardiovascular- (systolic and diastolic blood pressure), lipid- (high- and low-density lipoprotein, triglycerides, total cholesterol), inflammatory- (C-reactive protein, fibrinogen, insulin growth factor-1, tissue plasminogen activator) and diabetes- (glycated haemoglobin) related markers were examined in the 1958 British Birth cohort (n up to 5160). Interactions between each SNP and 25OHD concentrations were assessed using linear regression and the likelihood ratio test.

Results – After Bonferroni correction, none of the interactions reached statistical significance except for the interaction between the VDR SNP rs2239179 and 25OHD concentrations on waist-hip ratio (WHR) (P = 0.03). For every 1 nmol/L higher 25OHD concentrations, the association with WHR was stronger among those with two major alleles (−4.0%, P = 6.26e−5) compared to those with either one or no major alleles (−2.3%, P ≤ 8.201e−07, for both) of the VDR SNP rs2239179.

Conclusion – We found no evidence for VDR polymorphisms acting as major modifiers of the association between 25OHD concentrations and cardio-metabolic risk. Interaction between VDR SNP rs2239179 and 25OHD on WHR warrants further confirmation.

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Keywords: Vitamin D receptor; Metabolic traits; 25-hydroxyvitamin D; 1958 British Birth cohort; Waist-hip ratio

1. Introduction

Vitamin D is a hormone precursor. Low vitamin D status, measured by 25-hydroxyvitamin D [25OHD], has been shown to be associated with various metabolic traits such as obesity, diabetes, cardiovascular disease (CVD) risk factors and inflammatory diseases [1–3]. Metabolic actions related to vitamin D are mediated via binding of the active hormonal form, 1,25-dihydroxyvitamin D, to its nuclear receptor.
Vitamin D receptor (VDR) is ubiquitously expressed in a variety of body tissues, and hence, single nucleotide polymorphisms (SNPs) in the VDR gene may affect the risk of vitamin D-related metabolic traits, and could modify the efficacy of the receptor depending on the state of vitamin D deficiency or repletion [4].

Recently, a study in 1514 white individuals examined a range of vitamin D related variants and showed that two SNPs [rs7968585 (T→G) and rs2239179 (T→C)] in the VDR gene were able to modify the association between 25OHD concentrations and major clinical outcomes, a finding that was further replicated in a meta-analysis of three independent cohorts (n = 2727) [5]. The main outcome measure used in this previous study [5] was a composite outcome including incident hip fracture, myocardial infarction, cancer and mortality over long-term follow-up, hence providing limited insight as to the metabolic drivers of this association. In this paper, we use data from a large population-based study (n up to 5160) to examine interactions between these two VDR SNPs and 25OHD concentrations in relation to multiple metabolic traits, to evaluate whether evidence can be obtained for influences operating through an array of outcomes including obesity, blood pressure, lipids, inflammatory and diabetes related markers.

2. Methods

2.1. Study population

Study participants are from the 1958 British birth cohort (1958BC), which initially included all births in England, Scotland, and Wales during 1 week in March 1958 (n = 16,751) [6]. The main analyses were conducted in up to 5160 participants of European Ancestry with information on the two VDR SNPs, 25OHD and metabolic outcomes such as body mass index, waist circumference (WC), waist-hip ratio (WHR), high-(HDL) and low-density (LDL) lipoproteins, serum triglycerides, total cholesterol, c-reactive protein, insulin growth factor-1 (IGF-1), tissue plasminogen activator (tPA), glycated haemoglobin (HbA1c), systolic (SBP) and diastolic (DBP) blood pressures. The 45-year biomedical survey and genetic studies were approved by the South-East Multi-Centre Research Ethics Committee (Ref:01/144) and the joint UCL/UCLH Committees on the Ethics of Human Research (Ref:08/H0714/40).

2.2. Measurement of clinical and biochemical parameters

Weight and standing height, at 45 years of age, were measured without shoes and in light clothing by a trained nurse using standardized protocol and equipment; WC was measured by the nurse midway between the costal margin and iliac crest. Blood pressure was measured in a seated position, after 5 min rest, using an Omron 705CP automated sphygmomanometer with a large cuff for participants with a mid-upper arm circumference ≥ 32 cm; the measurement was repeated three times, and blood pressure was determined as the average of successful measurements.

Venous blood samples were drawn without prior fasting and posted to the collaborating laboratory. HbA1c was assayed using high-performance liquid chromatography standardized to the Diabetes Control and Complications Trial [6]. Triglycerides, LDL and HDL cholesisters were measured by standard auto-analyzer methodology. LDL cholesterol was calculated using the Friedewald formula [7]. As in a previous study of lipid measures in the 1958BC [8], HDL levels were corrected to allow for treatment effects amongst those taking lipid medications (n = 74) prior to the analysis. This was based on the assumption that commonly prescribed lipid-lowering medications increase HDL cholesterol by an average of 5%. The 25OHD was measured using automated application of an enzyme-linked immunosorbent assay (IDS OCEIA ELISA; IDS, Bolton, United Kingdom) and an analyser (BE2000; Dade-Behring, Milton Keynes, United Kingdom) with sensitivity of 5.0 nmol/L, linearity ≤ 155 nmol/L, and intra-assay CV 5.5–7.2% [9]. The 25OHD concentrations were standardized according to the mean of the values found by the Vitamin D External Quality Assurance survey (DEQAS).

2.3. Genotyping of the VDR polymorphisms

The genotype data for the two SNPs [rs2239179 and rs739837 (r² = 0.92 with rs7968585)] were obtained from the genome-wide platforms through two sub-studies [10,11], both using the 1958BC participants as population controls. The first sub-study included 3000 DNA samples randomly selected as part of the Welcome Trust Case Control Consortium and genotyped on the Affymetrix 6.0 platform [10]. The second sub-study was the Type 1 diabetes case-control study, which used 2500 DNA samples and genotyped using the Illumina Infinium 550 K chip through the JDRF/WT Diabetes and Inflammation Laboratory [11]. Both the SNPs included in the analysis had a call rate >98%, minor allele frequency >0.44 and were in Hardy-Weinberg equilibrium (P > 0.57).

2.4. Statistical analysis

The natural logarithm was used to transform all the metabolic measures to obtain a closer approximation of a normal distribution. Both the SNPs were coded additively for the minor allele. The genetic associations with the continuous outcomes were examined using linear and logistic regression models adjusting for gender and region (coded as Scotland, North, Middle, and South of England including Wales, and London). Season, in addition to gender and region, was adjusted when looking at 25OHD as outcome. Interactions between each SNP and 25OHD were assessed using linear and logistic regressions and the likelihood ratio test. According to World Health Organization recommendations, obesity was classified as BMI ≥ 30 kg/m². The use of medication was adjusted for in the models of HbA1c, LDL- and HDL-cholesterols. SBP and DBP values were corrected when participants were on blood pressure lowering medication, by increasing the DBP...
and SBP by 10 mmHg and 15 mmHg respectively. Hypertension was defined as SBP ≥ 140 mmHg, or DBP ≥ 90 mmHg, or current antihypertensive medication. Type 2 diabetes was defined as HbA1c ≥ 7 or known/self-reported type 2 diabetes. Multiple testing was corrected by using Bonferroni method \( P_{\text{interaction}} < 0.003 (= 0.05/17 \text{ outcomes}) \).

### 3. Results

None of the VDR polymorphisms were associated with any of the metabolic outcomes (Table 1). After correction for multiple testing, none of the VDR SNPs showed a significant interaction with 25OHD except for the borderline interaction between the SNP rs2239179 and 25OHD concentrations on WHR (corrected \( P = 0.03 \)) (Table 1). For every 1 nmol/L higher 25OHD concentrations, the association with WHR appeared stronger among those with two major ‘T’ alleles of the SNP rs2239179 (4.0% lower WHR, \( P = 6.26e^{-24} \)) compared to those with either one or no major alleles (2.3% lower WHR, \( P \leq 8.201e^{-07} \) for both).

None of the associations between the two VDR SNPs and the metabolic- and CVD- related traits such as obesity, type 2 diabetes and hypertension (as categorical outcomes) were statistically significant \( (P > 0.15) \) (Table 1). Also, the interactions between the VDR SNPs and 25OHD on obesity, type 2 diabetes and hypertension were not significant \( (P > 0.06) \) Table 1.

### 4. Discussion

Our study on British adults did not provide evidence for genetic variations in the VDR gene acting as major modifiers of the association between 25OHD concentrations and cardio-metabolic risk. This suggests, that the previous observations of VDR-25OHD interaction observed using a composite outcome (including incident hip fracture, myocardial infarction, cancer, and all-cause mortality), may have been driven by factors more closely related to longevity, bone metabolism/strength or cancer risk, rather than intermediate markers of cardiovascular risk.

Findings from our study are negative, with the only borderline interaction observed for VDR variant rs2239179 and 25OHD concentrations on WHR. In some studies, 25OHD concentrations have been associated with distribution patterns of adipose tissue [12,13] and, also in some clinical trials, calcium and vitamin D supplementation was related to a reduction in visceral fat [14] and body fat mass [15], respectively. Although the association between higher body mass and lower vitamin D status appears to be driven by obesity-related lowering of 25OHD con-
centrations rather than vice versa [1], this suggestion for a novel interaction in relation to body fat distribution warrants further replication.

The main strength of our study is in the large sample size and a population sample that is largely representative of the current white Caucasian population living in the UK. While limited information was available for intermediate phenotypes closely related to cancer risk or bone metabolism, the rich data available allowed for the examination of interaction across various metabolic and CVD-related outcomes. In conclusion, our study suggests that the two common VDR SNPs, which were previously suggested to modify the effects of 25OHD concentrations on major health outcomes [5], are unlikely to operate through alterations of cardiometabolic risk.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

**Acknowledgements and Funding**

We thank Alana Cavadino (UCL-ICH) for assistance in preparing the dataset. We are grateful to all the participants in the 1958 BC. Professor Ian Gibb, Dr Steve Turner, and Marie-Claude Fawcett (Royal Victoria Infirmary, Newcastle-upon-Tyne, UK) are acknowledged for carrying out the laboratory assays; and the Centre for Longitudinal studies, Institute of Education (original data producers) for providing the data. Research at the University College London, Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust benefits from R&D funding received from the NHS Executive.

Funding for the project was provided by the British Heart Foundation (grant PG/09/023). This work was undertaken at the Centre for Paediatric Epidemiology and Biostatistics.

**Author contributions:** KSV performed the statistical analysis and drafted the manuscript; CP provided assistance in the data aggregation and reviewed the manuscript; EH conceptualised the study and revised the manuscript.

**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2014.01.003.

**References**


