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Allelic variations in the vitamin D receptor gene, insulin secretion and parents' heights are independently associated with height in obese children and adolescents

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ABSTRACT

Polymorphisms in the *VDR* gene were reported to be associated with variations in intrauterine and postnatal growth and with adult height, but also with other traits that are strongly correlated such as the BMI, insulin sensitivity, insulin secretion and hyperglycemia. Here, we assessed the impact of *VDR* polymorphisms on body height and its interactions with obesity- and glucose tolerance-related traits in obese children and adolescents. We studied 173 prepubertal (Tanner's stage 1) and 146 pubertal (Tanner's stages 2–5) obese children who were referred for a weight-loss program. Three single nucleotide polymorphisms were genotyped: rs1544410 (*BsmI*), rs7975232 (*ApaI*) and rs731236 (*TaqI*). *BsmI* and *TaqI* genotypes were significantly associated with height in pubertal children, but the associations did not reach statistical significance in prepubertal children. In stepwise regression analyses, the lean body mass, insulin secretion, *BsmI* or *TaqI* genotypes and the father's and the mother's height were independently and positively associated with height in pubertal children. These covariables accounted for 46% of the trait variance. The height of homozygous carriers of the minor allele of *BsmI* was 0.65 z-scores (4 cm) higher than the height of homozygous carriers of the major allele ($P = .0006$). Haplotype analyses confirmed the associations of the minor alleles of *BsmI* and *TaqI* with increased height. In conclusion, VDR genotypes were significantly associated with height in pubertal obese children. The associations were independent from the effects of confounding traits, such as the body fat mass, insulin secretion, insulin sensitivity and glucose tolerance.

1. Introduction

The vitamin D endocrine system regulates multiple aspects of calcium metabolism and of cellular differentiation and replication in many target organs in addition to those directly involved in calcium homeostasis (bones, intestinal tract, kidneys and parathyroid glands). These target organs include the immune system, endocrine pancreas, liver, skeletal

Abbreviations: ANCOVA, analyses of covariance; AUC, area under the curve; BMI, body mass index; DM, diabetes mellitus; GWA, genome-wide association; HOMA, homeostasis model assessment; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; LD, linkage disequilibrium; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

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muscle and adipose tissue [1–3]. The actions of vitamin D are mediated by the binding of 1,25-(OH)2D3 to a specific cytosolic/nuclear vitamin D receptor (VDR), which is a member of the steroid/thyroid hormone receptor superfamily [3,4]. Frequent polymorphisms in the *VDR* gene were reported to be associated with a variety of physiological and pathological phenotypes in many populations. These phenotypes included a modulation of intrauterine and early postnatal growth [5,6] and, notably, adult height, which is a finding supported by a large meta-analysis [7]. Other studies showed associations with variations in body weight [8–10], insulin sensitivity [11–13], glucose-stimulated insulin secretion [14,15] and a susceptibility to type 1 [16] or type 2 diabetes [11].

Insulin secretion, insulin sensitivity, glucose tolerance and body fat mass are strongly related physiological traits. Insulin has a major impact on body composition and body growth in children [17], and epidemiological studies showed associations between insulin resistance, glucose intolerance, obesity and short stature in adulthood [18]. In this study, we assessed the impact of *VDR* polymorphisms on body height in a cohort of obese children and adolescents and its interactions with glucose tolerance, insulin secretion, insulin sensitivity and obesity-related traits.

2. Subjects and methods

2.1. Subjects

The study subjects consisted of 319 obese children and adolescents (BMI >95th percentile), 7 to 16 years of age, who were consecutively referred for weight reduction to the children's obesity outpatient clinic of the Hospital das Clínicas of the University of São Paulo (USP) Medical School, in São Paulo, Brazil. Subjects participated in a 20-week weight-loss program that included lifestyle and nutritional re-education and a balanced diet without weight-loss medications. The program was supported by nutritionists, physical therapists, psychologists and endocrinologists. Children with diagnosed endocrine or genetic diseases that are associated with or prone to obesity, and children under pharmacological treatment were excluded from the study. A full description of study subjects and clinical protocols was previously published [19]. The participants, individually and as a group, had a mixed ethnic background (African, Amerindian, Asian and European Caucasian of several different countries of origin), which reflects the Brazilian population [20]. The study was approved by the institutional Ethics Committee. The patients and their parents were given written and oral information about the research project, its purposes, procedures and potential risks, and had signed the consent forms.

2.2. Methods and computations

Height, body weight and BMI were expressed as z-scores and/or percentiles to allow pooled comparisons of values from children of different ages and sex. These parameters were determined according to the subject's class of age and sex from the curves provided by the Centers for Disease Control and Prevention, National Center for Health Statistics, United States (http://www.cdc.gov/growthcharts/). The puberty developmental stage was determined according to the criteria of Marshall and Tanner [21,22]. Body composition was analyzed by a bioelectrical impedance method with a Body Composition Analyzer equipment (BioQuantum RJL Systems, MI), and an adequate formula for age, weight and height was used to estimate lean and fat mass [23].

An oral glucose tolerance test (OGTT) was performed by giving a glucose solution of 1.75 g/kg of body weight up to a maximum of 75 g of glucose. Venous blood was withdrawn at 0, 30, 60, 90, 120 and 180 min afterwards for the measurement of glucose and insulin. Plasma glucose was determined by an automatic enzyme-based colorimetric method using hexokinase (Cobas Integra, Roche, Basel, Switzerland). Serum insulin was determined by Auto Delfia fluoroimmunoassay (Perkin-Elmer, Turku, Finland). A diagnosis of impaired fasting glucose (IFG), impaired glucose tolerance (IGT), or diabetes mellitus (DM) was made based on the fasting and 2 h plasma glucose levels during the OGTT according to most recent criteria [24]. The area under the curve (AUC) relating glucose levels and time during the OGTT was calculated by the trapezoidal rule. Glucose tolerance was expressed as $AUC_{glucose}$. Indices of insulin secretion and insulin sensitivity were computed from glucose and insulin values during the OGTT. The insulin secretion index was computed as $\Delta Insulin_{30\text{-}0\ min}/Glucose_{30\ min}$. This index was shown to present a good correlation (R^2 ~0.7) with insulin secretion determined during a glucose clamp [25]. Insulin sensitivity was assessed by Matsuda's composite insulin sensitivity index (ISI_{comp}), which represents a composite of both hepatic and peripheral tissue sensitivity to insulin and presents a good correlation with results of a euglycemic insulin clamp (R^2 ~0.73) [26]. Homeostasis model assessment (HOMA) indices of beta-cell function (%B) and insulin sensitivity (%S), based on fasting levels of glucose and insulin were computed with the HOMA Calculator v2.2 (available at http://www.dtu.ox.ac.uk/index.php?maindoc=/homa/index.php). HOMA is expressed as a percentage of the average values found in young fit subjects with an ideal body weight, who were taken as an absolute reference population [27]. Serum levels of 25-(OH)D were measured by chemiluminescent immunoassay (LIAISON 25OH Vitamin D Total Assay, DiaSorin, Stillwater, MN) in 137 children.

2.3. DNA studies

DNA was extracted from peripheral blood samples by standard procedures. There are at least five haplotype blocks with a high linkage disequilibrium (LD) and areas with a very low LD in the *VDR* gene (chr. 12q12-q14) in Caucasians. Blocks 1–4 are found in the promoter region while block 5 encompasses exons 4–9 and the 3' UTR [28]. We genotyped three single nucleotide polymorphisms (SNPs) located in haplotype block 5: rs1544410 (intron 8), rs7975232 (intron 8) and rs731236 (exon 9). The choice of the SNPs was based on previously reported associations with adult height [7], modulation of growth [5,6], and body weight and glucose homeostasis-related phenotypes [8–11,14–16]. These SNPs have been previously known by the names of the associated restriction enzymes (*Bsm*I, *Apa*I and *Taq*I, respectively) and this nomenclature was used throughout the manuscript. Genotyping was performed using Assay by Design (ABD) kits from Applied Biosystems (Life Technologies, Carlsbad, CA).