Genetically determined vitamin D reception in metabolically unhealthy obesity in children

Abstract. Background. Genomic effects of vitamin D are determined by conformational changes in the structure of the vitamin D receptor (VDR) determined by single nucleotide variants (SNV) of the VDR gene. The purpose is to study the association of the SNV of the VDR gene with metabolically unhealthy obesity (MUO) in children. Materials and methods. Two hundred and fifty-two obese children aged 6–18 years were examined. The main group (n = 152) was represented by patients with MUO. The control group (n = 100) consisted of children with metabolically healthy obesity. Whole genome sequencing (CeGat, Germany) was performed in 31 children of the main group and 21 controls. The level of serum 25-hydroxyvitamin D (Synevo, Ukraine) was measured in all patients. To verify the results, Spearman’s correlation coefficient (r) and p-value for each variable were calculated. Results. Five SNVs of the VDR gene were identified: rs2228570 (1 DNA copy number variation (CNV); c.2T>C in 94.23 %); rs731236 (2 CNV; c.11056T>C, c.1206T>C in 65.38 %); rs10783218 (2 CNV; c.296+8C>T, c.146+8C>T in 7.69 %); rs2228572 (2 CNV; c.57C>T, c.207C>T in 1.92 %); rs12721365 (2 CNV; c.1059C>T, c.909C>T in 1.92 % of patients). A correlation between SNV VDR and MUO was observed in the following genotypes: AA rs12721365 (r = 0.21), AA rs2228572 (r = 0.21), GG SNV rs731236 (r = –0.15) and GG rs2228570 (r = –0.31), p < 0.05. Conclusions. The genotypes AG SNV VDR rs12721365, rs2228572 are highly associated with the development of MUO.

Keywords: vitamin D receptor gene; next generation sequencing; analysis of single nucleotide gene variants; children; metabolically unhealthy obesity

Introduction
Vitamin D deficiency is characterized by epidemic prevalence in all countries of the world and is an obesogenic factor that causes increased lipogenesis and the development of insulin resistance [1, 10, 20]. Excessive accumulation of fat in adipocytes and a change in the range of products produced by them, in particular a decrease in the level of leptin), contributes to the formation of meta-inflammation [27]. Vitamin D realizes its influence through interaction with the vitamin D receptor (VDR), and therefore conformational changes in the structure of the VDR protein, which are determined by single nucleotide variants (SNV) of the VDR gene, predetermine the genomic effects of calcitriol [51]. The VDR gene is located on chromosome 12q13.1 and is expressed in more than half of the 400 human cell types [9]. Serum calcitriol concentration is a fundamental element that determines the expression of the VDR gene in visceral fat adipocytes [56] and a marker of oxidative stress and inflammation [27].

The most studied VDR genetic variants are the following SNVs: ApaI (rs7975232) and BsmI (rs1544410) located in intron 8; EcoRV (rs4516035), FokI (rs2228570), located in exon 2 and formerly known as rs10735810; TaqI (rs731236).
located in exon 9; Tru9I (rs757343) and CDX2 (rs11568820) [26]. SNVs of the VDR gene, such as ApaI, BsmI, TaqI, FokI, are considered to be the most highly associated with cardiometabolic disorders [55]. These SNVs are named according to the restriction enzymes by which they were identified: ApaI — Acetobacter pasteurianus, BsmI — Bacillus steatothermophilus, FokI — Flavobacterium okeanokoites, TaqI — Thermus aquaticus [45].

The FokI variant (rs2228570) is located in the second exon of the VDR gene and is a start codon polymorphism. This SNV of the VDR gene is characterized by a thymine-to-cytosine nucleotide substitution (T for C or T>t) and is the only non-synonymous SNV variant of the VDR gene. The aforementioned SNV of the VDR gene leads to a change in the amino acid sequence of the VDR protein — a threonine amino acid residue is replaced by a methionine residue. Start codon polymorphism predetermine the presence of two potential translation initiation sites: on the C allele, a new start codon (ATG) is located 9 bp after the common start site. In this connection, in carriers of the T allele, a full-length VDR protein is synthesized, consisting of 427 amino acid residues, and in carriers of the C allele, a VDR protein shortened by three amino acid residues, which contains 424 amino acid residues. The short isoform of the VDR protein differs from the full-length isoform in higher transcriptional activity [11, 13].

The ApaI (rs7975232) variant of the VDR gene is an A>C (or A>a) change, and the BsmI (rs1544410) variant of the VDR gene is an A>G (or B>b) change. ApaI (rs7975232) and BsmI (rs1544410) variants are located at the 3’ end of the VDR gene and are not associated with a change in the amino acid sequence of the VDR protein [32, 50].

The TaqI variant (rs731236) of the gene is a change C>T (or T>t), also does not cause an amino acid change in the encoded protein, but affects the stability of mRNA [45].

It has now been demonstrated that SNVs of the VDR gene are independent risk factors for the development of various diseases, such as prostate cancer, inflammatory bowel disease, osteopenia, tuberculosis [35], Parkinson’s disease [4], diabetes mellitus [36], polycystic ovary syndrome [48], steatohepatosis [8] and cardiovascular disease [28]. Also, data from previous cross-sectional, observational studies [52] indicate the presence of an inverse correlation between the level of vitamin D deficiency and the degree of glycemic control. Thus, it can be assumed that SNVs of the VDR gene can determine the development of a certain obesity phenotype.

The purpose of our study is to investigate the association of the SNV of the VDR gene with metabolically unhealthy obesity in children.

Materials and methods

All participants in our study gave written informed consent. The research protocols and methods were approved in accordance with the ethical standards of the Declaration of Helsinki 2013 and the Human Research Ethics Committee of the Dnipro State Medical University (ethical approval DSMU/EC/19/1107). Data collection time: January 2020 — February 2023.

Design: observational, analytical, longitudinal, cohort study.

Inclusion criteria: polygenic obesity (body mass index (BMI) ≥ 97th percentile), age of 6—18 years.

Exclusion criteria: monogenic and/or syndromic obesity, pregnancy.

Our study involved 252 obese children aged 6—18 years. To form the main observation group, we took into account the presence of abdominal obesity in a child and two of the following criteria: fasting glycaemia ≥ 5.6 mmol/l [16] and/or, according to the recommendations of the IDEFCIS study, the level of basal insulinemia, which was higher than 90th percentile [39]; high-density lipoprotein cholesterol ≤ 1.03 mmol/l or less than the 10th percentile for age [17]; an increase in triglycerides ≥ 1.7 mmol/l or more than the 90th percentile of the age norm; an increase in systolic and/or diastolic blood pressure above the 90th percentile for age, sex and height [21]. The abdominal type of obesity was determined according to the consensus of the International Diabetes Federation, based on the excess of the waist circumference over the 90th percentile for children [6].

Anthropometric measurements were made in a child in underwear and without shoes. Height (cm) was measured using Heightonic Digital Stadiometer® to the nearest 0.1 cm. Weight (kg) was measured using Tefal Bodysignal body composition analyzer (France).

Systolic and diastolic blood pressure were measured using a digital oscillometric device Dinamap ProCare (GE Healthcare).

Laboratory examination for the formation of observation groups for obesity phenotypes included general clinical methods. Blood samples were obtained after an overnight fast by venipuncture in vacutainer gel tubes, and serum was separated from cells by centrifugation in a certified laboratory Synevo (Ukraine) using an analyzer and a Cobas 6000 test system (Roche Diagnostics, Switzerland). Serum glucose was studied by the hexokinase method; triglycerides and high-density lipoproteins of blood plasma were evaluated by the enzymatic colorimetric method.

The level of basal insulin was analyzed using the immunochemical testing with electrochemiluminescence immunoassay. The level of basal insulin in the venous blood was considered normal at 2.6—24.9 μU/ml.

To study the level of 25-hydroxycholecalciferol (25(OH)D), an immunochemical method with chemiluminescence microparticle immunoassay and an Architect i2000 analyzer (ABBOTT, USA) were used. Reference values that indicated the risk of vitamin D insufficiency were 21—30 ng/ml, vitamin D deficiency — less than 20 ng/ml.

The sample population examined by whole genome sequencing (Illumina CSPro®, CeCat, Germany) consisted of 31 children of the main group and 21 controls and was qualitatively homogeneous compared to the general population. Average amount of DNA (μg) in samples was 0.875. Library Preparation: quantity used 50 μg. Library Preparation Kit: Twist Human Core Exome plus Kit (Twist Bioscience). Sequencing parameters: NovaSeq 6000; 2 × 100 bp.

Bioinformatic analysis — demultiplexing of the sequencing reads was performed with Illumina bcl2fastq (version 2.20). Adapters were trimmed with Skewer, version 0.2.2 [25]. DNA-Seq: trimmed raw reads were aligned to the human reference genome (hg19-cegat) using the Burrows-
Wheeler Aligner (BWA-mem version 0.7.17-cetag) [29]. ABRA version 2.18 and Genotype-Harmonizer v. 1.4.20 were used for local restructuring of readings in target regions to improve more accurate detection of indels in the genome during mutagenesis [14, 37].

Reference sequence was obtained from the National Center for Biotechnology Information, RefSeq database (http://www.ncbi.nlm.nih.gov/RefSeq) [43].

Statistical analysis of the results was carried out using a package of application programs Statistica 6.1 (No. AGAR909E415822FA) with help a personal computer based on an Intel Pentium 4. Depending on the test result, parametric and nonparametric statistical methods were used. Correlation analysis was applied to analyze 100 indicators of clinical, laboratory-instrumental and molecular genetic examinations in 252 children. To assess the relationship between quantitative traits, correlation analysis was used according to the Pearson method, and between qualitative traits, a non-parametric ranking method was used according to Spearman’s analysis (r). Only essential connections were taken into account (p < 0.05).

Results

The average age of children in the main and control groups was 12.35 ± 0.08 and 12.28 ± 0.13 years, respectively. The groups did not differ by gender. In the main group, the proportion of boys and girls was 56.14 ± 6.61 % and 43.86 ± 5.40 %; in the control group, it was 48.17 ± 6.86 % and 51.83 ± 5.70 %, respectively (p > 0.05).

In obese children, the average serum level of 25(OH)D was 21.70 ± 1.78 ng/ml. At the same time, the average level of 25(OH)D in the blood serum of patients with MUO was significantly lower than in children with metabolically healthy obesity (MHO): 14.57 ± 1.63 ng/ml vs. 28.82 ± 1.93 ng/ml, t = 5.64, p = 0.00061.

In obese children examined by whole genome sequencing, five SNVs of the VDR gene were identified: rs2228570 (1 DNA copy number variation (CNV): c.2T>C in 94.23 %; rs731236 (2 CNV: c.11056T>C, c.1206T>C in 65.38 %; rs10783218 (2 CNV: c.296+8C>T, c.146+8C>T in 7.69 %); rs2228572 (2 CNV: c.57C>T, c.207C>T in 1.92 %); rs12721365 (2 CNV: c.1059C>T, c.909C>T in 1.92 % of the examined patients). The distribution of genotype frequencies was in Hardy-Weinberg equilibrium in both groups.

Molecular genetic characteristics of the identified SNVs of the VDR gene are presented in Table 1.

Vitamin D deficiency correlated with the following SNV genotypes of the VDR gene: AA rs12721365 (r = 0.41), AA rs2228572 (r = 0.39) and GG rs2228570 (r = −0.27), p < 0.05.

Bioinformatic analysis of the results of whole genome sequencing showed that patients with various obesity phenotypes had associations with certain SNV genotypes of the VDR gene (Table 2, Fig. 1, 2).

The homozygous mutant genotype GG SNV rs2228570 occurred with the same frequency in both groups: it was registered in 35.5 % of children with MUO and in 38.1 % of those with MHO (p > 0.05). At the same time, according to the gnomAD browser, the frequency of the GG SNV rs2228570 genotype in the human population is 62–77 %.

Table 1. Characteristics of SNV types of the VDR gene

<table>
<thead>
<tr>
<th>SNV, ID</th>
<th>Position</th>
<th>GnomAD_maxPOP</th>
<th>Ref</th>
<th>Alt</th>
<th>Consequence</th>
<th>Base change</th>
<th>CADD</th>
<th>Raw Score</th>
<th>Clinical significance (gnomAD browser)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2228570 (FokI)</td>
<td>48272895</td>
<td>AFR</td>
<td>A</td>
<td>G</td>
<td>Initiator codon</td>
<td>c.2T&gt;C</td>
<td>p.?-M1?</td>
<td>23.8</td>
<td>3.09</td>
</tr>
<tr>
<td>rs10783218, 332626*</td>
<td>48272743</td>
<td>AFR</td>
<td>G</td>
<td>A</td>
<td>Splice region</td>
<td>c.296+8C&gt;T, c.146+8C&gt;T</td>
<td>2.76</td>
<td>0.01</td>
<td>Benign</td>
</tr>
<tr>
<td>rs2228572, 869884*</td>
<td>48272840</td>
<td>AFR</td>
<td>G</td>
<td>A</td>
<td>Synonymous</td>
<td>c.57C&gt;T, c.207C&gt;T</td>
<td>0.78</td>
<td>-0.19</td>
<td>Benign</td>
</tr>
<tr>
<td>rs12721365, 308880*</td>
<td>48240233</td>
<td>NFE</td>
<td>G</td>
<td>A</td>
<td>Splice region</td>
<td>c.1059C&gt;T, c.909C&gt;T</td>
<td>0.39</td>
<td>-0.29</td>
<td>Conflicting interpretations of pathogenicity</td>
</tr>
<tr>
<td>rs731236 (Taql), 308887*</td>
<td>48238757</td>
<td>NFE</td>
<td>A</td>
<td>G</td>
<td>Synonymous</td>
<td>c.1056T&gt;C, c.1206T&gt;C</td>
<td>0.15</td>
<td>-0.43</td>
<td>Benign</td>
</tr>
</tbody>
</table>

Notes: GnomAD_maxPOP — the frequency distribution of VDR mutations; AFR — African; NFE — non-Finnish European; Ref — reference allele; Alt — alternative allele; consequence — functional consequence of the variation in relation to the transcript; the nucleotide change and position relative to the coding sequence of the affected transcript in HGVS nomenclature: c. — CDS Position Reference Base > Alternative Base. Example: c.223A>T (c.1 — interpretation for DNA coding sequence; p.1 — protein sequence interpretation) [44]. This column is empty if the variant is intergenic; CADD — combined annotation dependent depletion; * — SNV VDR associated with MUO.
two fold decrease in the frequency of the GG SNV rs731236 genotype among children with MUO compared to the controls and general population (38 – 40%)

Among the children examined by us, the homozygous mutant genotype AA SNV rs10783218 was not found. An interesting fact is the absolutely identical occurrence of the heterozygous genotype AG SNV rs10783218 (12.9 %) and the homozygous wild genotype GG SNV rs10783218 (87.1 %) in the comparison groups.

A correlation between VDR and MUO genotypes was observed for AG SNV rs12721365 (r = 0.21), AG SNV rs2228572 (r = 0.21), GG SNV rs731236 (r = –0.15) and GG SNV rs2228570 (r = –0.31) genotypes, p < 0.05.

Discussion

Although the relationship between SNV of the VDR gene and metabolic disease remains poorly understood, previous studies have identified some positive associations demonstrating the potential impact of SNV VDR on the development of MUO and other diseases associated with a high risk of cardiometabolic disorders [5, 22].

Similar to X. Yan et al. [7, 55], the results of our study demonstrated the presence of vitamin D deficiency in children with obesity that was especially pronounced among children with MUO [41]. According to K. Xenos et al. [54], low vitamin D supply may be an independent predictor of obesity. Considering the results of M. Clemente-Postigo et al. [12], which showed that 25(О)D levels negatively correlated with the homeostatic model assessment for insulin resistance (r = –0.200; p = 0.032) and glucose (r = –0.295; p = 0.001), but not with BMI, we suggest that vitamin D deficiency has a more pronounced effect on carbohydrate metabolism than on adipogenesis.

As a result of whole genome sequencing, we have demonstrated for the first time a positive correlation between vitamin D deficiency and the genotypes AA SNV rs12721365; AA SNV rs2228572 of the VDR gene in obesity children. According to the results of our study, the wild AA FokI genotype (rs2228570), associated with vitamin D deficiency in obese children, is highly associated with the development of MUO. At the same time, the presence of the SNV rs2228570 mutant G allele is associated with a low risk of vitamin D deficiency in children with MUO. The GG rs2228570 genotype (base change: c1.2T>C) is accompanied by the production of a shorter variant of the VDR protein, which has a higher transcriptional activity [15]. Therefore, we assume that children with the GG SNV rs2228570 (FokI) genotype have a higher level of activation of vitamin D-associated signaling pathways, which reduces the risk of developing cardiometabolic disorders. However, there are studies demonstrating both the presence and absence of a significant relationship between the SNV genotypes of the VDR rs2228570 gene and the high cardiometabolic risk phenotype [38]. Thus, when examining 215 Chinese patients with metabolic syndrome and coronary heart disease (CHD), there was demonstrated a 2.61-fold

![Figure 1. Genotypes of the VDR gene in individuals with MHO (%)](image1)

![Figure 2. Genotypes of the VDR gene in individuals with MUO (%)](image2)

<table>
<thead>
<tr>
<th>Table 2. Comparative characteristics of the AF and CNV of the VDR gene in obesity phenotypes with AF in the world population and among non-Finnish Europeans</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>SNV (genotypes: HOM</em>/HET/HOM</em> )**</td>
</tr>
<tr>
<td>Popmax AF (HOM*), %</td>
</tr>
<tr>
<td>rs2288870 (GG/AG/AA)</td>
</tr>
<tr>
<td>rs10783218 (AA/AG/GG)</td>
</tr>
<tr>
<td>rs2228572 (AA/AG/GG)</td>
</tr>
<tr>
<td>rs12721365 (AA/AG/GG)</td>
</tr>
<tr>
<td>rs731236 (GG/AG/AA)</td>
</tr>
</tbody>
</table>

**Notes:** HOM* — homozygous variant (biallelic single nucleotide substitution); HET — heterozygous variant (single allelic single nucleotide substitution); Popmax AF — maximum population allele frequency in the genome (gnomAD browser); AF NFE — allele frequency for non-Finnish Europeans in the genome (gnomAD browser).
increase in the risk (95% confidence interval (CI) 1.12–6.1, p = 0.027) of developing cardiometabolic complications in individuals with the wild AA FokI genotype [24]. The authors also showed that the presence of the GG FokI mutant genotype predetermines a higher serum level of high-density lipoprotein cholesterol in adults and reduces the risk of CHD (p = 0.001). J. Maia et al. [33] demonstrated the potential protective effect of the GG SNV FokI genotype against coronary artery disease in postmenopausal women with type 2 diabetes mellitus (T2DM) in a recessive model. Also L. Ma et al. [30] showed that the incidence of the GG FokI mutant genotype was higher among Chinese T2DM patients without CHD, at 7%, compared to T2DM patients with CHD (4%) or healthy volunteers (3%), p < 0.0001.

At the same time, H. Fiaz et al. [19] received diametrically opposite data, which indicate that the GG (95% CI 1.63–17.2, p = 0.005) and AG FokI (95% CI 1.70–20.7, p = 0.005) genotypes are associated with a higher risk of arterial hypertension, by 5.29 and 5.94 times, respectively. The absence of significant differences in the occurrence of various FokI genotypes between patients with T2DM and healthy adults is shown in the works of N.A. Sattar [47], and I. Mahjouri [31], as well as M.T. Malecki et al. [34].

In our study, we identified SNV of the VDR TaqI gene (rs731236), but did not detect SNV ApaI, BsmI in obese children.

In contrast to the results of our work, which indicate a protective effect of the GG TaqI genotype for the risk of cardiometabolic disorders in children with obesity, D. Raljević et al. [42] in a study of 155 overweight Croatian patients (BMI = 28.5 ± 4.0 kg/m²) after myocardial infarction proved that the G/G TaqI genotype within the recessive model (A/+ vs. G/G: odds ratio = 0.31; 95% CI 0.11–0.83, p = 0.0016) is associated with a significantly higher risk of CHD and myocardial infarction.

According to other authors, the heterozygous AG TaqI genotype was more common in the group of T2DM patients with coronary artery disease compared to T2DM patients without heart damage [30]. At the same time, L. He et al. [24, 34] found no association between the TaqI genotype and increased cardiometabolic risk among T2DM patients, and R. Erasmus [18] found no relationship between vitamin D receptor SNV FokI and TaqI with the glycemic status of the probands.

While the GG SNV rs731236 and GG SNV rs2228570 genotypes of the VDR gene are associated with a low risk of developing MUO, the AA SNV rs12721365 and rs2228572 genotypes of the VDR gene are directly associated with the presence of MUO. For the first time, we presented data on the association of SNVs rs12721365 and rs2228572 of the VDR gene with MUO (there are no references to these SNVs of the VDR gene in the ClinVar database).

In children with obesity, the homozygous AA SNV rs10783218 genotype of the VDR gene was not identified. The frequency of the heterozygous genotype AG SNV rs10783218 of the VDR gene was the same and amounted to 12.9%, both in MHO and MUO. It should be noted that the rs10783218 variant of the VDR gene is not listed in the Human Gene Mutation Database [49] and classified solely by an automated scoring system, according to which it is characterized as a benign variant [23].

Conclusions

1. Obesity in children is usually accompanied by a decrease in the serum level of vitamin D. The degree of the latter is associated with the risk of cardiometabolic disorders and the development of MUO.

2. Various genotypes of some SNVs of the VDR gene are associated to varying degrees with the development of obesity and cardiometabolic disorders in children:
   - AG genotypes of both SNV rs12721365 and rs2228572 of the VDR gene are highly associated with the development of cardiometabolic disorders;
   - GG genotypes of both SNV rs2228570 and SNV rs731236 of the VDR gene are associated with a low risk of developing cardiometabolic disorders;
   - SNV rs10783218 of the VDR gene is not associated with the development of obesity in children.

References


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