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The frequency of allelic variants of the VDR gene and the level vitamin D in children under one year old in the Kazakh population

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Abstract

Introduction: The study of the genetic aspects of bone metabolism disorders in children is a theoretical and practical interest for pediatrics, especially according to the age and ethnic positions. There is a number of gene polymorphisms (primarily the vitamin D receptor (VDR) gene) that determine the norm and pathology of bone tissue formation. Calcium absorption worsens when there is no functional VDR and active forms of vitamin D. As a result the level of bone mineralization decreases. In children such disorders lead to the development of osteopenia.

Objective: To determine the frequency of allelic variants of the VDR gene (rs1544410, rs2228570) and to evaluate its relationship with the level of vitamin D in children under one year old in the Kazakh population.

Material and methods: 197 children under one year of age were examined for vitamin D by electrochemiluminescent immunoassay and genotyping of the VDR polymorphism (rs1544410, rs2228570) by PCR.

Results: It was found out that children with the C allele of the VDR rs2228570 gene have a reduced level of vitamin D by 1.84 times (95% CI 1.10 - 3.07) and CC - by 2.3 times compared with children with normal vitamin D levels.

Statistical analysis by the Kruskal-Wallis method showed that the serum level of vitamin D in AA carriers for the VDR rs1544410 was significantly reduced comparing to the level in GG and GA carriers (p=0.03).

Conclusion: The study confirms the need for further in-depth study of the genetic aspects of bone metabolism disorders in children for the development of personalized medicine.

Key words: vitamin D, VDR gene polymorphism, children, osteopenia

Introduction

Nowadays much attention is paid to the issues of bone mineral density in children. The results of a number of studies show that about 70-80% of the variability in bone mineral density in a population is determined by genetic factors [1,2].

There is a number of gene polymorphisms that determine the norm and pathology of bone tissue formation, the most relevant of which is the vitamin D receptor (VDR) gene [3,4].

The "VDR gene" (vitamin D receptor) encodes an intracellular vitamin D receptor, which is also a transcription factor. It is expressed in various tissues, mostly in the intestines, kidneys, parathyroid glands and bones - all these tissues and organs are integrated into the system for maintaining calcium homeostasis. In the absence of a functional VDR and an active form of vitamin D, calcium absorption worsens and, as a result, the level of bone mineralization decreases. In children such disorders lead to the development of osteopenia, in adults - to osteoporosis [5,6].

General statements about the role of genetic factors in osteopenic conditions cause no doubts, but there are still enough questions on the contribution of specific genes that regulate the growth and development of the skeletal system, especially from age and ethnic positions. The study of the genetic aspects of bone metabolism disorders in children, especially at an early age, is a theoretical and practical interest in pediatrics.

Purpose: To determine the frequency of allelic variants of the VDR gene (rs1544410, rs2228570) and to evaluate its relationship with the level of vitamin D in children under one year old in the Kazakh population.

Material and methods

197 children of Kazakh population under one year old were examined in the city of Aqtobe. The sample size was calculated according to the "Epi Info" program. The recruitment of children was carried out using the method of probability sampling.

Inclusion criteria: children from 0 to 12 months, of the I and II health groups, according to the order № 145 dated March 16, 2011, in a satisfactory condition at the time of the study, with informed consent signed by parents or legal representatives.

Exclusion criteria: children with hereditary and acquired diseases of the musculoskeletal system, registered in the dispensary account for severe chronic somatic diseases; disability due to other diseases; with genetic syndromes.

After a clinical examination all children underwent the venous blood sampling for molecular genetic analysis. Isolation of genomic DNA from the peripheral blood of the subjects was performed using "DNA-Blood-M-100" reagent kits from TestGen LLC (Russia). The principle of the method is based on the reversible binding of nucleic acids on the surface of magnetic particles.

Genotyping of the VDR polymorphism (rs1544410, rs2228570) was carried out by real-time polymerase chain reaction (PCR) on a DT-prime amplifier (DNA-technology, Russia) using commercial kits of reagents of LLC TestGen (Russia) by the method of fluorescent detection. The method is based on the degradation of oligonucleotide probes using synthetic analogues of oligonucleotides.

The process of DNA amplification consists in repeated cycles of thermal denaturation of DNA, annealing of primers with complementary sequences, and next completion of polynucleotide chains from these primers by Taq polymerase. Signal probes, containing FAM and HEX fluorescent labels, were introduced into the amplification mixture for each variant of the determined genetic polymorphism (mutation).

After the end of PCR, the duplexes, formed by the amplicons and signal probes, go through a round of thermal melting. As the result, the fluorescence level changes. It is fixed and presented in the form of a graph.

Genotyping was carried out at the Scientific and Practical Center of the WKMU named after Marat Ospanov.

The electrochemiluminescent immunoassay method was used to determine the concentration of vitamin D in blood samples (5 ml), It is a quantitative method of measuring an antigen or antibody based on changes in the electrochemiluminescence signal before and after the immunoreaction [7]. Vitamin D sufficiency was evaluated in accordance with the criteria of the National Program "Vitamin D Deficiency in Children and Adolescents of the Russian Federation: Modern Approaches to Correction", 2018. [8].

Nonparametric criteria were used for statistical processing of quantitative data. Using the Kruskal-Wallis test, the distribution of genotypes (GG, AG, AA/TT, CT, CC) for 2 polymorphisms of the VDR gene between groups with different levels of vitamin D was determined.

The Mann-Whitney test was used to perform pair-matched comparisons of groups (GG with AG, GG with AA, AG with AA and TT with CT, TT with CC, CT and CC). At the same time, the Bonferroni correction was held with the significance level of 0.017. The obtained data were processed by the statistical licensed program Statistica 10 and SPSS 25.

Results

A total of 197 children under one year old of the Kazakh population were examined for the content of vitamin D. It was found that the normal level of vitamin D was observed in 36 (18.3%), and hypovitaminosis - in 161 (81.7%) children.

Next, the distribution frequency of genotypes and alleles of the polymorphism of the VDR rs1544410 gene was determined. The results of the survey are presented in Table 1.

Table 1 Frequency distribution of genotypes and alleles of VDR rs1544410 polymorphism

VDR rs 1544410 polymorphism	Genotype frequency			Allele frequency	
	GG	GA	AA	G	A
	0,58	0,4	0,02	0,76	0,14

The results of studying the frequency distribution of genotypes and alleles of the VDR rs2228570 polymorphism are presented in Table 2.

Table 2 Frequency distribution of genotypes and alleles of VDR rs2228570 polymorphism

VDR rs2228570 polymorphism	Genotype frequency			Allele frequency	
	TT	TC	CC	T	C
	0,28	0,25	**0,47**	0,52	0,68

During the process of further research, the analysis of the frequency distribution of genotypes and alleles of the VDR rs1544410 depending on the level of vitamin D in the blood was made (Table 3).

Table 3 Frequency distribution of genotypes and alleles of the VDR rs1544410 depending on the level of vitamin D

rs1544410	**Vitamin D deficiency** (n = 161)	**Norm of vitamin D** (n = 36)	**χ2**	**p**	**OR (95% CI)**
G allele A allele	0.784 0.216	0.764 0.236	0.13	0.72	1.12(0.61 – 2.05) 0.89 (0.49 – 1.64)
G/G genotype G/A genotype A/A genotype	0.591 0.384 0.024	0.528 0.472 0.000	1.66	0.44	1.30 (0.63 – 2.67) 0.70 (0.34 – 1.44) 2.05 (0.11 – 38.86)

Table 4

Frequency distribution of genotypes and alleles of the VDR rs1544410 depending on the level of vitamin D

rs2228570	**Vitamin D deficiency** (n = 161)	**Norm of vitamin D** (n = 36)	**χ2**	**p**	**OR (95% CI)**
T allele *C* allele	0.378 0.622	0.528 0.472	5.49	0.02	0.54 (0.33 – 0.91) 1.84 (1.10 – 3.07)
T/T genotype *T/C* genotype *C/C* genotype	0.262 0.232 0.506	0.361 0.333 0.306	4.78	0.09	0.63 (0.29 – 1.35) 0.60 (0.28 – 1.32) 2.33 (1.08 – 5.04)

The results of the frequency distribution of genotypes and alleles of the VDR rs2228570 depending on the level of vitamin D are presented in Table 4.

At the next step, the distribution of the GG, AG, AA/TT, CT, CC genotypes of the VDR gene polymorphisms (rs1544410, rs2228570) and the level of vitamin D in blood serum in children under one year of age of the Kazakh population were compared using the Kruskal-Wallis nonparametric test. Comparison of VDR rs544410 genotypes and vitamin D levels is presented in Table 5.

Table 5

Relationship between VDR rs544410 genotypes and vitamin D levels

Dependent: Vitamin D 30–80 ng/ml	p (bilateral) for multiple comparisons; Vitamin D 30–80 ng/ml Group (independent) variable: VDR rs1544410 Kruskal-Wallis test p =0,0167		
	GG	GA	AA
	R:95,569	R:110,82	R:37,125
GG		0,209516	0,141243
GA	0,209516		0,038855
AA	0,141243	0,038855	

Discussion

An analysis of the frequency of distribution of genotypes and alleles of the VDR rs1544410 polymorphism, presented in Table 1, indicates that the GG genotype in children of the study group was found in 58% of cases compared with the GA and AA genotypes. According to the written above, the G allele frequency occurs more than the A allele. This fact is of a big interest, because a number of studies have shown that the GG genotype is responsible for reducing the risk of disorders of low bone mineral density, GA - intermediate risk, AA - increased risk [9, 10].

The result of the study of the distribution frequency of genotypes and alleles of the VDR rs2228570 polymorphism (Table 2) shows that the CC genotype (47%) and the C allele (68%) of the VDR rs2228570 were most common in children under one year of age in the Kazakh population.

The VDR rs2228570 polymorphism is currently under study. This polymorphism affects bone mineral density in children. According to literary sources, its results often vary depending on race and nationality [11].

Thus, a study among residents of Pakistan demonstrated the association of VDR polymorphisms with the appearance of bone metabolism disorders [12]. Similar associations of VDR polymorphisms are also observed in other groups: Egyptian (rs7975232, rs2228570 and rs1544410), French (rs10735810), Canadian (rs10735810), and Tunisian (rs10735810) [13]. Such studies provide a basis for studying the role of genetic factors in osteopenic conditions, depending on the ethnicity of the respondents.

Analysis of the frequency distribution of genotypes and alleles of the VDR rs1544410 gene depending on the level of vitamin D from Table 3 shows that the distribution of G and A alleles of the VDR rs1544410 polymorphism does not depend on the level of vitamin D. There were no statistically significant differences in the distribution of GG, GA and AA genotypes revealed.

Analysis of the frequency distribution of genotypes and alleles of the VDR rs2228570 gene depending on the level of vitamin D (Table 4) in groups of children with different levels of vitamin D revealed differences in the frequency of T and C alleles (p=0.02). A decrease in the level of vitamin D is observed in children with the C allele by 1.84 times and the CC genotype by 2.3 times.

When comparing VDR rs1544410 genotypes with vitamin D levels, it was noted that the level of vitamin D in blood serum in carriers of the AA genotype is reduced comparing to the level in carriers of the GG and GA genotypes. The results indicate that there are significant statistical differences between the AA and GA genotypes (p≥0.03) (Table 5).

When studying the relationship between VDR rs2228570 genotypes and vitamin D levels, it was found that the level of vitamin D in the blood serum of carriers of the TC, TT, and CC genotypes did not have statistically significant differences (p≥0.94). This appears to be due to weak link rather than insufficient statistical power.

Conclusion

The results of the molecular genotypic examination of 197 children under one year old in the Kazakh population made it possible to state the obvious predominance of the GG genotype of the VDR rs1544410 gene (58%) over the GA and AA genotypes, which generally corresponds to global data.

As part of the study, it was established that children with the C allele of the rs2228570 have a reduced level of vitamin D by 1.84 times (95% CI 1.10–3.07) and a CC genotype by 2.3 times compared with children with normal vitamin D content.

Statistical analysis by the Kruskal-Wallis method revealed that the level of vitamin D in blood serum in carriers of the AA genotype of the VDR rs1544410 gene was significantly reduced compared to the level in carriers of the GG and GA genotypes (p=0.03).

The study of the genetic aspects of bone metabolism disorders in children under one year of age is one of the premises for the development of personalized medicine, but requires further in-depth analysis.

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