® Postepy Hig Med Dosw (online), 2013; 67: 873-878
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2012.07.23
Accepted: 2013.04.10
Published: 2013.08.23

Effect of vitamin D receptor gene (VDR) polymorphism on body height in children – own experience*

Wpływ polimorfizmu genu dla receptora witaminy D (VDR) na wysokość ciała u dzieci - doświadczenia własne

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Elżbieta Jakubowska-Pietkiewicz[1, A, B, D, E, F, G], **Izabela Klich**[2, B, D], **Wojciech Fendler**[2, C, D, E], **Wojciech Młynarski**[2, C, D], **Danuta Chlebna-Sokół**[1, D]

[1] Department of Paediatric Propaedeutics and Bone Metabolism Diseases, Medical University, Lodz, Poland
[2] Department of Paediatrics, Oncology, Haematology and Diabetology, Medical University of Lodz, Poland

Summary

Genetic and environmental factors have an influence on the process of growth and development of the body. One of numerous genetic factors can be the vitamin D receptor gene (*VDR*). The study aimed at evaluating the relationship between *VDR* polymorphism and somatic parameters in children.

Patients and methods: The study group consisted of 395 children, aged 6–18 years. All the patients underwent gene typing using the PCR-RFLP method within polymorphic loci *Bsm*I (rs1544410), *Fok*I (rs2228570), *Apa*I (rs7975232) and *Taq*I (rs731236) of the *VDR* receptor gene. 294 children made up the control group in the study on the incidence of particular genotypes; in 161 patients somatic measurements of body weight and height were made with standard methods and skeletal densitometry (total body and spine programmes) examination was performed. Statistica 10.0 PL was used for statistical analysis.

Results: In patients with low bone mass a relationship between body height and *Fok*I *VDR* polymorphism was noted. The p-value was statistically significantly different in group I (p=0.002) and borderline significant in group III (p=0.09). None of the polymorphisms of the *VDR* receptor gene demonstrated any statistically significant differences in anthropometric values in the control group and in children with osteoporosis.

Summary: The presence of the F allele of *Fok*I polymorphism of the *VDR* receptor gene results in increased height, which is best observed in children with low bone mass. The FF genotype favours increased height in the study group of children from Łódź.

Keywords: **vitamin D receptor gene • VDR • children • body height • body weight**

*The study was partly financed by Ministry of Science and Higher Education grant No. 40605031/1860.
The study was funded by the TEAM programme "Polish Registry for Paediatric and Adolescent Diabetes – nationwide genetic screening for monogenic diabetes" financed by activity 1.2 of the Operational Programme "Innovative Economy" European Regional Development Fund and coordinated by the Foundation for Polish Science.
Wojciech Fendler received support from the Foundation for Development of Polish Pharmacy and Medicine and from the European Fund of Regional Development.

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1063747

Word count: 1859
Tables: 4
Figures: 1
References: 23

Author's address: Elżbieta Jakubowska-Pietkiewicz PhD; Department of Paediatric Propedeutics and Bone Metabolism Diseases, 36/50 Sporna St, 91-738 Lodz; e-mail: elzbieta.jakubowska-pietkiewicz@umed.lodz.pl

The vitamin D receptor belongs to a group of nucleus receptors and is a transcriptional factor. After binding with a ligand, i.e. calcitriol [1,25 $(OH)_2$ D], it forms a heterodimer with 9-cis retinoic acid receptor (RXR). The obtained complex binds to promoter loci which are dependent on the vitamin D receptor gene and inhibits or activates transcription. Variability of the vitamin D receptor gene results from its polymorphic loci: *Bsm*I, *Apa*I, *Fok*I, *Tru*I, *Eco*RV, Cdx2 [7,16]. The presence of *VDR* polymorphisms influences the quality of bone mineral density (osteoporosis, osteopenia, low bone mass), the size of bones and susceptibility to fractures [10,22]. In developmental age, when the basis for diagnosing low bone mass in densitometric examination is the Z-score (sum of standard deviation scores adjusted for sex and age), it is necessary to determine the biological age of all children, including basic anthropometric parameters, such as body weight and height. In serious cases of osteoporosis in children and adolescents, bone age should also be considered [1]. In some studies on the relationship between *VDR* polymorphisms and the incidence of osteoporosis or low bone mass, factors of biological development are taken into consideration [2,20]. The aim of the study was to investigate the relationship between the variability of the *VDR* receptor gene and body weight and height in children and adolescents.

Patients and methods

The study group consisted of 395 children, aged 6–18 years. All patients underwent genotyping using the PCR-RFLP method within polymorphic loci *Bsm*I (rs1544410), *Fok*I (rs2228570), *Apa*I (rs7975232) and *Taq*I (rs731236) of the *VDR* gene. The study was conducted in the Department of Immunopathology and Genetics in the Department of Paediatrics, Oncology, Haematology and Diabetes, at the Medical University of Łódź. Two hundred and ninety-four healthy children made up the control group in the study on the incidence of particular genotypes.

In 161 patients basic measurements of somatic development were made using standard methods. Body mass index (BMI) was calculated using the values of body weight and height (body weight (kg)/height $(m)^2$). All the obtained results were standardized for age and sex (Z-score: examined value- mean value/standard deviation) [15]. On the same day in all the children a skeletal densitometry examination was performed using dual energy X-ray absorption measurements (DXA), with the use of the Lunar Prodigy Advance device (GE Healthcare, Madison, US) with a paediatric programme. Two programmes were used: Total body and L2-L4 Spine. Bone mass density (BMD) was measured (g/cm^2, Z-score) in both densitometric projections. The studied patients were divided into three groups depending on the DXA examination result: I – the control group, with Z-score ranging from +1.0 to -1.0; II – patients with low bone mass, Z-score ranging from -1.0 to -2.0; and III – patients with osteoporosis, Z-score<-2.1. The studies were carried out in the Regional Centre for Menopause and Osteoporosis of Clinical Hospital No. 3 of the Medical University of Łódź. Analysis of variance was used for comparisons of multiple groups with Tukey's HSD test for post-hoc comparisons. Student's t-test was used for pairwise comparisons when respective genotype models of polymorphism effect (dominant/recessive) were ascertained. Statistica 10.0 Pl statistics package (StatSoft,

Table 1. Primer pairs and PCR product conditions used for amplification of DNA regions spanning the analyzed polymorphic loci

Polymorphisms	Numer in NCBI	Primer pairs
*Fok*I	rs2228570	5′ CCC TGG CAC TGA CTC TGG CTC TG 3′ 5′ GAA ACA CCT TGC TTC TTC TCC CTC C 3′
*Bsm*I	rs15444410	5′ GCG ATT CGT AGG GGG GAT TCT G 3′ 5′TCT CCA TTC CTT GAG CCT CCA GTC C 3
*Apa*I	rs7975232	5′ CAC GGA GAA GTC ACT GGA GGG C 3′ 5′TCA TCT TGG CAT AGA GCA GGT GG 3′
*Taq*I	rs731236	

Table 2. Z-sore body mass, height and BMI index in three examination group of children

Somatic parameter	Group I (n=30)		Group II (n=91)		Group III (n=40)		p
	X	SD	X	SD	X	SD	
Age	12.9	3.29	12.6	3.39	14.68*	3.07	<0.001
Z-score body mass	0.69*	1.24	-0.52	1.14	-0.86	1.15	<0.001
Z-score body height	0.72*	1.22	-0.31	1.48	-0.78	1.21	<0.001
Z-score BMI	0.33*	1.27	-0.52	1.05	-0.64	1.25	<0.05

* statistically significant in post-hoc comparisons with all other groups p<0,05

Table 3. Mean values, standard deviations and p-values for normalized anthropometric measurements and carriage of polymorphic alleles

Z-score	*Bsm*I				p
	bb, Bb		bb		
	Z-Score	SD	Z-Score	SD	
Body mass	-0.3358	1.31	-0.6747	0.94	0.3038
Body height	-0.1802	1.48	-0.5841	1.11	0.2799
BMI	-0.3739	1.22	-0.5264	0.95	0.6218
	*Fok*I				
	ff, Ff		FF		
Body mass	-0.3873	1.15	-0.3026	1.41	0.6800
Body height	-0.3990	1.27	0.0471	1.61	0.0663
BMI	-0.2793	1.23	-0.4970	1.10	0.2588
	*Apa*I				
	aa. Aa		AA		
Body mass	-0.3832	1.23	-0.3898	1.31	0.9749
Body height	-0.1838	1.45	-0.3648	1.42	0.4443
BMI	-0.4202	1.08	-0.3456	1.36	0.7042
	*Taq*I				
	TT. Tt		tt		
Body mass	-0.4060	1.28	-0.5000	1.00	0.7718
Body height	-0.2069	1.41	-0.4082	1.21	0.5764
BMI	-0.4466	1.16	-0.4205	0.95	0.9293

Tulsa, USA) was used for the purpose of statistical analysis. A threshold of p<0.05 was established as statistically significant. The Institutional Bioethics Committee of the Medical University of Łódź gave consent for the study (No. RNN/72/05/KE as of March 2005). Informed consent forms were collected from parents of all participants.

Molecular methods

PCR reactions were performed in volumes of 20 ml: containing 0.1-0.5 ng of genomic DNA, 8 mM of dNTP, 2-3 mM of $MgCl_2$, 2U of Taq DNA polymerase (TIB Molbiol, Poznan, Poland), using sets of specific oligonucleotide primers spanning the respective polymorphic loci under conditions listed in table 1. An initial period of 10 minutes at 95°C for initial denaturation and 10 minutes at 72°C for final elongation were used in all protocols.In each reaction stages of 10 minutes at 95 °C for initial denaturation and 10 minutes at 72 °C for final elongation were used. Following PCR, 6 ml of the product were digested using 0.2 U of respective enzymes and matching reaction buffers. Samples were incubated overnight at 37°C and separated using 2% agarose gel electrophoresis with ethidium bromide staining. Unique band patterns corresponding to each polymorphism were used to determine the genotype of a given patient.

Results

Table 2 presents characteristic features of the study groups: group I (Z-score ranging from -1.0 to 1.0, n=30, group II (patients with low bone mass, n=91), group III (children with osteoporosis, n=40). The average age of the patients differed significantly between the groups. Children with osteoporosis were older than those with low bone mass and those from the control group. Z-scores

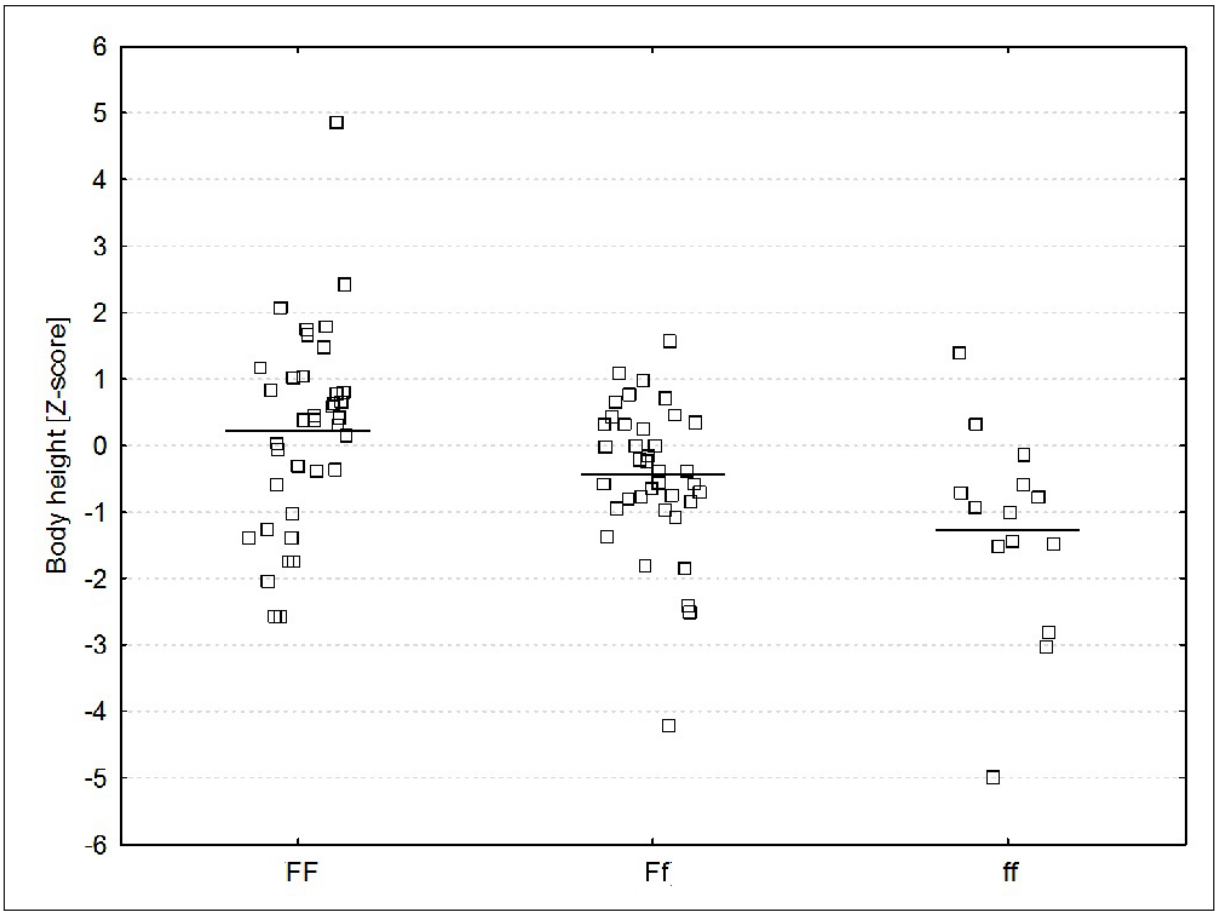

Figure 1. Z-score body height and FokI genotype in children with low bone mass (II)

Table 4. Values of statistical significance (p)from analysis of variance (ANOVA)* for comparing normalized anthropometric parameters with particular genotypes in three groups of patients

Antropometric parameter	*Bsm*I	*Fok*I	*Apa*I	*Taq*I
Group I (control)				
Body mass	0.6233	0.8899	0.6371	0.6381
Body height	0.2347	0.2921	0.9690	0.2357
BMI	0.8399	0.5121	0.4166	0.9759
Group II (osteopenia)				
Body mass	0.8788	0.2313	0.7506	0.9352
Body height	0.9832	**0.0023**	0.8365	0.8567
BMI	0.8791	0.5365	0.7342	0.9478
Group III (osteoporosis)				
Body mass	0.2049	0.3992	0.7981	0.1783
Body height	0.7630	0.2254	0.6296	0.8621
BMI	0.0807	0.5817	0.7805	0.1115

of BMI were significantly greater in group I than those in groups II and III. Similar observations were made for Z-scores of weight and height (table 2). No statistically significant differences were noted for weight, height and BMI depending on genotypes at all three analyzed loci (table 3). Differences in body height were however close to statistically significant depending on the genotype of the *Fok*I locus, with FF homozygotes showing marginally greater than all other genotypes (p=0.07).

Results of subgroup comparisons of age and sex-standardized anthropometric parameters between particular genotypes in patients differing by bone mineral density category are presented in table 4. In group I (the control group) and group III (children with osteoporosis), none of the polymorphisms for the *VDR* receptor gene resulted in statistically significant differences in the values of any of the anthropometric parameters. In patients from group II (low bone mass) a relationship between body height and *Fok*I polymorphism was observed (p=0.002). Z-score values for body height of individuals with different *Fok*I genotypes in group II are depicted in figure 1. In view of the statistically significant differences between the three genotypes one may conclude that in patients with low bone mass ff genotype favours decreased body height.

Discussion

Vitamin D and its receptor influence calcium-phosphate metabolism and bone metabolism [2, 10,20,22]. The patients were divided according to bone mass criteria, introduced before [1]. The authors of the study pointed out the relationship between polymorphism for the *VDR* receptor gene and anthropometric measurements.

According to recently published papers, *VDR* has pleiotropic properties in cells of the immune and renin-angiotensin systems as well as neoplastic cells (it influences proliferation, differentiation and apoptosis) [6,7,11]. In consequence, there is a stronger relationship between the *VDR* receptor gene and rheumatoid arthritis, diabetes, hypertension, obesity, metabolic syndrome, Leśniowski-Crohn's disease, and neoplasms of the alimentary tract and skin [6,19]. Activity of vitamin D and its receptor in overall growth cannot be described as pleiotropic. Some studies suggest that there is a relationship between *VDR* polymorphisms and somatic development indices [2, 20], taken into consideration together with densitometry examination results, which are the basis for diagnosing low bone mass. In the adult population low BMI index is a confirmed factor contributing to the development of osteoporosis. Thus, it is important to make anthropometric measurements [3,8,13].

The conducted analysis did not prove that there is a relationship between *Bsm*I, *Apa*I and *Taq*I polymorphisms of the *VDR* receptor gene and somatic development indices. A relationship between *Fok*I polymorphism and body height in the patients from group II (low bone mass) was noted. FF genotype contributed to increased body height in all the children, irrespective of their sex and age. The obtained results do not correspond to the results of Tao, who concluded that TT genotype of *Taq*I polymorphism resulted in greater body weight and increased height only in girls in preadolescence. Such an association was not observed in boys [20]. Also Suarez et al. claims that the relationship between *VDR* polymorphism and somatic development indices is different for boys and girls [18]. Young healthy girls (2 years old) with BB genotype (*Bsm*I polymorphism) were characterized by greater body weight and increased height. Boys with the same genotype were characterized by lower body weight; they were also shorter and their BMI was lower as well. These associations with *VDR* genotype were also observed at birth and at 10 months of age in the longitudinal analysis of 145 selected full-term babies homozygous for *Bsm*I polymorphism. The authors concluded that the *VDR* genotype may influence intrauterine and early postnatal growth [18]. Lorentzon et al. described the relationship between *Bsm*I and *Taq*I polymorphisms of the *VDR* receptor gene and somatic development indices in a group of healthy Caucasian boys. Boys with the BB genotype had lower body weight at birth and grew more slowly until adolescence than their peers who had *Bb* and *bb* genotypes. In adolescence and post-adolescence the genotype contributed to decreased height (and smaller area of humeral bone, femoral bone and total body projection) [14]. Baroncelli's analysis of 209 children in preadolescence did not show any relationship between *VDR* polymorphism and somatic development indices [2]. Similar conclusions were drawn by Gunnes et al., who studied a group of 73 healthy girls and boys, aged 8–16.5 [5]. Also studies on Japanese girls, aged 12–15, did not show statistically significant associations of *VDR* polymorphism with body weight, height and BMI [9].

Relationships between body height and *VDR* genotype for *Fok*I, *Apa*I and *Taq*I polymorphisms were however observed in a group of Spanish children with bone neoplasms. Ninety-four patients (58 with bone sarcoma and 36 with Ewing's sarcoma) were taller (in comparison to the control group) and Ff genotype of *Fok*I polymorphism appeared more frequently [17]. In the studies presented here the relationship between *Fok*I polymorphism and body height in children with low bone mass was similar.

In the Polish adult population, according to the EPOLOS study, *Bsm*I polymorphism of the *VDR* receptor gene contributes to increased body height in women in premenopausal age. Such an observation was not made in other age groups in women or in the male population [12].

The findings obtained in a group of males with metabolic syndrome, originating from the Wrocław area, indicate that there is a relationship between the BB genotype of *Bsm*I polymorphism and BMI index. The genotype contributed to the increase of BMI index [4]. In post-menopausal women with metabolic syndrome no statistically significant relationship between *VDR* genotype and somatic measurements was noted [21]. In a study of 1873 white patients Xiong et al. found a within-family associa-

tion with height at *Bsm*I and *Taq*I loci (p=0.048 and 0.039, respectively). Subjects with bT haplotype were on average 1% taller than those without it. The authors suggested that *VDR* may be associated with adult height variation in white populations [23].

The influence of *VDR* polymorphisms on somatic development is one of many genetic factors determining both growth and development as well as the involution processes. Moreover, it depends on many environmental factors (geographical, social, economic, cultural). Therefore, the results obtained in various populations are different.

Conclusions

1. The presence of the F allele of *Fok*I polymorphism of the *VDR* receptor gene favours increased body height, which is best observed in children with low bone mass.

2. FF genotype favours increased body height in the studied group of children from Łódź.

3. Variability of the vitamin D receptor gene might be connected with overall growth in children and adolescents.

References

[1] Baim S., Leonard M.B., Bianchi M.L., Hans D.B., Kalkwarf H.J., Langman C.B., Rauch F.: Official Positions of the International Society for Clinical Densitometry and executive summary of the 2007 ISCD Pediatric Position Development Conference. J. Clin. Densitom., 2008; 11: 6-21

[2] Baroncelli G.I., Federico G., Bertelloni S., Ceccarelli C., Cupelli D., Saggese G.: Vitamin D receptor genotype does not predict bone mineral density, bone turnover, and growth in prepubertal children. Horm. Res., 1999; 51: 150-156

[3] Czerwiński E., Badurski J.E., Marcinowska-Suchowierska E., Osieleniec J.: Current understanding of osteoporosis according to the position of the World Health Organization (WHO) and International Osteoporosis Foundation. Ortop. Traumatol. Rehabil., 2007; 9: 337-356

[4] Filus A., Trzmiel A., Kuliczkowska-Płaksej J., Tworowska U., Jędrzejuk D., Milewicz A., Mędraś M.: Relationship between vitamin D receptor BsmI and FokI polymorphisms and anthropometric and biochemical parameters describing metabolic syndrome. Aging Male, 2008; 11: 134-139

[5] Gunnes M., Berg J.P., Halse J., Lehmann E.H.: Lack of relationship between vitamin D receptor genotype and forearm bone gain in healthy children, adolescents, and young adults. J. Clin. Endocrinol. Metab., 1997; 82: 851-855

[6] Holick M.F.: The influence of vitamin D on bone helalth across the life cycle. Vitamin D epidemic and its health consequences. J. Nutrition, 2010; 4: 2739s-2748s

[7] Holick M.F., Chen T.C.: Vitamin D deficiency: a worldwide problem with health censequences. Am. J. Clin. Nutr., 2008; 87 (Suppl. 1): 1080S-1086S

[8] Kanis J.A.: WHO Scientific Group on the Assessment of Osteoporosis at Primary Health Care Level. Technical Report. WHO Press, 2007

[9] Katsumata K., Nishizawa K., Unno A., Fujita Y., Tokita A.: Association of gene polymorphisms and bone density in Japanese girls. J. Bone Miner. Metab., 2002; 20: 164-169

[10] Kelly P.J., Morrison N.A., Sambrook P.N., Nguyen T.V., Eisman J.A.: Genetic influences on bone turnover, bone density and fracture. Eur. J. Endorcinol., 1995, 133: 265-271

[11] Köstner K., Denzer N., Müller C.S., Klein R., Tilgen W., Reichrath J.: The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature. Anticancer Res., 2009; 29: 3511-3536

[12] Kruk M., Jaworski M., Łukaszkiewicz J., Karczmarewicz E., Biliński P., Czerwiński E., Lewiński A., Marcinowska-Suchowierska E., Milewicz A., Spaczynski M., Uitterlinden A., Lorenc R.S. i grupa EPOLOS: Wpływ polimorfizmu BsmI genu VDR na wysokość ciała u dorosłych mężczyzn i kobiet w populacji polskiej- badanie EPOLOS. XVIII Wielodyscyplinarne Forum Osteoporotyczne. Postępy w diagnostyce i leczeniu osteoporozy. Materiały Zjazdowe, 19.05.2007, Warszawa; 33-34

[13] Lorenc R., Głuszko P., Karczmarewicz E., Misiorowski W., Księżopolska-Orłowska K., Franek E., Horst-Sikorska W., Jabłoński M., Jaworski M., Goncerz G., Męczekalski B., Olszynski W., Pluskiewicz W., Przedlacki J., Skalska A., Trznadel-Morawska I., Więcek A.: Zalecenia postępowania diagnostycznego i leczniczego w osteoporozie. Aktualizacja 2011. Medycyna Praktyczna: Reumatologia, wyd. spec. 2011, 1: 11-40

[14] Lorentzon M., Lorentzon R., Nordström P.: Vitamin D receptor gene polymorphism is associated with birth height, growth to adolescence, and adult stature in healthy caucasian men: a cross-sectional and longitudinal study. J. Clin. Endocrinol. Metab., 2000: 85: 1666-1670

[15] Malinowski A., Chlebna-Sokół D.: Dziecko łódzkie. Metody badań i normy rozwoju biologicznego. Wyd. Ankal, Łódź 1998

[16] Nejentsev S., Godfrey L., Snook H., Rance H., Nutland S., Walker N.M., Lam A.C., Guja C., Ionescu-Tirgoviste C., Undlien D.E., Ronningen K.S., Tuomilehto-Wolf E., Tuomilehto J., Newport M.J., Clayton D.G., Todd J.A:. Comparative high-resolution analysis of linkage disequilibrium and tag single nucleotide polymorphisms between populations in the vitamin D receptor gene. Hum. Mol. Genet., 2004: 13: 1633-1639

[17] Ruza E., Sotillo E., Sierrasesúmaga L., Azcona C., Patino-Garcia A.: Analysis of polymorphisms of the vitamin D receptor, estrogen receptor, and collagen Ialpha1 genes and their relationship with height in children with bone cancer. J. Pediatr. Hematol. Oncol., 2003: 25: 780-786

[18] Suarez F., Zeghoud F., Rossignol C., Walrant O., Garabédian M.: Association between vitamin D receptor gene polymorphism and sex-dependent growth during the first two years of life. J. Clin. Endocrinol. Metab., 1997: 82: 2966-2970

[19] Śledzińska K., Góra-Gębka M., Kamińska B., Liberek A.: Plejotropowe działanie witaminy D3 ze szczególnym uwzględnieniem jej roli w chorobach układu pokarmowego u dzieci. Med. Wieku Rozw., 2010; 14: 59-67

[20] Tao C., Yu T., Garnett S., Briody J., Knight J., Woodhead H., Cowell C.T.: Vitamin D receptor alleles predict growth and bone density in girls. Arch. Dis. Child, 1998; 79: 488-494

[21] Tworowska-Bardzińska U., Lwow F., Kubicka E., Łaczmański Ł., Jedrzejuk D., Dunajska K., Milewicz A:. The vitamin D receptor gene Bsml polymorphism is not associated with anthropometric and biochemical parameters describing metabolic syndrome in postmenopoausal women. Gynecol. Endocrinol., 2008; 24: 514-518

[22] Uitterlinden A.G., Pols H.A., Burger H., Huang Q., Van Daele P.L., Van Duijn C.M., Hofman A., Birkenhäger J.C., Van Leeuwen J.P.: A large-scale population-based study of the association of vitamin D receptor gene polymorphisms with bone mineral density. J. Bone Miner. Res., 1996; 11: 1241-1248

[23] Xiong D.H., Xu F.H., Liu P.Y., Shen H., Long J.R., Elze L., Recker R.R., Deng H.W.: Vitamin D receptor gene polymorphisms are linked to and associated with adult height. J. Med. Genet., 2005; 42: 228-234

The authors have no potential conflicts of interest to declare.