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ORIGINAL ARTICLE

The Association Between CYP2R1 rs10741657 Polymorphisms and Bone Variables, Vitamin D, and Calcium in Iranian Children and Adolescents: A Cross-Sectional Study

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Abstract

Osteoporosis is a common disorder with a strong genetic component. Bone mineral density (BMD), vitamin D, and calcium levels declining are a main contributor of osteoporosis and fragility fractures. This cross-sectional study designed to explore the possible link between CYP2R1 rs10741657 polymorphism and BMD of the total hip, lumbar spine and femoral neck, vitamin D, and calcium in Iranian children and adolescents. 247 children and adolescents (127 girls and 120 boys) between 9 and 18 years old from Kawar (an urban area located 50 km east of Shiraz, the capital city of the Fars province in the south of Iran) were randomly selected based on age-stratified systematic sampling and recruited for genetic analysis. The polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method was used for genotyping CYP2R1 rs10741657. Anthropometric, biochemical, and bone mineral density (BMD) parameters were also measured. The results specified that in the dominant [$P < 0.0001$, − 2.943 (− 4.357–1.529)] and over-dominant [$P < 0.0001$, 2.789 (1.369–4.209)] models, vitamin D concentration significantly differed between genotypes. The highest vitamin D levels were displayed for participants carrying the rs10741657 AG genotype (16.47 ng/ml). In regard to calcium, in a dominant model [$P = 0.012$, 0.194 (0.043–0.345)] and over-dominant model [$P = 0.008$, 0.206 (− 0.357–0.055), there was a significant association. AG genotype displayed the highest (9.96 mg/dl) and GG genotype the lowest (9.75 mg/dl) calcium values. This study reported the association of CYP2R1 rs10741657 polymorphisms with calcium and vitamin D levels in Iranian children and adolescents.

Keywords Osteoporosis · Polymorphism · Children · Vitamin D · Calcium

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Introduction

Bone mineral density (BMD) declining is a main contributor of osteoporosis and fragility fractures (Liu et al. 2012). As reported in epidemiological studies, a 10% increase in peak bone mass in the pediatric period is associated with a 50% reduction in subsequent fracture risk at adult period (Zhu and Zheng 2021). The maximum bone mass attainment occurs in childhood and post-peak bone loss rate affecting BMD in adults (Hansen et al. 1991). Genetic and non-genetic determinants (physical activity, calcium and vitamin D intake, weight, smoking, and alcohol consumption) are responsible for BMD (Zhu and Zheng 2021). Twin and family-based studies on osteoporosis have designated that about 60–85% of the BMD is predisposed hereditarily (Zhu and Zheng 2021). Among non-genetic factors, Vitamin D is a well-established endocrine hormone which regulate intestinal calcium absorption and ionization of calcium (Björk et al. 2018). A meta-analysis and systematic review-confirmed vitamin D deficiency is closely linked to calcium homeostasis and bone mineralization (Reid et al. 2014; Borel et al. 2015).

Vitamin D is a prohormone that its synthesis and activation is initiated in the skin and finalized in the kidney. In the first step, provitamin D3 (7-dehydrocholesterol) is converted to previtamin D3 (cholecalciferol) within the skin after exposure to UV light. After that in the liver, 25(OH)D3 (the main circulating form of vitamin D) is produced by the hydroxylation process which is mainly conducted by the cytochrome P450 enzyme, 25-hydroxylase (CYP2R1). The final step occurs in the kidney where 1alpha-hydroxylase (CYP27B1) add another hydroxyl moiety to 25(OH)D3 and produce vitamin D3 active metabolite $1,25(OH)_2D3$. Binding of $1,25(OH)_2D3$ and its cell surface receptor regulates downstream pathways, such as cell proliferation, differentiation, and apoptosis (Torkko et al. 2020). Variation at the gene level of enzymes involving vitamin D metabolism can affect vitamin D levels (Berry and Hyppönen 2011).

A hereditary form of vitamin D deficiency and rickets in children is as a result of polymorphisms in the main enzyme participate in vitamin D hydroxylation in the liver (CYP2R1) (Molin et al. 2017; Thacher et al. 2015). Additionally, CYP2R1 SNPs; rs10741657 has been identified as one of the most important genetic determinants of low vitamin D levels in large-scale studies (Wang et al. 2010; Manousaki et al. 2017). CYP2R1 rs10741657 is participating in regulating gene expression and activity, maintaining of 25-hydroxylase (Thacher et al. 2015).

According to our best information, the association between BMD changes and CYP2R1 gene polymorphism in the children and adolescent is not well elucidated. In one study, an association was detected for CYP2R1 (rs11023374) with hip BMD in MrOS Sweden in elderly men. Another study in postmenopausal Chinese women displayed no relation between CYP2R1 rs10766197 and BMD (Li et al. 2016). Our study goal is to inspect the association of CYP2R1 rs10741657 with vitamin D, calcium, and BMD in children and adolescent in the city located in the south of Iran. Since in this region of Iran, the population has enough sunlight exposure and receive adequate vitamin D from nutrition, but a deficiency

of vitamin D is prevalent. So, we proposed that polymorphisms in the vitamin D metabolic pathway may affect the serum concentration of vitamin D, calcium, and BMD (as a consequence of vitamin D deficiency) in this region of Iran. Accordingly, we directed a cross-sectional study to explore the possible link between CYP2R1 rs10741657 polymorphism and vitamin D, calcium, and BMD of the total hip, lumbar spine, and femoral neck in Iranian children and adolescents.

Material and Methods

Study Population

In this cross-sectional study, 247 children and adolescents (127 girls and 120 boys) between 9 and 18 years old from Kawar (an urban area located 50 km east of Shiraz, the capital city of the Fars province in the south of Iran) were randomly selected based on age-stratified systematic sampling and recruited for genetic analysis. To be eligible for the study, children and adolescents did not have systemic problems (e.g., thyroid complications, diabetes, renal problem, adrenal deficiency), history of developed postponed puberty, and using any drugs (e.g., anticonvulsants or steroids). Ethical approval for this study was obtained from the ethics committee of Shiraz University of Medical Sciences. An informed consent was gained from participants and their parents. The informed consent was also signed by their parents.

SNP Selection

The criteria of CYP2R1 rs10741657 SNP selection from The National Center for Biotechnology Information Single Nucleotide Polymorphism (SNP) Consortium Database (www.ncbi.nlm.nih.gov/SNP) were as follows:

(1) rs10741657 is a common (minor allele frequency ≥ 0.1 according to TOPMED) (https:, , www.ncbi.nlm.nih.gov, snp, rs10741657horizontal_tab=true.) and functional polymorphism (affect the activity of vitamin D 25-hydroxylase) located in the 5′-untranslated region of gene CYP2R1. (2) The significant association of CYP2R1 rs10741657 with vitamin D deficiency and its biological impact in vitamin D metabolism was recognized in earlier studies. (3) CYP2R1 rs10741657 association with BMD was not investigated in children and adolescents and also in the Iranian population.

Genotyping of the CYP2R1 gene.

Blood samples for genotyping were collected after 10–12 h overnight fasting in tubes containing EDTA as an anticoagulant and kept at − 70 °C until extraction. Genomic DNA was isolated and purified from the whole blood by Cinnagen Kit DNPTM protocol (DNG plus DNA Extraction Kit, Cinnagen Company, Tehran, Iran). The polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method was used for genotyping CYP2R1 rs10741657.

Amplification of 276 base pairs (bp) was done by PCR using forward primer (FP): 5'- Forward:5′ GCTTACGACTCATTCACAC 3′ and reverse primer (RP) 5′ CAG

CTCCAATGTCATCTTC 3′. After amplification, the PCR product was digested with 3 units of *MnII* restriction enzyme (New England Biolabs) at 37 ◦C overnight in a total volume of 20 µl. The homozygous "GG" genotype gave rise to 3 bands of 118-bp, 106-bp, and 52-bp fragment size, while infrequent "AA" genotype gave rise to 2 bands of 158-bp and 118-bp fragment size and the heterozygous genotype "AG" yielded 4 bands of 158-bp, 118-bp, 106-bp, and 52-bp fragment size.

Anthropometric Parameters

Children's weight and height measurement was conducted using a standard scale and a wall-mounted meter (Seca, Hamburg, Germany), respectively. The weight was rounded to the nearest 0.1 kg, and height was rounded to the nearest 0.5 cm. Body mass index (BMI) and pubertal stages calculation was presented in the previous study (Montazeri-Najafabady et al. 2017).

Measurement of BMD Variables

BMC in grams, bone area (BA) in square centimeters, BMD in g/cm2 were quantified by the Hologic system DXA (Discovery QDR, USA). The variation coefficient was 2.4% for the femoral neck, 0.51% for the lumbar spine, and 1% for the total body (based on the quantities in ten children). Total body less head (subtotal) including arms, ribs, spine, pelvis, and legs of both right and left side, total spinal involved L1, L2, L3 and L4, and total femoral involved neck, trochanteric, and intertrochanteric were measured and expressed in BA (cm2), BMC (g), and BMD (g/cm2). Densitometry studies were implemented when the child was wearing special clothing without shoes.

Assessments of Biochemical Parameters

All the blood samples were taken by an experienced technician and stored in the Shiraz Endocrinology Research Center after 8–12 h of fasting. Serum calcium was checked by colorimetric assay with an auto-analyzer (Biosystems SA, Barcelona, Spain). The concentration of vitamin D was checked by high-performance liquid chromatography (Young Lee 9100, South Korea) in ng/ml.

Statistical Analysis

All statistical analyses were accomplished using IBM SPSS Statistics v 22.0 for Windows. Quantitative variables were presented as mean ± standard deviation. One sample Kolmogorov–Smirnov test was used to check the demographic, clinical, and DXA parameters for normal distribution. If the data had normal distribution, we used T test for the boys and girls separately and if the data had no normal distribution, we used Mann–Whitney test. Differences Between dependent variables in three genotype groups were estimated using parametric (one-way ANOVA test with Bonferroni correction in the case of multiple comparisons)/

nonparametric (Mann–Whitney U or Kruskal–Wallis) tests for continuous variables. The generalized linear model for vitamin D (non-normal distribution) and linear regression for calcium (normal distribution) was performed to find their association and CYP2R1 rs10741657 SNP in three genetic models (Dominant, recessive, over-dominant) when adjusted for age and sex. P values less than 0.05 were considered statistically significant.

Results

Basic Characteristics

Table 1 shows the baseline characteristics of participants according to the sex. The mean ± SD of age, weight, height, BMI, total area, total BMC, total BMD, vitamin D, and calcium were similar between girls and boys, and no significant variation was observed.

CYP2R1 rs10741657 Genotypes and Allele Frequency

Genotypes and allele frequency of CYP2R1 (rs10741657) are presented in Table 2. G allele was the major allele with the frequency of 60.7% and A allele was the minor allele with the frequency of 39.3%. GG, AG, and AA genotype frequency were 38.9%, 43.7%, and 17.4%, respectively. We observed no difference in the frequencies of the genotypes and alleles of the rs10741657 polymorphism between girls and boys.

Table 1 Basic characteristic of the studied population. The values are presented as mean ± SD

Data	Girls (127)	Boys (120)	P value
Age (year)	13.39 (2.9)	13.9 (2.6)	0.17
Weight (kg)	43.23 (13.6)	42.2 (14.5)	0.61
Height (cm)	153.6 (14.5)	153.7 (15.6)	0.98
BMI (kg/m^2)	17.8 (3.3)	17.5 (3.6)	0.52
Total area (cm^2)	1610 (295)	1610 (314)	0.98
Total BMC (g)	1444 (457)	1428 (462)	0.80
Total BMD (g/cm2)	0.877 (0.12)	0.866 (0.12)	0.56
Serum Calcium (mg/dl)	9.8 (0.47)	9.8 (0.62)	0.80
Serum Vitamin D (ng/ml)	14.8 (5.1)	15.1 (5.5)	0.85

T test was used for age, weight, height, BMI, and calcium

Mann–Whitney was used for Total area, Total BMC, Total BMD, and Serum Vitamin D

Table 2 Genotypes and allele frequency of CYP2R1 (rs10741657) in studied population

Data	Gender		*P* value
	Boys ($n=120$)	Girls ($n=127$)	
Genotype			0.13
GG	48 (48%)	48 (37.9%)	
AG	46 (45%)	62 (48.8%)	
AA	26 (7%)	17 (13.3%)	
Allele			
G	142 (59.1%)	158 (62.2%)	0.48
A	98 (40.9%)	96 (37.8%)	

Chi-square test was used for obtaining genotypes and allele frequency

Table 3 Effect of CYp2R1 (rs10741657) polymorphism on Bone variables, vitamin D, and calcium in our studied population

Data	Genotype			*P* value
	GG (96)	AG (108)	AA (43)	
Total body				
BMC (g)	1388.98 (449.62)	1468.21(469.05)	1370.15 (379.90)	0.331
BMD (g/cm^2)	0.86 (0.12)	0.87 (0.12)	0.85 (0.11)	0.428
Area (cm^2)	1577.08(292.16)	1632.64(314.40)	1575.71(250.08)	0.343
Lumbar Spine				
BMC (g)	38.26 (15.23)	40.07 (17.04)	37.41 (11.68)	0.565
BMD (g/cm^2)	0.81 (0.16)	0.83 (0.18)	0.82 (0.16)	0.555
Area (cm2)	45.71 (10.87)	46.16 (11.40)	44.62 (7.31)	0.733
BMAD	0.19 (0.03)	0.20 (0.03)	0.20 (0.03)	0.495
Femur				
Neck BMC (g)	3.31 (0.86)	3.49 (0.97)	3.32 (0.71)	0.313
Neck BMD (g/cm^2)	0.69 (0.12)	0.72 (0.14)	0.76 (0.46)	0.259
Neck area (cm^2)	4.74 (0.51)	4.79 (0.50)	4.77 (0.65)	0.791
Neck BMAD	0.14 (0.02)	0.14 (0.02)	0.14 (0.02)	0.599
Calcium *	9.75 (0.56)	9.96 (0.51)	9.78 (0.56)	0.020
Vitamin D *	13.36 (3.2)	16.47 (6.73)	15.17 (4.1)	<0.0001

LSD post hoc analysis was done for significant P values. P values less than 0.05 were shown in bold

One-way ANOVA test with Bonferroni correction in the case of multiple comparisons was used to explore the differences between dependent variables in three genotype groups parametric

*Post Hoc for vitamin D: AA vs AG = 0.178, AA vs GG = 0.067, and AG vs GG = < 0.0001

Post Hoh for Calcium: AA vs AG = 0.073, AA vs GG = 0.793, and AG vs GG = 0.008

The Effect of CYP2R1 rs10741657 Polymorphism on Bone Variables, Vitamin D, and Calcium

One-way ANOVA results for the difference between bone variables, vitamin D, and calcium, in three genotype groups of CYP2R1 rs10741657 is displayed in Table 3. We perceived no significant variation between genotype frequency and total BMC ($P=0.331$), total BMD (0.428), total area (0.343), lumbar spine BMC (0.565), lumbar spine BMD (0.55), lumbar spine area (0.733), lumbar spine BMAD (0.495), neck BMC (0.313), neck BMD (0.259), neck area (0.791), and neck BMAD (0.599). There was a significant association between vitamin D ($P=<0.0001$) and calcium ($P=0.020$) concentrations and genotype frequencies. In the case of vitamin D, post hoc analysis indicated a significant difference when AG compared to GG ($P=<0.0001$). For the rs10741657 polymorphism, highest vitamin D levels were displayed for participants carrying the rs10741657 AG genotype (16.47 ng/ml), intermediate levels were revealed in participants carrying the rs10741657 AA genotype (15.17 ng/ml), and lowest levels were presented in participants carrying the rs10741657 GG genotype (13.36 ng/ml). Similar to vitamin D, LSD post hoc analysis for pairwise comparison between genotypes and calcium revealed a significant association between AG and GG ($P=0.08$). AG genotype displayed the highest (9.96 mg/dl) and GG genotype displayed the lowest (9.75 mg/dl) calcium values.

Association Between CYP2R1 rs10741657 Genetic Models and Vitamin D and Calcium

In Table 4, generalized linear model was used for analyzing the effects of CYP2R1 rs10741657 genotypes (in three genetic models), on vitamin D (dependent variable) adjusted for age and sex. Linear regression was performed for analyzing the effects of CYP2R1 rs10741657 genotypes (in three genetic models) on calcium (dependent variable) adjusted for age and sex. The results specified that in the dominant (GG vs AG/AA) [$P<0.0001$, − 2.943 (− 4.357–1.529)] and over-dominant (AG vs AA/GG) [$P<0.0001$, 2.789 (1.369–4.209)] models, vitamin D concentration significantly

Table 4 The association of CYP2R1 (rs10741657) dominant (GG vs AG/AA), recessive (AA vs AG/GG), and over-dominant (AG vs AA/GG) genetic models with vitamin D and calcium adjusted for age and sex

Data	Models		
	Dominant P value, OR (95% CI)	Recessive P value, OR (95% CI)	Over-dominant P value, OR (95% CI)
Vitamin D	**< 0.0001**, -2.943 (-4.357–1.529)	0.565, 1.192 (-2.872–5.256)	**< 0.0001**, 2.789 (1.369–4.209)
Calcium	**0.012**, 0.194 (0.043–0.345)	0.675, 0.091 (-0.336–0.517)	**0.008**, 0.206 (-0.357–0.055)

Generalized linear model was used for analyzing the effects of genotypes (in three models), age, and sex on vitamin D (dependent variable). Linear regression for analyzing the effects of genotypes (in three models), age, and sex on calcium (dependent variable). For each genetic model, one regression analysis was conducted. *P* values < 0.05 are shown in bold

differed between genotypes. In regard to calcium, in a dominant model [$P=0.012$, 0.194 (0.043–0.345)] and over-dominant model [P = 0.008, 0.206 (− 0.357–0.055)] there was a significant association. In the recessive model (AA vs AG/GG) for both vitamin D [$P=0.565$, 1.192 (− 2.872–5.256)] and calcium [$P=0.675$, 0.091 (− 0.336–0.517)], no significant association was observed.

Discussion

In this cross-sectional study, polymorphism in CYP2R1 rs10741657 was associated with vitamin D and calcium concentrations in dominant (GG vs. AG/GG) and over-dominant models (AG vs. GG/AA) when adjusted for age and sex in Iranian children and adolescents. No significant association was observed between CYP2R1 rs10741657 polymorphism and bone variables including area, BMC, and BMD of total body, lumbar spine, and femur.

The human cytochrome P450 family 2 subfamily R member 1 (CYP2R1) gene known as a member of the CYP450 enzyme superfamily is placed at 11p15.2. CYP2R1 enzyme is a key physiological enzyme for converting vitamin D into 25-hydroxyvitamin D [25(OH)D] (Shinkyo et al. 2004).

Two genome-wide association study (GWAS) in 30,000 subjects of European descent from 15 cohorts or in 496 unrelated healthy Caucasian subjects revealed that rs10741657 was significantly associated with vitamin D (Wang et al. 2010; Bu et al. 2010). CYP2R1 rs10741657 polymorphism is oriented in the non-coding region 5′-untranslated region (Ramos-Lopez et al. 2007). In addition, polymorphism in CYP2R1 led to Proline Leucine substitution at amino acid 99 in the CYP2R1 protein and (Nissen et al. 2014) linked to the diminished expression and activity of 25-hydroxylases, with direct effect on the serum 25(OH) D level (Thacher et al. 2015).

Kotur et al. analyzed the different populations for CYP2R1 rs10741657 G allele frequencies and displayed that Serbian had the lowest G allele occurrence (0.58) among European populations, and African population had the highest G allele frequency (0.73). Italian and Spanish populations had the maximum frequency of the G allele (0.66 and 0.69, respectively) among Europeans (Kotur et al. 2021). The minor allele frequency of CYP2R1 rs10741657 in Arabs, south Asian, and southeast Asian was 0.31, 0.36, and 0.42, respectively (Elkum et al. 2014). The minor allele frequency of CYP2R1 rs10741657 based on GWAS by Wang et al. 2010 (11) was 0.4. The minor allele frequency in Iranian children and adolescents based on the outcomes of our study was about 39.3.

Also, we detected that CYP2R1 rs10741657 polymorphism was associated with the change in vitamin D concentration. We spotted that individuals carrying two risk allele (G allele) of CYP2R1 rs10741657 had the lowest value of vitamin D and calcium compared to non-carriers (AA genotype) or carriers of one G allele (AG genotype). The participants with GG genotype had serum vitamin D and calcium levels below the reference values.

We found that individuals with GG genotype had 2.94 times lower odds of vitamin D concentration compared to AA/AG genotypes in a dominant model after

adjustment for age and sex. In addition, individuals with one A allele (AG) had 2.78 times higher odds of vitamin D concentration compared to non-carriers (GG). It seems that the positive effect of A allele on 25(OH) D concentration was greater than the negative effect of G allele.

As mentioned earlier, CYP2R1 rs10741657 G allele results in reduced CYP2R1 synthesis, which likely leads to a decrease in the conversion rate of cholecalciferol to vitamin D (Hindy et al. 2018). In agreement with our findings, Lafi et al., in Jordanian (Lafi et al. 2015) and Hassanein et al., in Egyptian population (Hassanein et al. 2014) indicated that polymorphisms in CYP2R1 were associated with vitamin D deficiency. Also, rs10741657 was associated with variation in vitamin D values among in 872 participants of the German Asthma Family Study (Wjst et al. 2006). In another study, Ramos-Lopez et al. confirmed strong association between the rs10741657 genotype and serum levels of vitamin D in 203 German patients with diabetes (Ramos-Lopez et al. 2007). Another recent GWS study investigated the vitamin D modulating genes in early childhood for a cohort of 761 healthy Finnish children who supplemented with vitamin D and reported the role of CYP2R1 rs10741657 in response to supplementation (Kämpe et al. 2019).

Hypermethylation in CpG sites might be a reason for the lower 25(OH)D level in rs10741657 "GG" genotype compared to "AA" and "AG" genotypes (Harishankar et al. 2021). Our findings revealed that the presence of the A allele of CYP2R1 rs10741657 (located in the promoter region of the gene) enhances enzyme production. Because the G allele at rs10741657, which was more frequent in our population, was associated with lower vitamin D. It could be postulated that this allele previously conferred an evolutionary advantage to prevent vitamin D toxicity (Moon et al. 2017).

Lasky-Su et al. (Lasky-Su et al. 2012) performed a meta-analysis of 1164 subjects from two groups of Caucasian and Costa Rican asthmatic children and found that rs10741657 was significantly associated with vitamin D levels.

Only one study investigates the association of CYP2R1 rs10741657 polymorphism in which no association was reported (Björk et al. 2018). In this research, we observed that was significantly associated with calcium levels. Individuals with GG genotype showed lower calcium values compared to AG/AA genotype in a dominant model adjusted for age and sex p value = 0.012, odds ratio (CI 95%) 0.194 (0.043–0.345). In over-dominant model, p value was 0.008 with the odds ratio (CI 95%) of 0.206 (− 0.357–0.055). Vitamin D and calcium homeostasis relation are essential for optimal skeletal health (Khazai et al. 2008). Calcium active absorption from the small intestine is dependent on the presence of optimal vitamin D levels. The latest studies have displayed that a minimum concentration of vitamin D of 32 ng/mL is required to provide optimum protection against fractures and intestinal absorption of calcium (Khazai et al. 2008). Calcium intake in the absence of vitamin D is approximately 10% to 15% of dietary calcium, which increases to 30% to 40% in the presence of sufficient vitamin D (Holick 2007). So it seems that the effect of CYP2R1 rs10741657 polymorphism on vitamin D conversion could be as a subsequent directly affecting serum calcium concentration.

In the current study, we did not observe any relation between bone parameters and CYP2R1 rs10741657 polymorphism. Like our research findings, Trummer

et al. showed no association between SNP rs10741657 and five-year follow-up of fracture incidence in a cohort of 342 subjects in Austria (Trummer et al. 2019). A possible explanation is that bone variables could be controlled by other mechanisms than only the levels of vitamin D. In contrast, Bjork et al. indicated that in the CYP2R1 haplotypes are associated with levels of vitamin D and bone mineral density in the MrOS Sweden study (4). They concluded that the mechanistic action of CYP2R1 on the bone cell surface is mediated by the local conversion of vitamin D depending on the CYP2R1 genotype rather than by an effect on circulating vitamin D levels (4).

This is a former study on association of CYP2R1 rs10741657 polymorphism and vitamin D, calcium, and BMD of the total hip, lumbar spine, and femoral neck in Iranian children and adolescents. This is the primary study that reported the association of CYP2R1 rs10741657 polymorphisms with calcium levels in children and adolescents.

As limitations, we did not explore the mechanism of action of CYP2R1 rs10741657 polymorphisms on vitamin D and calcium levels. Also, investigating the haplotype analysis of CYP2R1 could be better for understanding the association of this gene with vitamin D concentration which will be the topic of our future work. Regarding the relatively small sample size, the statistical potency for detecting associations of CYP2R1 rs10741657 variants with vitamin D, calcium and bone variables was limited. Although a significant difference in calcium and vitamin D levels across the three genotypes, it may not translate into clinical significance because of complex polygenic architecture of BMD. So that, investigating the association of other haplotypes that is in linkage disequilibrium with CYP2R1 loci could be helpful in guiding clinicians for detecting bone loss based on these differences.

Author contributions All authors contributed to the study conception and design, material preparation, data collection and analysis, and manuscript writing. All authors read and approved the final manuscript.

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Data availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to [restrictions, e.g., their containing information that could compromise the privacy of research participants].

Declarations

Competing interests The authors declare no competing interests.

Conflict of interests The author(s) declare(s) that they have no competing interests.

Ethical approval All procedures performed in the study were in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for the experiments involving humans. The research protocol was evaluated and confirmed by the Ethics Committee of Shiraz University of Medical Sciences (ethic code: IR.SUMS.REC.1400.211).

Consent to participate Written informed consent forms for the use of samples were obtained from all participants.

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