Environmental Science and Pollution Research (2023) 30:106059–106067
https://doi.org/10.1007/s11356-023-29698-w

RESEARCH ARTICLE

Association between ADAMTS14_rs4747096 gene polymorphism and bone mineral density of Chinese Han population residing in fluorine exposed areas in ShanXi Province, China

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Received: 24 April 2023 / Accepted: 31 August 2023 / Published online: 19 September 2023

Abstract

This study aimed to investigate the effects of fluorine and ADAMTS14_rs4747096 on bone mineral density (BMD). The survey was explored in a cross-sectional case–control study conducted in Shanxi, China. The BMD was measured by an ultrasonic bone mineral density instrument. The urine fluoride concentration was detected using the fluoride ion electrode. ADAMTS14_rs4747096 polymorphism was examined by multiplex polymerase chain reaction (PCR) and sequencing. The multinomial logistic regressions found that the urine fluoride was a risk factor for osteopenia (OR = 1.379, 95% CI: 1.127–1.687, $P = 0.0018$), osteoporosis (OR = 1.480, 95% CI: 1.1138–1.926, $P = 0.0035$), and rs4747096 AG + GG genotype increased the risk of osteoporosis (OR = 2.017, 95% CI: 1.208–3.369, $P = 0.0073$). In addition, the interaction between urine fluoride and rs4747096 polymorphism on the risk of decreased BMD also was observed. The study suggests that fluoride exposure and mutation G allele in ADAMTS14_rs4747096 may be risk factors for the decrease of BMD. And there is an interaction between the two influencing factors.

Keywords Bone mineral density · ADAMTS14_rs4747096 · Fluoride · Osteopenia · Osteoporosis

Introduction

Bone mineral density (BMD) is a complex numerical variable, and it is a measurable and powerful index predicting osteopenia and osteoporosis (Deng et al. 2013). Osteoporosis is recognized as the most prevalent bone disorder in the world (Coughlan and Dockery 2014). It is a widespread and common disease in older adults and is a major public health problem worldwide (Lane et al. 2000). Meanwhile, BMD measurement criteria was established by the World Health Organization (WHO) to diagnose osteoporosis before incident fractures (Srivastava and Deal 2002). Abnormal BMD is an early manifestation of these bone diseases (Kazakia and Majumdar 2006, Sun et al. 2020). Therefore, the influencing factors of BMD have been widely focused. BMD is affected by the cumulative and interactive effects of genetic and environmental factors. Among these factors, genetics account for 40–70% of the influence (Shoukry et al. 2015; Yao et al. 2021). Environmental factors such as fluoride, cadmium(Cd), lead(Pb), phthalates, and perfluoroalkyl and polyfluoroalkyl substances (PFASs) are expected to have adverse effects on BMD(Goyer et al. 1994). These environmental factors regulate bone turnover by altering the activity of osteoblasts and osteoclasts, thereby affecting BMD (Elonheimo et al. 2021).

Fluoride is a bone-loving tissue element. It is widely found in the soil, rocks, and water throughout the world, with high concentrations especially in areas with recent/past pyroclastic activity or geological uplift (Everett 2011). Although adding fluoride to drinking water has been carried out in many areas of the world for the reduction of dental caries in past years (Lan et al. 1995), long-term

Responsible Editor: Lotfi Aleya

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ingestion of excess fluoride can harm human and animal health (Dhar and Bhatnagar 2009). Studies have shown that high fluoride deposition causes bone resorption and calcium level alterations in bone tissue, resulting in an imbalance in the bone mineral metabolism and abnormal bone turnover (Joshi et al. 2011; Mou et al. 2011). And chronic exposure to excess fluoride can disturb bone mineralization and alter BMD (Sun et al. 2020). It is estimated that at least 25 countries on all continents, including Asia, Africa, Europe, and North and South America, are at risk of exposure to high fluoride (Yadav et al. 2019). Therefore, it is critical to investigate the relationship between fluoride exposure and BMD.

In addition to environmental factors, genetic factors also play an important role in BMD. Single-nucleotide polymorphisms (SNPs) are one of the most prevalent heritable variants and occur at a frequency of approximately 1 per 1000 base pairs in human genome (Fan et al. 2022). It has been reported that RREB1 polymorphisms (rs475011) may contribute to increase BMD in elderly Chinese women (Feng et al. 2021). Ichikawa et al. found that the polymorphism of ALOX12 gene might be a protective factor for spine BMD (Ichikawa et al. 2006). Moreover, it has been discovered that common LRP5 polymorphisms influence BMD variation in the general population (Koay et al. 2004). These results suggest that some SNPs in bone regulation genes are associated with BMD.

The ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family contains 20 members which play an important role in bone-related diseases (Kevorkian et al. 2004; Yu et al. 2015). ADAMTS-2, ADAMTS-3, and ADAMTS-14 promote the formation of collagen fibers (Li et al. 2022b). ADAMTS18 may be related to BMD and increased the risk of osteoporosis (Xiong et al. 2009). ADAMTS14 gene polymorphism was associated with knee osteoarthritis, and ADAMTS14_rs4747096 may be a diagnostic marker and therapeutic target for knee osteoarthritis (Ma et al. 2018). However, the association of SNPs in ADAMTS14_rs4747096 with the risk of BMD has not been reported. Therefore, we conducted a case–control study in high fluorine exposed areas in Shanxi Province, China, to investigate the effects of fluorine and ADAMTS14_rs4747096 on BMD.

Materials and methods

Participants and data collection

In 2018, a cross-sectional case–control study was conducted in lv Liang city, Shanxi Province, People's Republic of China, an area with high fluoride drinking water before the water improvement according to long-term monitoring by the Shanxi Institute of Endemic Disease Prevention and Control. The questionnaires were completed by professionally trained investigators. The questionnaires included population information (age, gender, occupation, education, drink, smoke, etc.), and clinical examination (blood pressure, height, weight, bone mineral density, etc.). The study was confirmed by the Ethics Committee of Chinese Center for Disease Control and Prevention, Center for Endemic Disease Control (hrbmu-ecdc20210303). All participants signed informed consent forms.

Patients had to be (1) > 20 years; (2) born and grew up in their village for more than 16 years; (3) diagnosed with osteopenia or osteoporosis; (4) Han people (to avoid different polymorphisms distribution between ethnics). Exclusion criteria for patients included (1) bone-related diseases such as bone tumors, (2) information loss, (3) unable to answer the question correctly, (4) hemolysis samples, and (5) rheumatoid or rheumatism diseases. The control group was a healthy population homogeneous to the case group. Urine samples were stored at − 20 °C until analysis.

Criteria for grading bone mineral density (BMD)

Bone mineral density of calcaneus was measured by the ultrasonic bone densitometer (Yang et al. 2022). BMD *t*-value was used to classify the severity of osteoporosis in patients. *T* value ≥ -1 SD was considered normal, − 2.5 SD < *T* value < -1.0 SD was reduced bone mass, and *T* value ≤ -2.5 SD was osteoporosis (Kanis et al. 2008; Tsai et al. 2021).

Measurement of fluoride concentration

All urine samples were stored at − 20 °C until use. Before the determination, 5 mL urine and 5 mL buffer solution were mixed. Then, the concentration of urine fluoride was determined by fluoride ion selective electrode (Yingke Crystal Materials Company, Hunan, China) based on China's urinary fluoride detection-fluoride electrode method WS/T89-2006. The final fluorine concentration was calculated by averaging the two measurements for each sample.

ADAMTS14_rs4747096 genotyping

All blood samples were stored at − 80 °C until they were analyzed. The genomic DNA was isolated from the blood samples using the DNA extraction kit (ENLIGNTEN Mag Blood DNA Extraction Kit (1000 T)). ADAMTS14_rs4747096 was detected by multiple PCR and Genotype sequencing, and was performed by the Beijing Novogene Biological Information Technology Co., Ltd (https://www.novogene.com/).

ADAMTS14_rs4747096 were followed:

rs4747096 forward: GATGTCTGGGAACTTGGGAC
rs4747096 reverse: CTTCATAACTGAGGTATCTGAGGC

Determination of serum calcium and serum vitamin D

Serum samples were stored at − 80 °C. Before testing, take 200 μL sample and thaw in refrigerator at 4 °C. Serum calcium and vitamin D were detected using automatic biochemical analyzer 3100 (Hitachi High-Technologies Corporation, Japan) for evaluating intake level. The test kit is from Meikang Biotechnology Co., LTD. Serum calcium and vitamin D were categorized according to the definition of the kit specification. Serum calcium was defined 2.25–2.75 mmol/L as the normal range. Serum vitamin D was defined 25(OH)D < 30 ng/mL as deficient, 30–100 ng/mL as adequate, and > 100 ng/mL as excessive.

Diagnosis of diabetes mellitus

Whole blood samples were obtained at − 80 °C until they were analyzed. HbA1c was tested by Automatic Biochemical Analyzer (Hitachi 3100, Japan) using Glycosylated hemoglobin A1c kits by Micon Biological Co., LTD, followed the kit instruction and the manual of Analyzer. Based on the International Diabetes Federation "IDF DIABETES ATLAS: Ninth edition 2019," participants with HbA1c ≧ 6.5% were diagnosed as diabetics.

Determination of biochemical indexes

Serum samples were stored at − 80 °C. Before testing, take 200 μL sample and thaw in refrigerator at 4 °C. In accordance with the kit instruction, GSH, GSSH, TNF-α, IL1-β, IL-6, and INF-γ were tested using MILLIPLEX MAP kits (Millipore Corporation, Billerica, USA).

Statistical analysis

All statistical analyses were conducted using the SAS (version 9.4). Pearson's chi-square test was used for categorical variables; Kruskal–Wallis test was used for ordered multiple categorical variables. ANOVA test was used for continuous variables which conform to homogeneity of variance and normality. Testing for deviation from Hardy–Weinberg equilibrium (HWE) was performed within the control participants using Pearson's chi-square test. Multiple logistic regressions were performed to examine the association of genotypes with BMD. Odds ratio (OR) and 95% confidence intervals (95% CI) were used to assess the risk. $P < 0.05$ was considered statistically significant for all analysis.

Results

Participants' characteristics

In total, we recruited 943 individuals, including 296 controls, 515 with osteopenia, and 132 with osteoporosis. Their characteristics including age, gender, urine fluoride, smoke, drink, education, blood calcium, vitamin D, and diabetes mellitus are listed in Table 1. At the same time, indexes of antioxidant, oxidative stress, and inflammatory biomarkers are listed in Table S1.

Patients with osteoporosis or osteopenia were significantly older than controls (58.12 ± 10.49, 66.03 ± 9.19 vs 53.55 ± 10.47, $F = 67.38$, $P < 0.0001$). The proportion of gender ($\chi^2 = 21.67$, $P < 0.0001$), smoke ($\chi^2 = 10.04$, $P = 0.0066$), drink ($\chi^2 = 12.71$, $P = 0.0018$), education ($\chi^2 = 39.62$, $P < 0.0001$), or diabetes mellitus ($\chi^2 = 10.40$, $P = 0.0342$) was observed significant difference among these three groups. Compared with control individuals, patients with osteopenia or osteoporosis exhibited higher urine fluoride (UF) ($F = 30.72$, $P < 0.0001$; Fig. 1). Meanwhile, it was found that UF was a risk factor for osteopenia (OR = 1.379, 95% CI: 1.127–1.687, $P = 0.0018$) and osteoporosis (OR = 1.480, 95% CI: 1.1138–1.926, $P = 0.0035$) by using the multinomial logistic regressions (Table S2). There was no statistical significance in the blood calcium ($P = 0.5996$) and vitamin D ($P = 0.2336$) among these groups. The indexes of antioxidant oxidative stress GSH/GSSG ($P = 0.55$) was no statistical significance, and the SOD values were in the normal range. GSH/GSSG is the main dynamic index of cell redox status. There was no statistical significances of the inflammatory biomarkers TNF-α ($P = 0.11$), IL1-β ($P = 0.81$), IL-6 ($P = 0.72$), and INF-γ ($P = 0.23$).

Allele, genotype, and genetic model for ADAMTS14_rs4747096

Testing for deviation from Hardy–Weinberg equilibrium (HWE) was performed within the control participants, and the hypothesis of HWE could not be rejected for each of the ADAMTS14_rs4747096 ($P > 0.05$). The association of ADAMTS14_rs4747096 with decreased BMD was analyzed after adjusting for age (continuous), gender, smoke, drink, and urine fluoride, culture, and diabetes. Individuals with AG genotype increased 2.145 times osteoporosis risk than individuals with AA genotype in a co-dominant model (OR = 2.145, 95% CI: 1.263–3.645, $P = 0.0048$). Individuals with at least one mutation allele (AG genotype and GG genotype) had a 2.017 times higher risk of osteoporosis than individuals with the AA genotype in dominant model

Table 1 Descriptive the general characteristics of osteoporosis cases, osteopenia cases and control ($n = 943$)

	BMD			χ^2/F	P value
	Control	Osteopenia	Osteoporosis		
Age (continuous, years)	53.55 ± 10.47	58.12 ± 10.49	66.03 ± 9.19	67.38	**< 0.0001**
Gender					
Male	87 (29.39)	202 (39.45)	26 (19.70)	21.67	**< 0.0001**
Female	209 (70.61)	310 (60.55)	106 (80.30)		
UF (mg/L)					
< 1.6	217 (73.31)	322 (62.52)	77 (58.33)	12.97	**0.0015**
≥ 1.6	79 (26.69)	193 (37.48)	55 (41.67)		
UF (continuous, mg/L)	1.138 (0.771,1.620)	1.363 (0.902,1.891)	1.460 (1.082,2.301)	30.72	**< 0.0001**
Smoke					
Yes	49 (16.78)	115 (22.73)	15 (11.54)	10.04	**0.0066**
No	243 (83.22)	391 (77.27)	115 (88.46)		
Drink					
Yes	42 (14.38)	119 (33.47)	18 (13.85)	12.71	**0.0018**
No	250 (85.62)	388 (76.53)	112 (86.15)		
Education					
Never	12 (4.17)	22 (4.38)	12 (9.60)	36.87	**0.0001**
Primary school	73 (25.35)	161 (32.07)	57 (45.60)		
Junior high school	178 (61.81)	248 (49.40)	50 (40.00)		
Senior high school or above	25 (8.68)	71 (14.14)	6 (4.80)		
Blood calcium					
< 2.25 mmol/L	41 (13.80)	61 (11.84)	16 (12.12)	1.0228	0.5996
2.25–2.75 mmol/L	256 (86.20)	451 (87.57)	116 (87.88)		
> 2.75 mmol/L	0 (0.00)	3 (0.58)	0 (0.00)		
Vitamin D					
< 30 ng/mL	8 (2.70)	17 (3.30)	3 (2.27)	5.5702	0.2336
30 ~ 100 ng/mL	241 (81.42)	403 (78.25)	115 (87.12)		
> 100 ng/mL	47 (15.88)	95 (18.45)	14 (10.61)		
Diabetes mellitus					
Yes	25 (8.89)	40 (8.03)	16 (12.59)	10.40	**0.0342**
No	256 (91.11)	458 (91.97)	111 (87.41)		

Control; bone mineral density > −1. Osteopenia; −2.5 = < bone mineral density = < −1. Osteoporosis; bone mineral density < −2.5. Bold indicates statistical significance at 0.05 level. Continuous variables were expressed as mean ± standard deviation ($\bar{x} \pm s$) for normality. Categorical variables were presented as number (proportion/ percentage)

UF urine fluoride

(OR = 2.017, 95% CI: 1.208–3.369, $P = 0.0073$). In over-dominant model, individuals with mutation heterozygous (AG genotype) increased 1.944 times risk of osteoporosis than individuals with other genotypes (OR = 1.944, 95% CI: 1.192–3.170, $P = 0.0077$). Similarly, the log-additive model also showed statistically significant (OR = 1.525, 95% CI: 1.060–2.196, $P = 0.0231$) in the osteoporosis group. The AIC, SC, and −2LogL values (1462.9, 1587.8, and 1410.9) in dominant genetic model were the smallest among these five genetic models, suggesting dominant genetic model best fitted this study. (Table 2).

Risk factors of BMD by logistic regression stepwise analysis

Because many factors had inter-correlated effects, logistic stepwise regression analysis was used to identify the variables that contributed significantly to the presence of osteopenia and osteoporosis. Analysis revealed that ADAMTS14_rs4747096 AG + GG genotypes and female were the risk factors for the susceptibility to osteoporosis after adjusting for different variables, while urine fluoride was a risk factor for the susceptibility to osteopenia after

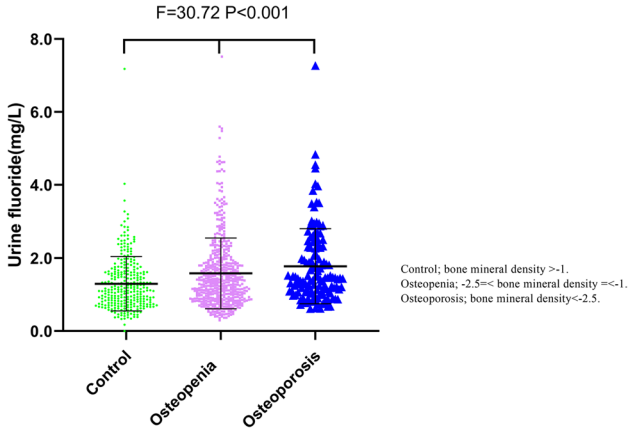

Fig. 1 The pictures of urine fluoride level in each group

adjusting for different variables except model 5. Age was a risk factor for both osteopenia and osteoporosis.

In these models, model 4 was the best (AIC = 1353.9, SC = 1440.3, and − 2Log = L1317.9) as shown in the Table S3 and Table 3. After adjustment, the risk of osteopenia increased by 1.038-times for every year of age rise and by 1.444-times for every unit of urine fluoride increase. Meanwhile, the risk of osteoporosis increased to 1.135-time with the increase in age; female participants had a 2.19-fold increase in the risk of osteoporosis than males (OR = 3.19, 95% CI: 1.456–6.671); the participants with ADAMTS14_rs4747096 AG + GG genotype had a higher risk of osteoporosis.

Interaction of ADAMTS14_rs4747096 SNP and the level of urine fluoride

The interaction between UF (mg/L) and rs4747096 SNP on the risk of BMD was further evaluated and shown in Table 4. The interaction between UF ≥ 1.6 (mg/L) and AG + GG genotype increased the risk of osteopenia to 1.739 times, and the risk of osteoporosis to 2.842 times. The interaction between UF < 1.6 (mg/L) and AG + GG genotype increased the risk of osteoporosis to 2.077 times. And degree of education had no influence on these above interactions (Table S4).

Discussion

The decrease of BMD is the early manifestation of osteoporosis, and maintaining normal BMD is of great significance to reduce the prevalence of osteoporosis and improve the quality of life (Gao et al. 2020). BMD is influenced by many factors, including environmental factors (Koay et al. 2004). Fluoride is a widely element distributed in the natural environment, which promotes two processes of bone metabolism:

Table 2 The genetic model of ADAMTS14_rs4747096 adjusted for confounding factor

Genetic model	Genotype	BMD			OR_1 (95% CI)	P_1 value	OR_2 (95% CI)	P_2 value	AIC	SC	−2LogL
		Control	Osteopenia	Osteoporosis							
Co-dominant	AA	145 (48.99)	235 (45.63)	49 (37.12)	1.0 (ref)		1.0 (ref)				
	AG	117 (39.53)	237 (46.02)	69 (52.27)	1.274 (0.911–1.782)	0.1576[a]	**2.145 (1.263–3.645)**	**0.0048**			
	GG	34 (11.48)	43 (8.35)	14 (10.61)	1.584 (0.687–3.652)	0.4855[a]	0.825 (0.480–3.652)	0.2807	1486.5	1611.3	1434.5
Dominant	AA	145 (48.99)	235 (45.63)	49 (37.12)	1.0(ref)		1.0 (ref)				
	AG + GG	151 (51.01)	280 (54.37)	83 (62.88)	1.171 (0.850–1.612)	0.3335[a]	**2.017 (1.208–3.369)**	**0.0073**	**1462.9**	**1587.8**	**1410.9**
Recessive	GG	34(11.49)	43 (8.35)	14 (10.61)	1.0(ref)		1.0 (ref)				
	AA + AG	262 (88.51)	472 (91.65)	118 (89.39)	0.786 (0.468–1.319)	0.8322[a]	1.125 (0.521–2.428)	0.0895	1513.9	1638.6	1641.9
Overdominant	AA + GG	179 (60.47)	278 (53.98)	63 (47.73)	1.0 (ref)		1.0 (ref)				
	AG	117 (39.53)	237 (46.02)	69 (52.27)	1.316 (0.955–1.815)	0.0935[a]	**1.944 (1.192–3.170)**	**0.0077**	**1479.9**	**1604.6**	**1427.9**
Additive	For each variant allele (G) increase				1.039 (0.818–1.319)	0.7551	**1.525 (1.060–2.196)**	**0.0231**	**1522.8**	**1638.0**	**1474.8**

Bold indicates statistical significance at 0.05 level. a: Adjusted for age (continuous), diabetes mellitus, gender, smoke, drink, and urine fluoride, culture. P_1, OR_1: osteopenia vs control; P_2, OR_2: osteoporosis vs control

Table 3 Logistic regression stepwise analyses the effect of urinary fluorine concentration, ADAMTS14_rs4747096 SNP on osteoporosis risk

Variable	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)	Model 4 OR (95%CI)	Model5 OR (95%CI)	Model 6 OR (95%CI)	Model 7 OR (95%CI)
Age (continuous, years)	–	**1.125 (1.098, 1.152)***	**1.133 (1.103, 1.165)**	**1.135 (1.104, 1.168)**	**1.128 (1.096, 1.161)**	**1.130 (1.100–1.162)**	**1.138 (1.109, 1.167)**
Gender[b]							
Male			1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
Female	–	–	**2.561 (1.401, 4.681)**	**3.192 (1.447, 7.044)**	**3.116 (1.456, 6.671)**	**3.023 (1.471, 6.211)**	**3.113 (1.523, 6.364)**
UF[c]							
< 1.6 (mg/L)	1.0 (ref)	1.0(ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)		
≥ 1.6 (mg/L)	**1.931 (1.374, 2.714)**	1.435(0.897,2.297)	1.393 (0.829, 2.339)	1.392 (0.823, 2.352)	1.324 (0.793, 2.211)	–	–
Smoke[d]							
Yes				1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
No	–	–	–	1.401 (0.558, 3.515)	1.455 (0.596, 3.553)	1.457 (0.622, 3.415)	1.408 (0.610, 3.254)
Drink[e]							
Yes				1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
No	–	–	–	0.520 (0.201, 1.346)	0.510 (0.204, 1.279)	0.539 (0.225, 1.288)	0.541 (0.228, 1.280)
Diabetes mellitus[f]							
Yes			1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
No	–	–	1.231 (0.551, 2.750)	1.232 (0.537, 2.827)	0.410 (0.039, 4.266)	1.240 (0.584, 2.633)	1.267 (0.598, 2.683)
Education							
Never					1.0 (ref)	1.0 (ref)	
Primary school	–	–	–	–	1.044 (0.380, 2.865)	1.078 (0.413, 2.812)	–
Junior high school	–	–	–	–	0.897 (0.325, 2.481)	0.935 (0.355, 2.460)	–
Senior high school or above	–	–	–	–	1.045 (0.262, 4.161)	1.073 (0.288, 3.997)	–
SNP							
AA	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
AG + GG	**1.594 (1.143, 2.222)**	**1.605 (1.045, 2.610)**	**1.762 (1.060, 2.929)**	**1.876 (1.117, 3.148)**	**2.069 (1.247, 3.434)**		
AIC	2937.5	1646.6	1373.7	**1353.9**	1463.0	1603.6	1627.5
SC	2966.6	1685.4	1441.2	**1440.3**	1587.8	1709.2	1694.9
-2LogL	2925.5	1630.6	1345.7	**1317.9**	1411.0	1559.6	1599.5

Bold indicates statistical significance at 0.05 level

Table 4 The interaction of ADAMTS14_rs4747096 SNP and the level of urine fluoride on risk of abnormal bone mineral density

	Wald$_1$ χ^2	OR$_1$	OR$_1$ (95% CI)	P_1 value	Wald$_2$ χ^2	OR$_2$	OR$_2$ (95% CI)	P_2 value
UF < 1.6 (mg/L) * AA genotype	-	-	-	-				
UF ≥ 1.6 (mg/L) * AG + GG genotype	**4.6765**	**1.739**	**1.053–2.870**	**0.0306**	**7.0401**	**2.842**	**1.314–6.147**	**0.0080**
UF ≥ 1.6 (mg/L) * AA genotype	0.3888	1.182	0.699–2.000	0.5329	0.4627	1.347	0.571–3.178	0.4963
UF < 1.6 (mg/L) * AG + GG genotype	0.0936	1.065	0.712–1.592	0.7597	**4.4033**	**2.077**	**1.049–4.110**	**0.0359**

Wald$_1$ χ^2, P_1, OR$_1$, OR$_1$ (95% CI): control vs osteopenia; Wald$_2$ χ^2, P_2, OR$_2$, OR$_2$ (95% CI): control vs osteoporosis; Bold indicates statistical significance at 0.05 level. Adjusted for age (continuous), diabetes mellitus, gender, smoke, and drink

bone formation and resorption (Yang et al. 2016). It has an ability to improve BMD, and can be used in the therapy of osteoporosis, especially in the lumbar spine, and increasing bone formation (Haguenauer et al. 2000; Kleerekoper and Mendlovic 1993; Prestwood et al. 1995). At the same time, it is reported that excess fluoride consumption can lead to abnormal bone turnover (Jiang et al. 2018; Mou et al. 2011), bone loss, and an increased chance of osteoporosis (Krishnamachari and Krishnaswamy 1973; Lane 2006; Li et al. 2022a; Lv et al. 2016). Therefore, the effect of fluoride on bone metabolism is double-sided. It is essential to explore the relationship between the fluoride exposure and BMD in the population living in that area where drinking fluoride contains high fluoride. According to national standards WS/T 256–2005 (China), people whose urine fluoride are over 1.6 mg/L are considered to have excessive fluoride in the body. In this study, we observed that urinary fluoride level (continuous, mg/L) was related to the decreased in BMD, which is consistent with previous studies that found the association of levels of fluoride exposure with risk of decreased BMD (Krishnamachari and Krishnaswamy 1973; Li et al. 2022a; Lv et al. 2016). Therefore, high fluoride might be a risk factor for BMD decrease and it is vital to reduce the content of fluoride in drinking water in this area.

Moreover, it has been reported that a decrease in BMD is related to some gene SNPs (Gao et al. 2020; Kang et al. 2020; Sun et al. 2020; Zhang et al. 2020). ADAMTS14, having procollagen N-endopeptidase activity, is identified as one of expression signatures for responsiveness of IGR-CaP1 cells to the bone microenvironment (Al Nakouzi et al. 2012). It is frequently co-expressed with ADAMTS2, which is involved in type I collagen maturation (Bekhouche and Colige 2015). Type I collagen is the major organic component in bone, providing a stable template for mineralization (Terajima et al. 2014). ADAMTS14_rs4747096 located in exon. After splicing, changing glutamic acid (GAA) to glycine (GGA) affects the biological properties (Ma et al. 2018; Poonpet et al. 2013). This study evaluated the effect of ADAMTS14_rs4747096 on the risk of osteopenia or osteoporosis in the overall study participants of Shanxi Province, China. We selected this model for analysis because ADAMTS14_rs4747096 was best fitted with the dominant genetic model, and we found that the AG + GG genotypes were associated with a higher risk of osteoporosis. Therefore, G allele of ADAMTS14_rs4747096 SNP might be a risk factor for osteoporosis. It suggests that we may able to conduct further research on the relationship between ADAMTS14 and BMD, especially the significance of rs4747096.

Genetic variables influence the susceptibility of trabecular bone to both anabolic and catabolic stimuli (Yang et al. 2018), indicating that BMD is regulated by the combination of environmental and genetic factors. In this research, ADAMTS14_rs4747096 AG + GG genotype was a risk factor for the susceptibility to osteoporosis, while urine fluoride was a risk factor for osteopenia. The interaction effect suggested that the risk was further increased in the combination of fluoride exposure and AG + GG genotype. Hence, the likelihood of a BMD decline can be enhanced by both the AG + GG genotype and fluoride exposure. Aging and female are risk factors for osteoporosis and associated fractures (Kelsey 1989; Wang et al. 2009). Similarly, we also found age was a risk factor for the decrease of BMD, and female was a risk factor for osteoporosis. However, we did not find the association of decrease in BMD with some potential risk factors, such as smoke, drink, diabetes mellitus, blood calcium, blood vitamin D levels, antioxidant oxidative stress GSH/GSSG and SOD, the inflammatory biomarkers TNF-α, IL1-β, IL-6, and INF-γ (Agas et al. 2017; Labouesse et al. 2014; Napoli et al. 2017; Papaioannou et al. 2009; Yokota et al. 2021). It might be caused by the following reasons: (1) Blood calcium and vitamin D levels were uniformly distributed among the groups in this study; (2) the population may have good living habits, the smoking and drinking population accounted for a smaller proportion in the total population; (3) we excluded bone correlation diseases that may associated with antioxidant oxidative stress and inflammatory biomarkers, such as heumatoid or rheumatism diseases.

To our knowledge, this is the first epidemiologic study investigating the possible links between ADAMTS14 polymorphism, fluoride exposure level, and risk of BMD in a population. ADAMTS14 polymorphism could be used to test for disease vulnerability in the general population. However, there were two limitations in our study. First, as a result of the complexity of the pathogenesis of decreased BMD, we only focused on the relationship between rs4747096 and BMD. More research is necessary to determine whether the occurrence of the disease is simultaneously influenced by a number of genes or polymorphism sites. Second, literature reports that a healthy diet, in particular, with adequate intakes of calcium and vitamin D, and adequate physical activity throughout life can help determine one's risk for osteoporosis (Weaver et al. 2016). In this survey, food intake was investigated; because conditions were limited, we did not conduct a precise dietary survey to accurately calculate daily calcium intake. However, this survey was conducted in the rural areas of central China, where the living habits, lifestyles, and the dietary structure of the respondents were similar. Meanwhile, there no difference was observed in blood vitamin D and calcium levels between the different groups. Our study showed that high fluoride exposure and G allele of ADAMTS14 rs4747096 might be the risk factor for BMD, and there is an interaction between the two influencing factors. Our finding supports the hypothesis that genetic variations in genes may affect BMD. Additional research is warranted to validate these findings in a larger sample and

to study the role of genes and/or polymorphic sites in the pathogenesis of BMD.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-023-29698-w.

Author contribution Ming Qin: conceptualization, methodology, visualization, and writing–original draft. Yue Gao: writing modification. Meichen Zhang: writing modification. Juahua Wu: resources. Yang Liu: resources. Yuting Jiang: resources. Xiaodi Zhang: investigation. Xin Wang: investigation. Yanmei Yang: writing–review and editing, and supervision. Yanhui Gao: conceptualization, methodology, writing-review and editing, supervision, and project administration.

Funding This work was supported by the National Key R&D Program of China (2022YFC2503000), Natural Science Foundation of China (82273751).

Data availability Not applicable.

Declarations

Ethics approval Applicable.

Consent to participate Applicable.

Consent for publication Applicable.

Competing interests The authors declare no competing interests.

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