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*Original Article*

# VDR gene polymorphism and osteoporosis risk in Chinese Mulam people

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**Abstract:** Background: Osteoporosis is a chronic disease caused by multiple factors, and the vitamin D receptor (VDR) gene plays a significant role in bone metabolism. Objectives: To genotype three VDR single nucleotide polymorphisms (SNPs) - rs7975232, rs2228570, and rs1544410 - and investigate their associations with osteoporosis risk in the Mulam ethnic population of China. Methods: A total of 384 middle-aged and elderly people over 45 years of age from the Mulam ethnic group in Luocheng County, Hechi City, Guangxi Province, China, were selected as the study subjects. The bone mineral density (BMD) was determined and three VDR gene loci were genotyped. Results: Significant sex-based differences in BMD were observed among middle-aged and elderly individuals of the Mulam ethnic group, with males exhibiting higher BMD values than females (P<0.01). BMD exhibited a progressive decline with advancing age, paralleled by an age-dependent increase in the prevalence of abnormal bone mass (osteopenia/osteoporosis). The CT genotype at the VDR rs1544410 locus was strongly associated with osteopenia risk (OR=3.414, 95% CI=1.894, 6.154, Chi-square value =17.995, P<0.001). A statistically significant difference was observed between alleles C and T. The T allele at the rs1544410 locus may act as a risk allele, and its presence is significantly associated with an increased risk of bone loss (OR=3.414, 95% CI=1.849, 6.302, Chi-square value =17.05, P<0.001). Carrying the AGT gene haplotype showed a higher risk (OR=4.719, 95% CI=1.298-17.161, P=0.009). Conclusions: The rs1544410 of the VDR gene is associated with the risk of osteoporosis in Chinese Malam people. AGT haplotypes had a higher risk.

**Keywords:** Mulam people, VDR gene, polymorphism, osteoporosis, BMD, Broadband Ultrasound Attenuation (BUA)

## Introduction

Osteoporosis is characterized by reduced bone density, degradation of bone tissue, altered bone microarchitecture, diminished bone strength, and fracture [1, 2]. Osteoporosis is often termed a "silent disease" in its early stages due to the absence of fractures or symptoms despite progressive BMD loss, making early detection challenging without screening [3, 4]. Osteoporosis's primary clinical symptoms in its middle and late phases are pain and fracture, which not only cause suffering for the patient but also place a strain on families and society [5, 6]. Osteoporosis has grown to be a severe public health issue due to the associated morbidity, mortality decreased life quality, and high treatment costs [7-9].

The diagnosis of osteoporosis is primarily grounded in the World Health Organization (WHO) diagnostic framework utilizing dual-energy X-ray absorptiometry (DXA)-derived BMD measurements. Established in 1994, the WHO diagnostic threshold defines osteoporosis as a BMD T-score of ≤-2.5 standard deviations (SD) below the mean value for young healthy adults of the same sex and ethnicity [10]. According to WHO, T-score ≥-1.0 is normal bone mass, -Osteopenia: -2.5<T-score <-1.0 is osteopenia,

T-score ≤-2.5 is osteoporosis [10]. Despite being the gold standard for osteoporosis diagnosis, DXA faces limitations in community-based screening due to high costs and poor portability. Quantitative ultrasound (QUS) has evolved into a significant bone assessment modality since its pioneering development by Langton et al. By analyzing low-frequency broadband ultrasound parameters - speed of sound (SOS) and BUA-QUS evaluates comprehensive bone strength characteristics including density, trabecular architecture, and elastic modulus. Ultrasound-bone interactions (absorption/scattering) induce detectable signal modifications: SOS correlates with bone elasticity, while BUA reflects trabecular structural integrity. The calcaneus is the preferred site for QUS measurements due to its high content of cancellous bone, which exhibits heightened sensitivity to bone mass changes compared to other anatomical regions. With advantages of portability, cost-effectiveness, radiation-free operation, and user-friendliness, QUS is widely adopted in primary care, health screenings, and research settings for early osteoporosis detection. Studies confirm strong consistency between QUS and DXA-derived bone density measurements, coupled with QUS's unique capacity to evaluate bone biomechanical properties, making it particularly advantageous for large-scale bone abnormality screening in community and field investigations [11-13].

China has the world's fastest aging population and the largest elderly population. In total, 249.49 million people in China were over 60 by the end of 2018, making up 17.9% of the country's total population. It is anticipated that 300 million people in China will be over 60 by 2025, by which time China will become a super-aged country [14]. Osteoporosis is on the rise every year due to the aging of society and the growth in average life expectancy. With the arrival of an aging society and the increase in the incidence of osteoporosis, the medical costs of osteoporotic fractures for the elderly in China are expected to double by 2035 [15]. This means that China's elderly will face severe challenges in terms of health, financial burden, and social security.

The incidence of osteoporosis and osteoporotic fractures depends on peak bone mass and bone loss rate, which are influenced by a combination of genetic, environmental, and lifestyle factors, including age, sex, nutritional status, physical activity, medication use, and comorbidities [16]. Genetic factors significantly affect bone mass, accounting for 40-75% of the inter-individual variation [17, 18]. VDR gene polymorphism may be associated with BMD [19]. Few research reports exist on the Mulam ethnic group in Guangxi.

The relationship between VDR gene polymorphism and osteoporosis is a current research hotspot. The VDR gene is the effector gene for vitamin D and has significant physiological effects. Vitamin D is a steroid derivative. Its activated form 1,25-(OH)2D3 works in conjunction with VD and VDR and has biological activities such as regulating blood calcium and tissue cell differentiation [20]. The VDR gene has nine exons and eight introns and is found on chromosome 12 (12Q13-14) [21]. At present, there are more than 20 known VDR gene polymorphisms. In addition, 3 polymorphic loci may be closely related to osteoporosis, namely rs7975232, rs1544410, and rs2228570 [22]. VDR gene polymorphism is associated with osteoporosis risk, but there are differences between races and genders [23]. Kow M et al. found that in white British men, rs7975232 and rs1544410 of the VDR gene were not associated with osteoporosis [24]. A meta-analysis showed that polymorphisms in rs7975232 and rs1544410 may affect the risk of osteoporosis in Caucasians, whereas polymorphisms in rs1544410 may affect the risk of osteoporosis in Asians [23]. The VDR gene is crucial in regulating bone metabolism, and various studies have suggested a possible link between osteoporosis and VDR gene polymorphism. However, there is still no clear conclusion on this relationship due to inconsistent results from different studies. Some studies have shown that VDR gene polymorphism is connected to decreased BMD in postmenopausal females, while others have found no significant association. Similarly, according to some research, VDR gene variants may increase the risk of osteoporosis in specific ethnic groups, while others have not found any significant association. Therefore, it is essential to carry out further research based on diverse ethnic groups in order to better comprehend the link between VDR gene polymorphism and osteoporosis. There are currently

few investigations on the connection between osteoporosis and VDR gene variation in the Mulam ethnic group of China. This study aimed to genotype three VDR polymorphisms (rs7975232, rs2228570, and rs1544410) and investigate their associations with osteoporosis risk in the Mulam ethnic population of China.

## Materials and methods

### *Subjects*

We recruited 384 Chinese Mulam minority individuals over 45, comprising 165 men and 219 women, from Guangxi Luocheng Mulam Ethnic Autonomous County. Every subject has been a resident for over three generations. Participants were stratified into five-year age intervals (e.g., 45-49, 50-54 years), with those aged ≥70 years classified as a single category. All participants provided written informed consent, and the study protocol was approved by the Ethics Committee of Youjiang Medical University for Nationalities (Approval No. 2020040901).

### *Inclusion and exclusion criteria*

Inclusion: Members 45 years of age and older, as well as members of the Mulam ethnic minority (for at least three generations), are included.

Exclusion: All conditions that impact bone metabolism within a year are excluded, including heart disease, hypertension, liver and renal insufficiency, diabetes, ovariectomy, thyroid, parathyroid, endocrine, blood system, connective tissue illness, and drugs.

### *Measurement of BMD*

The Achilles Express (GE, Fairfield, IA, USA) was used to assess the BMD of the right calcaneus in the study population. The device was calibrated using a standard template (DXA), and all subjects were tested independently by a professional. Test method: Preheated the instrument for 30 min before the test, and operated it as prompted after passing the test. Inputted the name, age, sex, and other pertinent information of the subject before measurement. Sprayed alcohol evenly between the right foot of the subject and the contact film of the instrument and fixed the right foot in the instrument test slot before the test. The measurement indexes included BUA (dB/MHz) and T score. According to the Diagnostic Guidelines of Primary Osteoporosis in China [25], the investigation population was divided into the following three categories: T-score ≥-1.0 is normal bone mass, - Osteopenia: -2.5<T-score <-1.0 is osteopenia, T-score ≤-2.5 is osteoporosis. This is consistent with the WHO diagnostic criteria described above.

### *SNP genotyping of the VDR gene*

The SNPs genotyping assay of the VDR gene was performed using the KASP genotyping technology on the LGC IntelliQube platform of Nanning Virkai Biotechnology Co. in Guangxi, China. This technology designs primers based on terminal base-specific matching for the SNP loci of the target gene. The specifically matched primers were used to perform high-precision two-allele typing of the specified SNPs by competitive allelic characterization PCR. The main steps were as follows: ① Primer design: The SNP sequence of the gene was analyzed to design proper specific primers. ② PCR reaction system: According to the number of samples to be tested and SNP sites to be tested, the corresponding MasterMix and AssayMix were mixed and transferred to a 96-well 2 ml deephole plate. ③ After setting the corresponding conditions, the IntelliQube instrument (LGC, Britain) was used to add Mix and DNA template successively into the holes in the PCR plate of 384 samples, and the PCR plate was sealed. ④ The procedure of KASP genotyping PCR amplification was as follows: Stage I was 94°C pre-denaturation for 15 min. Stage II was 94°C 20 s, 65-57°C (each cycle was decreased by 0.8°C) 1 min, a total of 10 cycles. Stage III was 94°C 20 s, 59°C 1 min, a total of 27 cycles. ⑤ IntelliQube instrument (LGC, Britain) was used to perform fluorescence scanning for PCR amplification products and read the fluorescence signal. The IntelliQube scan data was analyzed, and the specific genotypes of genes were found according to the analysis results.

### *Data analysis*

The data was processed using SPSS 26.0 [26]. The significance level was set at $\alpha=0.05$, with *p*-values below this threshold showing statisti-

**Table 1.** Measurements of BUA in middle-aged and elderly Mulam people

| Age Groups | BUA (mean ± SD, g/cm$^2$) | | t | P |
|---|---|---|---|---|
| | Male | Female | | |
| 45-49 | 117.00±13.68 | 112.51±12.25 | 1.20 | 0.234 |
| 50-54 | 116.00±14.90 | 111.66±11.48 | 1.05 | 0.298 |
| 55-59 | 117.79±11.55 | 108.45±13.45 | 2.74 | 0.008 |
| 60-64 | 118.29±14.12 | 107.09±11.87 | 4.10 | <0.001 |
| 65-69 | 115.22±11.03 | 112.73±18.93 | 0.59 | 0.559 |
| ≥70 | 114.85±13.19 | 100.24±12.52 | 5.13 | <0.001 |
| F | 0.40 | 4.60 | | |
| P | 0.851 | <0.001 | | |

cal significance. The comparisons between two or more groups were made using t-test, chi-square test, and ANOVA. The changes in BMD and abnormal bone mass rate with age were analyzed using line graphs. The chi-square test was used to detect whether the polymorphic genotypes were in Hardy-Weinberg equilibrium (P>0.05 was in accordance with the Hardy-Weinberg law of genetic equilibrium). The chi-square test was used to compare the differences between the normal group and the osteoporosis group at each VDR gene locus, and the chi-square test was also used to compare the differences between the normal group and the osteoporosis group for each VDR gene haplotype. The analysis of LD parameters D' among multiple groups, as well as the haploid frequency, was conducted using the SHEsis online platform (http://analysis.bio-x.cn/myAnalysis.php).

## Results

### *Results of BUA measurements for the middle-aged and elderly Mulam People*

The BUA measurements of middle-aged and elderly Mulam individuals were found to follow a normal distribution. The results are presented in **Table 1**, which includes the F-values and *p*-values from the one-way ANOVA, as well as the t-values and *p*-values from two-sample t-tests. From **Table 1**, it is evident that the BUA of middle-aged and elderly Mulam individuals was compared by gender across different age groups. The analysis shows that BUA values for males and females differed significantly in the age groups of 55-59, 60-64, and ≥70 (P<0.01), with males exhibiting higher BUA values than females in these age categories. The difference was not statistically significant in BUA among the middle-aged and elderly Mulam men of different age groups (F=0.40, P=0.851). There were significant differences in BUA among middle-aged and elderly Mulam women across different age groups (F=4.60, P<0.001). Post hoc multiple comparisons were conducted by LSD method and the findings are displayed in **Table 2**. Women in the ≥70 year age group had lower BUA levels compared to other age groups.

The trends of BUA in middle-aged and elderly Mulam individuals are illustrated in **Figure 1**. The data indicates that the BUA of Mulam men decrease gradually with age, resulting in a relatively smooth curve. In contrast, for middle-aged and elderly Mulam women, the curve remains smooth between the ages of 45 and 65, but shows a significant decline after the age of 70.

### *Prevalence of abnormal bone mass rate among the middle-aged and elderly Mulam people*

The 297 cases with normal bone mass were used as the normal bone mass group (control group), and the 49 cases with decreased bone mass and 38 cases with osteoporosis total ing 87 cases were combined as the abnormal bone mass group (osteopenia group or case group). The prevalence of bone mass abnormality among the middle-aged and elderly people of the Mulam ethnic group was 22.66%, with 21.82% (36/165) of bone mass abnormality in men and 23.29% (51/219) of bone mass abnormality in women.

**Table 3** showed that the difference was not statistically significant in abnormal bone mass rate in Mulam men and women of different age

**Table 2.** Multiple Comparisons of female BUA by LSD method

| Age Group | Age Group | Mean Difference | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 45-49 | 50-54 | 0.85702 | 3.21575 | 0.790 | -5.4817 | 7.1958 |
| | 55-59 | 4.05765 | 3.09949 | 0.192 | -2.0520 | 10.1673 |
| | 60-64 | 5.41786 | 2.75650 | 0.051 | -0.0157 | 10.8514 |
| | 65-69 | -0.21857 | 3.32264 | 0.948 | -6.7680 | 6.3309 |
| | ≥70 | 12.26895* | 3.00524 | 0.000 | 6.3451 | 18.1928 |
| 50-54 | 45-49 | -0.85702 | 3.21575 | 0.790 | -7.1958 | 5.4817 |
| | 55-59 | 3.20063 | 3.37337 | 0.344 | -3.4488 | 9.8501 |
| | 60-64 | 4.56083 | 3.06122 | 0.138 | -1.4733 | 10.5950 |
| | 65-69 | -1.07560 | 3.57948 | 0.764 | -8.1313 | 5.9801 |
| | ≥70 | 11.41193* | 3.28697 | 0.001 | 4.9328 | 17.8911 |
| 55-59 | 45-49 | -4.05765 | 3.09949 | 0.192 | -10.1673 | 2.0520 |
| | 50-54 | -3.20063 | 3.37337 | 0.344 | -9.8501 | 3.4488 |
| | 60-64 | 1.36021 | 2.93886 | 0.644 | -4.4328 | 7.1532 |
| | 65-69 | -4.27622 | 3.47541 | 0.220 | -11.1268 | 2.5744 |
| | ≥70 | 8.21130* | 3.17333 | 0.010 | 1.9561 | 14.4665 |
| 60-64 | 45-49 | -5.41786 | 2.75650 | 0.051 | -10.8514 | 0.0157 |
| | 50-54 | -4.56083 | 3.06122 | 0.138 | -10.5950 | 1.4733 |
| | 55-59 | -1.36021 | 2.93886 | 0.644 | -7.1532 | 4.4328 |
| | 65-69 | -5.63643 | 3.17331 | 0.077 | -11.8915 | 0.6187 |
| | ≥70 | 6.85110* | 2.83927 | 0.017 | 1.2544 | 12.4478 |
| 65-69 | 45-49 | 0.21857 | 3.32264 | 0.948 | -6.3309 | 6.7680 |
| | 50-54 | 1.07560 | 3.57948 | 0.764 | -5.9801 | 8.1313 |
| | 55-59 | 4.27622 | 3.47541 | 0.220 | -2.5744 | 11.1268 |
| | 60-64 | 5.63643 | 3.17331 | 0.077 | -0.6187 | 11.8915 |
| | ≥70 | 12.48753* | 3.39162 | 0.000 | 5.8021 | 19.1730 |
| ≥70 | 45-49 | -12.26895* | 3.00524 | 0.000 | -18.1928 | -6.3451 |
| | 50-54 | -11.41193* | 3.28697 | 0.001 | -17.8911 | -4.9328 |
| | 55-59 | -8.21130* | 3.17333 | 0.010 | -14.4665 | -1.9561 |
| | 60-64 | -6.85110* | 2.83927 | 0.017 | -12.4478 | -1.2544 |
| | 65-69 | -12.48753* | 3.39162 | 0.000 | -19.1730 | -5.8021 |

*Statistically significant difference.

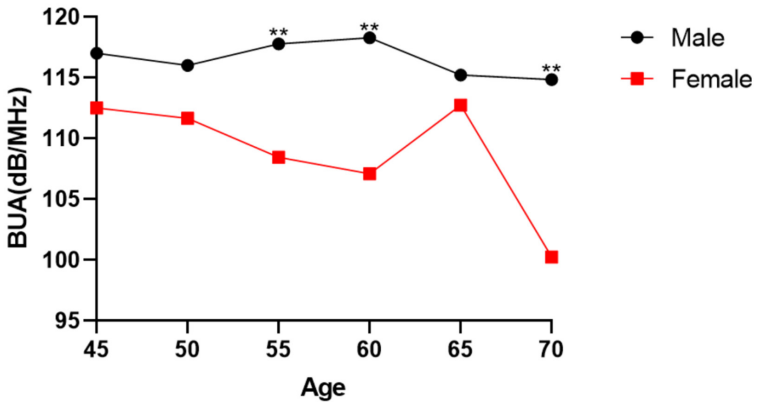


**Figure 1.** Trends of BUA in Mulam middle-aged and elderly people (male vs. female in the same age group, **P<0.01).

groups (P>0.05). The difference was not statistically significant in abnormal bone mass rate among the Mulam men in different age groups (P>0.05). The abnormal bone mass rate among Mulam women varies significantly by age group (Chi-square =27.02, P<0.001). Further two-by-two comparisons were conducted by the Bonferroni Chi-square split with an adjusted test level: $\alpha' = \frac{0.05}{6\,(6-1)/2+1} = \frac{0.05}{22} = 0.0023$,

**Table 3.** Comparison of abnormal bone mass rate in different age groups

| Age Groups | Male | | Female | | Chi-square | P |
|---|---|---|---|---|---|---|
| | Normal (n, %) | Osteopenia (n, %) | Normal (n, %) | Osteopenia (n, %) | | |
| 45-49 | 15 (93.8) | 1 (6.3) | 39 (94.6) | 2 (5.4) | - | 1.000 |
| 50-54 | 12 (85.7) | 2 (14.3) | 25 (86.2) | 4 (13.8) | 0.000 | 1.000 |
| 55-59 | 22 (91.7) | 2 (8.3) | 27 (81.8) | 6 (18.2) | 0.450 | 0.502 |
| 60-64 | 29 (76.3) | 9 (23.7) | 38 (71.7) | 15 (28.3) | 0.243 | 0.622 |
| 65-69 | 21 (77.8) | 6 (22.2) | 21 (80.8) | 5 (19.2) | 0.072 | 0.788 |
| ≥70 | 30 (65.2) | 16 (34.8) | 18 (48.6) | 19 (51.4) | 2.308 | 0.129 |
| Chi-square | 9.910 | | 27.02 | | | |
| P | 0.078 | | <0.001 | | | |

**Table 4.** Pairwise comparisons of abnormal bone mass rate of females

| Age Group | Age Group | Chi-square | P |
|---|---|---|---|
| 45-49 | 50-54 | 0.773 | 0.379 |
| | 55-59 | 2.118 | 0.146 |
| | 60-64 | 8.562 | 0.003 |
| | 65-69 | 2.137 | 0.144 |
| | ≥70 | 21.350 | 0.000 |
| 50-54 | 45-49 | 0.773 | 0.379 |
| | 55-59 | 0.0151 | 0.902 |
| | 60-64 | 2.216 | 0.136 |
| | 65-69 | 0.032 | 0.858 |
| | ≥70 | 10.101 | 0.001 |
| 55-59 | 45-49 | 2.118 | 0.146 |
| | 50-54 | 0.0151 | 0.902 |
| | 60-64 | 1.129 | 0.288 |
| | 65-69 | 0.055 | 0.815 |
| | ≥70 | 8.359 | 0.003 |
| 60-64 | 45-49 | 8.562 | 0.003 |
| | 50-54 | 2.216 | 0.136 |
| | 55-59 | 1.129 | 0.288 |
| | 65-69 | 0.759 | 0.384 |
| | ≥70- | 4.925 | 0.026 |
| 65-69 | 45-49 | 2.137 | 0.144 |
| | 50-54 | 0.032 | 0.858 |
| | 55-59 | 0.055 | 0.815 |
| | 60-64 | 0.759 | 0.384 |
| | ≥70 | 6.680 | 0.009 |
| ≥70 | 45-49 | 21.350 | 0.000 |
| | 50-54 | 10.101 | 0.001 |
| | 55-59 | 8.359 | 0.003 |
| | 60-64 | 4.925 | 0.026 |
| | 65-69 | 6.680 | 0.009 |

the results are displayed in **Table 4**. **Table 4** showed that the abnormal bone mass rate in women in ≥70 age group was lower than 45-49 and 50-54 age groups.

Trends of abnormal bone mass rate (osteopenia rate) in Mulam people are shown in **Figure 2**. The abnormal bone mass rate among the Mulam people had a certain relationship with age, and the abnormal bone mass rate increased gradually with age, with the abnormal bone mass rate of men rising from 6.3% to 34.8%. For women, the rate increases from 5.4% to 51.4%.

*Distribution of VDR genotypes and alleles among the middle-aged and elderly Mulam population*

The SNP loci of the VDR gene were examined, and three polymorphisms (rs7975232, rs2228570, rs1544410) in the VDR gene were found in Mulam people, which followed Hardy-Weinberg's law of genetic equilibrium after the Chi-square text (P>0.05). Rs1544410 locus CT genotype is associated with the risk of osteopenia, a risk factor for bone loss (OR=3.414, 95% CI=1.894, 6.154, Chi-square value =17.995, P<0.001). The difference achieved statistical significance compared to alleles C and T. The T gene at the rs1544410 locus may be a risk gene, and there is an increased risk of bone loss with the T gene at the rs1544410 locus (OR=3.414, 95% CI=1.849, 6.302, Chi-square value =17.05, P <0.001). Shown in **Table 5**.

*Linkage imbalance analysis of Mulam people's VDR gene polymorphism*

Analysis using the SHEsis online platform indicated no evidence of linkage disequilibrium among the SNPs rs7975232, rs2228570, and

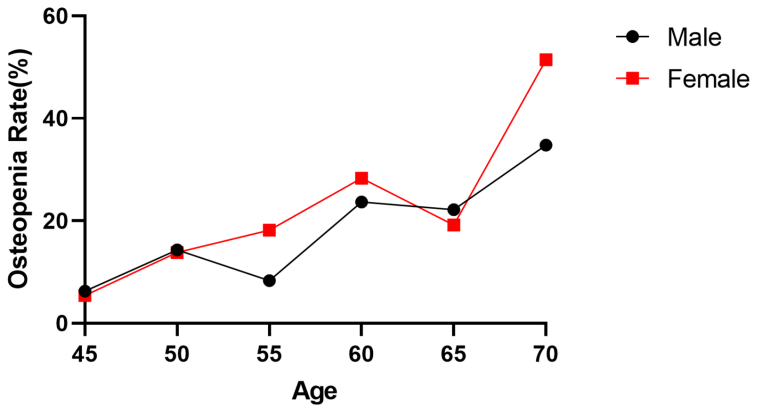


**Figure 2.** Trends of abnormal bone mass rate in middle-aged and elderly Mulam people.

rs1544410, as detailed in **Table 6** and illustrated in **Figure 3**. Six haplotypes were produced (shown in **Table 7** for haplotype frequencies). Among the six identified haplotypes, the AGT haplotype showed the strongest association with an elevated risk of osteoporosis. A statistically significant difference was observed between the case and control groups, with AGT haplotype carriers in the case group exhibiting a significantly higher disease risk compared to controls (OR=4.719, 95% CI=1.298-17.161, P=0.009).

## Discussion

*Sex-specific differences and age-specific changes in BUA and abnormal bone mass rate*

BUA is an indicator of BMD and can predict the risk of fracture [27]. The BUA of Mulam people displayed a downward trend with age, and the BUA of women was lower than that of men in most of the age groups, and the incidence of osteoporosis increased with age, which is in line with the findings of earlier research [28, 29]. Women aged 55 years and older exhibit significantly lower levels of BUA compared to men in the same age group. This puts them at a higher risk of developing osteoporosis, which is often linked to hormonal changes during menopause. Estrogen can help promote bone formation and prevent bone loss [30]. The minor increase of BUA in women in the 65-69 age group in this study may be related to a selective bias because of the small sample size. The prevalence of bone mass abnormality among the Mulam middle-aged and elderly people is 22.66%, among which the prevalence of bone mass abnormality among males is 21.82% (36/165) and that of females is 23.29% (51/219). The highest prevalence of bone mass abnormality in men is 34.8% in the ≥70 age group, and the highest prevalence in women is also in the ≥70 age group, reaching 48.6%, which is lower than the prevalence of bone mass abnormality in the same age group in Guangxi Zhuang nationality [29, 31]. This phenomenon may be linked to the warm climate and abundant rainfall in Luocheng County, Guangxi, where the Mulam people primarily engage in agricultural labor. Such regular physical activity likely contributes to bone health through moderate, sustained exercise.

*The correlation of osteoporosis with VDR gene single nucleotide polymorphism*

The association between VDR gene polymorphisms and osteoporosis risk remains controversial across diverse populations, with inconsistent findings highlighting the complexity of genetic and environmental interactions. In our study of the Mulam ethnic group, the CT genotype at rs1544410 emerged as a significant risk factor for bone loss, while no associations were observed for rs7975232, or rs2228570. This contrasts with prior studies in other populations. For instance, in postmenopausal Saudi women, the heterozygous AC genotype of rs7975232 was linked to elevated osteoporosis risk [32], a finding absent in our cohort. Similarly, Thai research identified the T allele of rs2228570 as a susceptibility variant [33], yet no such association was detected in the Mulam population. Conversely, in postmenopausal women from Malta, the GG homozygotes for rs1544410 exhibited the highest lumbar and femoral BMD values, suggesting a protective role for the G allele in this population [34]. Yuri Sakamoto (2021) found that the AA genotype of rs1544410 correlated with lower bone ultrasound scores in Japanese women [35], partially aligning with our observation of

**Table 5.** Distribution of genotype and allelic gene frequencies in VDR SNPs

| Loci | Genotype | Normal | Osteopenia | OR (95% CI) | Chi-square value | P |
|---|---|---|---|---|---|---|
| rs7975232 | AA | 34 | 7 | 1 | | |
| | CA | 122 | 42 | 0.598 (0.247-1.450) | 1.314 | 0.252 |
| | CC | 141 | 38 | 0.764 (0.314-1.858) | 0.354 | 0.552 |
| Allelic gene | A | 190 | 56 | 1 | | |
| | C | 404 | 118 | 1.009 (0.703-1.449) | 0.002 | 0.961 |
| rs2228570 | AA | 79 | 24 | 1 | | |
| | AG | 154 | 44 | 1.063 (0.603-1.874) | 0.045 | 0.832 |
| | GG | 64 | 19 | 1.023 (0.515-2.032) | 0.004 | 0.948 |
| Allelic gene | A | 312 | 92 | 1 | | |
| | G | 282 | 82 | 1.014 (0.723-1.422) | 0.007 | 0.936 |
| rs1544410 | CC | 278 | 68 | 1 | | |
| | CT | 19 | 19 | 3.414 (1.894-6.154) | 17.995 | <0.001 |
| Allelic gene | C | 575 | 155 | 1 | | |
| | T | 19 | 19 | 3.414 (1.849-6.302) | 17.058 | <0.001 |

**Table 6.** Degree of LD between three sites D' values

| Cite | rs2228570 | rs1544410 |
|---|---|---|
| rs7975232 | 0.030 | 0.021 |
| rs2228570 | - | 0.111 |

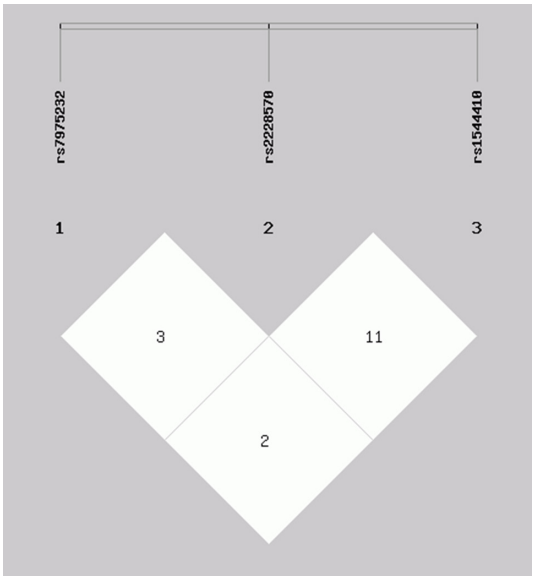


**Figure 3.** LD and haplotype block structure of the VDR gene (the values in the cells representing the logarithm of odds score for D').

the T allele's risk role. However, the Maltese study's identification of GG homozygotes as protective [34] complicates this narrative, indicating that rs1544410's impact on BMD may vary not only by allele but also by gene-environment interactions. This phenomenon indicates that the population-specific effects of VDR polymorphisms, where the same locus may confer risk or protection depending on ethnic background or environmental context.

Haplotype analysis further revealed that the AGT combination (rs7975232-A, rs2228570-G, rs1544410-T) conferred a 4.719-fold increased osteoporosis risk in the Mulam group. Mechanistically, these genetic variations might modulate bone metabolism through crosstalk with pathways such as Wnt/β-catenin signaling and sex hormone regulation, which are known to interact with vitamin D receptor activity [36]. However, the exact biological mechanisms remain elusive, particularly regarding how rs1544410-T influences osteoblast or osteoclast function.

This cross-sectional study, while providing novel insights into the Mulam population, has limitations. The sample size of 384, though statistically powered, may lack granularity to detect subtle genetic effects. Additionally, unmeasured confounders - such as serum vitamin D levels, dietary calcium intake, and physical activity - limit our ability to explore gene-environment interactions. Future longitudinal studies tracking rs1544410-T carriers over time could clarify its predictive value for osteoporosis incidence. Expanding cohorts to include multi-ethnic populations and integrat-

**Table 7.** Distribution of haplotype frequencies for VDR polymorphisms

| Haplotype | Case | Control | Chi-square value | *P* | OR (95% CI) |
|---|---|---|---|---|---|
| AAC | 0.163 | 0.163 | 0.036 | 0.849 | 1.045 (0.661-1.654) |
| AGC | 0.017 | 0.150 | 0.812 | 0.368 | 0.789 (0.472-1.321) |
| AGT | 0.031 | 0.007 | 6.697 | 0.009 | 4.719 (1.298-17.161) |
| CAC | 0.316 | 0.337 | 0.043 | 0.836 | 0.962 (0.668-1.386) |
| CAT | 0.039 | 0.025 | 1.203 | 0.273 | 1.667 (0.663-4.196) |
| CGC | 0.295 | 0.318 | 0.067 | 0.784 | 0.950 (0.655-1.376) |

ing functional assays (e.g., VDR expression profiling in bone tissue) would help disentangle population-specific genetic effects from environmental influences. Furthermore, investigating how the AGT haplotype interacts with local factors like traditional diets or sunlight exposure in the Mulam community may uncover unique risk modifiers.

## Conclusion

This study revealed significant sex- and age-related differences in BMD among middle-aged and elderly Mulam individuals. Men exhibited higher BMD values compared to women, and both sexes showed a progressive decline in BMD with advancing age, accompanied by an increasing prevalence of abnormal bone mass (osteopenia/osteoporosis). The CT genotype at the VDR rs1544410 locus was identified as a genetic risk factor for osteoporosis in middle-aged and elderly Mulam people, with the T allele serving as a susceptibility variant. Carriers of the AGT haplotype faced a higher osteoporosis risk.

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## Disclosure of conflict of interest

None.

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