Factors relating to bone mineral density in young and middle-aged patients with ankylosing spondylitis

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Abstract

Background: Ankylosing spondylitis (AS) is a common chronic progressive rheumatic disease. The aim of this study was to explore factors influencing abnormal bone mineral density (BMD) in young and middle-aged patients with AS.

Methods: From July 2014 to August 2018, hospitalized patients with AS and health examinees in the health examination center of our clinics, ranging in age from 20 to 50 years, were monitored. The BMD of the lumbar spine and femoral neck of AS patients and those of a healthy control group were measured using dual-energy X-ray absorption. The BMDs of AS patients were compared with respect to age, course of disease, iritis, smoking habits, sex, height, weight, body mass index (BMI), medication use, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), platelet volume, platelet count, uric acid (UA), alkaline phosphatase (AKP), and calcium ion levels. Single-nucleotide polymorphisms (SNPs) related to BMD were screened using genome-wide association analysis.

Results: There was no statistical difference in the proportion of abnormal bone masses between the different body parts. The BMD of all bones in AS patients was lower than that in healthy controls ($P < 0.05$). Additionally, BMD was correlated with serum calcium and CRP in AS patients ($P < 0.05$), but not with age, platelet volume, platelet count, ESR, UA, AKP, height, weight, and BMI. The incidence of abnormal bone mass in AS patients was correlated with sex ($P < 0.05$), but not with medication use, iritis, or smoking. BMD of the lumbar spine in AS patients did not correlate linearly with the course of the disease, but BMD of the femoral neck correlated linearly with the course of the disease ($P < 0.05$). BMD was correlated with multiple SNPs in patients with AS. Lumbar BMD was correlated with rs7025373 and rs7848078. Femoral head BMD was correlated with 3:102157365, 3:102157417, rs1252202, rs1681355, rs3891857, rs7842614, and rs9870734, suggesting that genetic factors play a role in BMD in patients with AS.

Conclusions: The proportion of abnormal bone mass in AS patients was higher than that in healthy individuals of the same age. The factors related to BMD in patients with AS are gender, CRP, and blood calcium. The BMD of the femoral neck of AS patients decreases with the course of the disease, but BMD of the lumbar spine is not related to the course of the disease. BMD in AS patients is associated with multiple SNPs.

Keywords: Ankylosing spondylitis; Bone mineral density; Genome-wide association analysis

Introduction

Ankylosing spondylitis (AS) is a common chronic progressive rheumatic disease with a prevalence rate of approximately 0.29% in the Chinese population.[1] Morbidity is high in young and middle-aged people, often involving the sacroiliac, spinal, and hip joints. Early clinical manifestations include chronic inflammatory lower back pain, which can be unilateral or intermittent and can gradually develop into persistent bilateral pain leading to ankylosis and deformation of the spine. Peripheral joint involvement is common and often involves the hip, knee, and other joints. It can lead to joint ossification, often

resulting in joint stiffness, which further affects the ability to work and live an active life. Although periarticular ossification is a pathological feature of AS, a large number of AS patients with osteoporosis/osteopenia have been observed clinically. The epidemiological data vary greatly. Many reports suggest that bone mineral density (BMD) is related to age, course of disease, iritis, smoking, sex, height, weight, body mass index (BMI), medication use, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), platelet volume, platelet count, uric acid (UA), alkaline phosphatase (AKP), calcium ion levels, and other factors.[2-9] Whether such information can be easily

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obtained and whether it can be an effective factor for predicting osteoporosis/osteopenia is worth discussing. Because AS is closely related to genetic factors, genome-wide association analysis (GWAS) has been used to analyze the difference in single-nucleotide polymorphisms (SNPs) between AS patients and healthy people, and many susceptibility loci have been found.[9] However, there is no conclusive evidence regarding the relationship between SNP polymorphisms and BMD in patients with AS. In this study, we aimed to analyze the effects of age, course of disease, iritis, smoking, sex, height, weight, BMI, medication, ESR, CRP, platelet volume, platelet count, UA, AKP, and calcium ion levels on BMD in 193 patients with AS. We also screened disease-related SNPs using GWAS technology in order to find an effective predictor of osteopenia/osteoporosis. This method can be used to intervene and prevent the occurrence of abnormal bone masses as early as possible.

Methods

Ethical approval

This study was approved by the ethics committee of Shanghai Changzheng Hospital, Second Military Medical University (No. 2017SL046), and written consent was obtained from all participants.

Population and study design

A total of 248 consecutive individuals (193 patients and 55 healthy donors) were recruited. All patients fulfilled the modified New York criteria for AS[10] and were recruited from the hospitalized patients' clinic in the Department of Rheumatology and Immunology, Shanghai Changzheng Hospital, Second Military Medical University. Healthy donors were volunteers recruited from the Health Examination Center of Shanghai Changzheng Hospital, Second Military Medical University. The BMD of the lumbar spine and femur, ESR, CRP, platelet volume, AKP, calcium ion level, platelet count, blood UA, age at admission, course of disease, iritis, smoking, sex, height, weight, BMI, and medication status were measured. All patients and healthy donors were excluded from the study if they had a fracture, joint replacement, diabetes mellitus, or another rheumatic immune disease that could cause secondary osteoporosis or interfere with BMD testing. There was no significant difference in sex or age between the AS and healthy groups ($P > 0.05$).

Definition

GWAS was used to detect SNPs related to BMD in 87 patients with AS. According to the World Health Organization definition, for premenopausal women and men under 50 years old, it is suggested to use the Z-value of the same race to judge the BMD level (Z-value = [BMD measurement value–mean BMD of the same race, sex, and age] / standard deviation of BMD of the same race, sex, and age). A Z-value≤–2.0 is regarded as "lower than the expected range of the same age group" or low bone mass, that is, abnormal bone mass.[11]

BMD measurement and DNA extraction

The BMD data (Z-value) of the AS patients and healthy control group were measured. The instrument used was a DPX-type BMD instrument produced by the LUNAR Company of the United States. Blood samples from 87 patients were coded, and the DNA was extracted according to the manufacturer recommendations (Axygen DNA extraction kit, Union City, CA, USA) for exon sequencing.

Genotyping

Samples were genotyped using the HumanCoreExome-24v1-0 Chip (Illumina, San Diego, CA, USA) according to the manufacturer instructions. Bead intensity data were processed and normalized for each sample using GenomeStudio (V2.0). Data for successfully genotyped samples were extracted, and the genotypes were called. In addition, we applied the following filters to the AS study: Filter 1: remove chromosomes MT and XY; Filter 2: Drop genotypes with call rates below Tcr = 98%; Filter 3: Replace NC with the most frequent genotype of the whole population; Filter 4: remove SNPs with minor allele frequency (MAF) less than Tmaf = 0.01 in a federated setting. Finally, 270,475 genomic loci were evaluated as feature spaces in this study.

Data analysis

Statistical analysis of measurement data was carried out using SPSS22.0 software (IBM Corp, Armonk, NY, USA). The measurement data were analyzed using the t-test, Mann-Whitney U test, chi-square test, and linear regression analysis. Differences were considered statistically significant at $P < 0.05$. Quality control was performed on mitochondrial and XY chromosomes, as well as poor quality regions harboring SNPs with call rates below 98%. These regions were excluded from further analysis. Samples with call rates below 98% were also removed. Nocall regions were imputed with the most common genotypes among the populations. Non-homogeneous regions were then selected by eliminating the loci with the same genotype and low MAF (<0.01). A generalized linear model and a mixed linear model were adopted for GWAS analysis.

Results

Disease and control groups characteristics

There was a total of 193 AS patients (158 males and 35 females), with an average age of 32.6 ± 8.3 years. On the other hand, there was a total of 55 individuals in the healthy control group (42 males and 13 females), with an average age of 31.5 ± 5.4 years. There was no significant difference between the AS and healthy groups ($P > 0.05$).

Proportion and location of abnormal bone mass

Among the 193 patients with AS, 47 (24.4%) had an abnormal bone mass. The proportions of abnormal bone masses in the different body parts are shown in Table 1.

There was no statistical difference in the proportion of abnormal bone masses between the different body parts ($P = 0.569$).

BMD comparison between AS patients and healthy controls

The BMD of AS patients was significantly different from that of the healthy control group in the lumbar spine and femur ($P < 0.05$) (Table 2).

Analysis of BMD and related factors in AS patients

BMD was correlated with sex ($P = 0.008$), serum calcium ($P = 0.002$), and CRP ($P < 0.001$), but not with others (Table 3).

Relationship between BMD and course of AS

Femoral head BMD was linearly correlated with the course of AS ($P = 0.021$), but lumbar spine BMD was not linearly correlated with the course of AS ($P = 0.066$) [Figures 1 and 2].

GWAS

Using the general linear model and mixed linear model, the SNP information was subjected to correlation analysis with phenotypic data, including the BMD (g/cm^2) of the lumbar vertebrae, femoral neck, and femoral head. There

was no significant site using exon chip data [Figures 3 and 4].

There are two significant variants associated with BMD in the lumbar spine (L1–L4). The results are presented in Table 4 and Figure 5.

There were seven significant correlations between BMD and the genotype in the femoral head. The results are presented in Table 5 and Figure 6.

Table 3: Analysis of abnormal bone mass in AS patients related influencing factors.

Influencing factors	Statistics	P values
Male	6.957[*]	0.008
Iritis	2.341[*]	0.126
Smoking	1.529[*]	0.216
cDMARDs	1.367[*]	0.242
NSAIDs	0.017[*]	0.895
GC	0.047[*]	0.828
Age	−1.233[†]	0.219
ESR	1.330[†]	0.185
CRP	−3.482[‡]	<0.001
Platelet count	1.382[†]	0.169
Platelet volume	−1.962[†]	0.053
AKP	0.591[†]	0.555
UA	1.460[†]	0.146
Ca	−3.107[†]	0.002
Height	1.000[†]	0.336
Weight	−0.540[†]	0.927
BMI	−0.465[†]	0.799

AS: Ankylosing spondylitis; cDMARDs: conventional diseasemodifying anti-rheumatic drugs; NSAIDs: nonsteroidal anti-inflammatory drugs; GC: glucocorticoid; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; AKP: alkaline phosphatase; UA: uric acid; Ca: calcium ion levels; BMI: body mass index. [*] χ^2 values. [†] t values. [‡] Z values.

Table 1: Proportions of abnormal bone masses in the different body parts, n/N (%).

Body parts	Abnormal bone mass
Lumbar spine 1	14/190 (7.4)
Lumbar spine 2	20/190 (10.5)
Lumbar spine 3	14/190 (7.4)
Lumbar spine 4	23/191 (12.0)
Lumbar spine 1–4	19/190 (10.0)
Femoral neck	12/187 (6.4)
Femoral head	18/182 (9.9)
Total	47/193 (24.4)

Table 2: BMD comparison between AS patients and healthy controls (g/cm^2, mean ± SD).

Body parts	AS patients (n = 193)	Healthy control (n = 55)	t	P values
Lumbar spine 1	1.01 ± 0.18	1.08 ± 0.16	−2.897	0.008
Lumbar spine 2	1.07 ± 0.19	1.15 ± 0.16	−3.115	0.003
Lumbar spine 3	1.09 ± 0.18	1.19 ± 0.15	−4.405	<0.001
Lumbar spine 4	1.07 ± 0.20	1.17 ± 0.16	−4.572	<0.001
Lumbar spine 1–4	1.06 ± 0.18	1.15 ± 0.15	−4.047	<0.001
Femoral neck	0.97 ± 0.21	1.04 ± 0.22	−2.601	0.038
Femoral head	0.95 ± 0.18	1.03 ± 0.18	−3.596	0.004

AS: Ankylosing spondylitis; BMD: Bone mineral density; SD: Standard deviation.
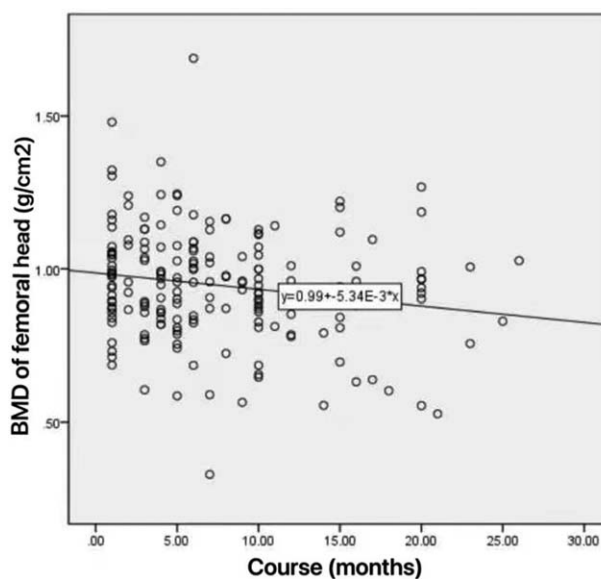

Figure 1: Femoral head BMD and scatter plot of the course of AS. AS: Ankylosing spondylitis; BMD: Bone mineral density.

Discussion

The proportion of bone mass abnormality is higher in patients with AS because of the abnormal function of osteoclasts and osteoblasts caused by periarticular inflammation. An analysis of 1051 Chinese patients with AS showed that the proportions of osteoporosis and osteopenia were 34% and 33.78%, respectively.[2] Another

analysis on 504 Chinese AS patients was performed, where the proportion of osteoporosis was 9.7% and the proportion of osteopenia was 57.5%.[3] Some studies have shown that femoral BMD in early AS patients has decreased,[12] and the low BMD is related to the formation of new vertebral osteophytes.[13] This study focused on bone mass abnormalities in young and middle-aged patients with AS, where the proportion of bone mass abnormalities was found to be 24.35%. Because of the Z-value, it is impossible to compare the results with those reported previously. However, our study suggests that the proportion of abnormal bone mass in young and middle-aged AS patients is significantly higher than that in age-matched healthy individuals. This indicates that BMD testing is necessary for AS patients of any age to detect abnormal bone mass as soon as possible so as to start clinical intervention earlier.

The main risk of abnormal bone mass is that it increases the risk of fracture in patients with AS, and BMD reduction in AS patients is an important risk factor for vertebral fracture.[14] A total of 292 patients with AS were monitored for two years. Among these patients, 59 (20%) had vertebral fractures at baseline, 15 had new fractures, and seven had an increased severity of fractures. However, most of the fractures were mild and located in the mid-thoracic and thoracolumbar regions of the spine. Additionally, the lower BMD of the hip joint was a risk factor for fracture.[15] Similarly, Mehmet found that
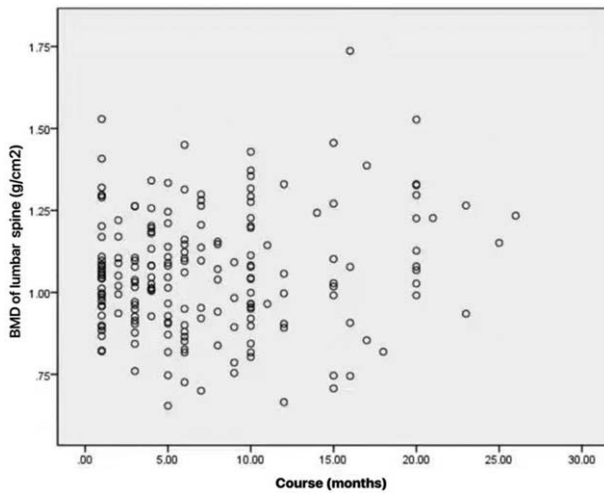

Figure 2: Lumbar spine BMD and scatter plot of the course of AS. AS: Ankylosing spondylitis; BMD: Bone mineral density.
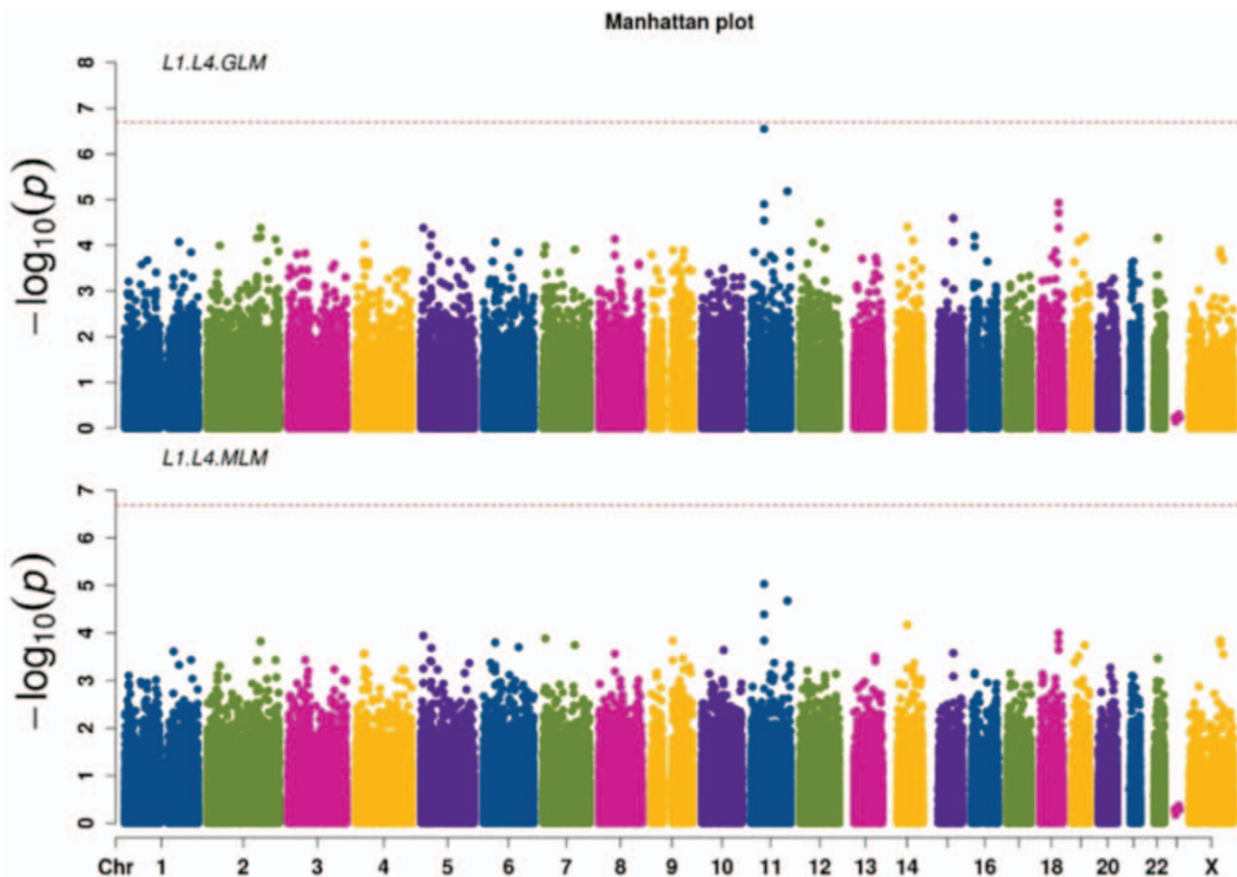

Figure 3: Comparisons of BMD (g/cm²) for lumbar vertebrae with exon chip data showing no significant site. BMD: Bone mineral density; GLM: General linear model; MLM: Mixed linear model.
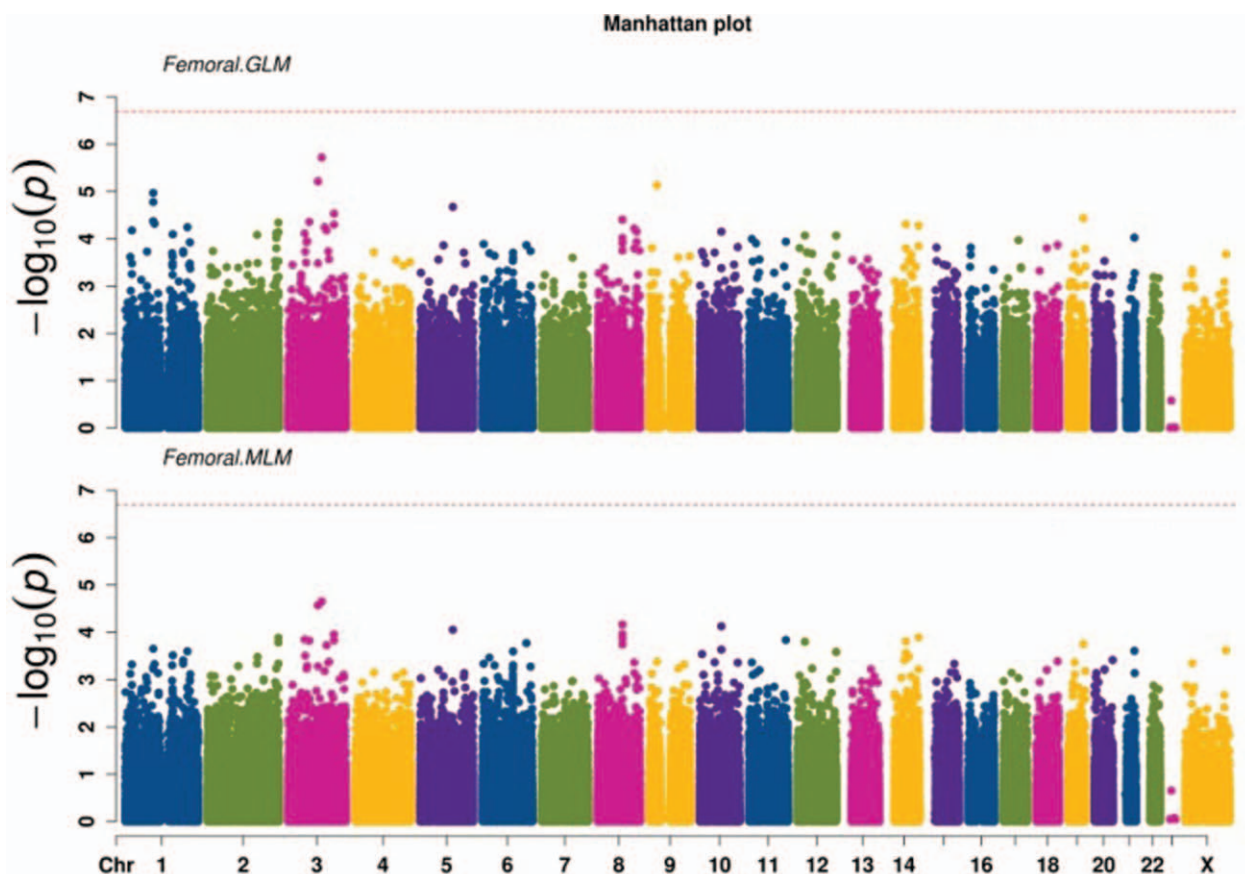
Figure 4: Comparisons of BMD (g/cm²) for femoral neck and femoral head with exon chip data showing no significant site. BMD: Bone mineral density; GLM: General linear model; MLM: Mixed linear model.

Table 4: Suggestive significant loci associated with BMD in lumbar vertebrae (L1–L4).

SNP	Chr	Position	L1.L4.cor	L1.L4.P value
rs7025373	9	107439675	−0.519069352	2.54E-06
rs7848078	9	107430373	0.519069352	2.54E-06

SNPs: single nucleotide polymorphisms; Chr: chromosome.

vertebral fractures were significantly associated with decreased BMD in the femur and lower lumbar spine.[4] From these data, we can infer that AS is a serious cause of bone fracture due to abnormal bone mass, which cannot be underestimated in clinical practice. Timely diagnosis and treatment of AS patients with abnormal bone mass is of great significance in preventing fractures. This study suggests that BMDs of all bones are significantly lower than those of healthy people of the same age. Moreover, it is suggested that AS may involve both the lumbar spine and femoral neck. Young and middle-aged patients are involved in greater intensity of labor and activity. Therefore, the risk of fracture is significantly higher than that of other age groups, and the risk of disability after lumbar and femoral neck fractures is very high. Therefore, abnormal bone mass in young and middle-aged patients with AS needs to be identified as early as possible in order to be alert to the risk of lumbar and femoral neck fractures.

The causes of bone mass abnormalities in AS patients are not yet fully understood, but the presence of many inflammatory factors can affect bone formation and bone resorption. The increased levels of interleukin-1, interleukin-6, and tumor necrosis factor in AS patients are strong stimulators of osteoclast-mediated bone resorption.[16] Our study found that BMD was correlated with blood CRP in patients with AS ($P = 0.042$), suggesting that CRP was significantly increased in patients with abnormal bone mass. This suggests that high levels of inflammation in AS patients may be associated with bone destruction, which is consistent with the results of van der Weijden.[5] However, this study did not find any correlation between ESR and bone mass abnormalities, which may be related to many factors affecting ESR, including occult infection and micro-fracture. In addition, it has been reported that UA can affect BMD in AS patients.[17] However, this study did not find any correlation between them, possibly because there is a lower proportion of abnormal UA levels in young and middle-aged patients.
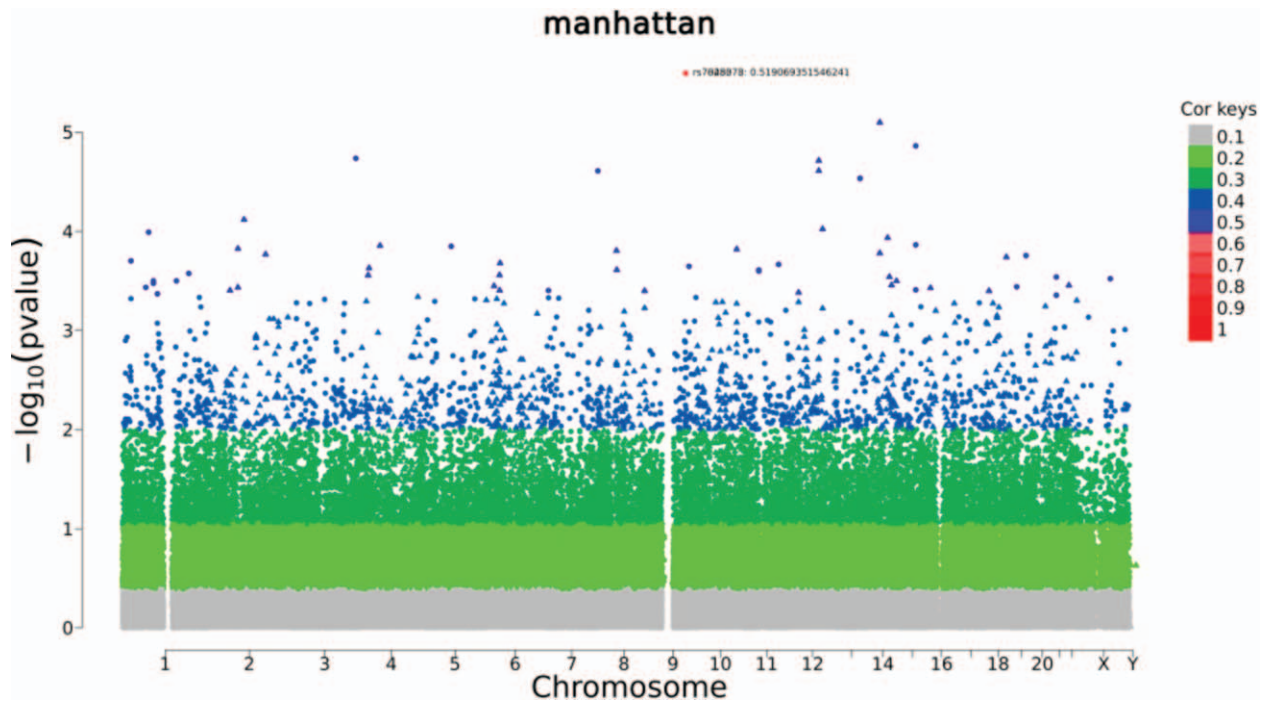
Figure 5: BMD and genotype Manhattan map of the lumbar spine (L1–L4): the abscissa represents the chromosome; the ordinate represents -log10 *P* value; and color represents correlation, with gray being no correlation, green weak, blue moderate, and red strong correlation. There are two suggestive significant variants associated with BMD in the lumbar spine (L1–L4). BMD: Bone mineral density.

Table 5: Suggestive significant loci associated with BMD in the femoral head.

SNP	Chr	Position	Correlation	*P* value
3:102157365	3	102157365	0.545365904	6.06E-07
3:102157417	3	102157417	−0.545365904	6.06E-07
rs1252202	8	85470451	−0.54228833	7.21E-07
rs1681355	8	85509648	−0.523023894	2.06E-06
rs3891857	3	102141451	−0.545365904	6.06E-07
rs7842614	8	85526604	−0.54228833	7.21E-07
rs9870734	3	102124339	0.545365904	6.06E-07

SNPs: single nucleotide polymorphisms; Chr: chromosome.

This study also found that BMD was associated with serum calcium levels ($P = 0.002$) in patients with AS. These suggest that patients with abnormal bone mass have lower calcium levels than those with normal bone mass, which is different from postmenopausal or senile osteoporosis. However, no relevant studies have been conducted in retrospective studies. Therefore, it is not clear whether abnormal bone mass in AS patients is related to blood calcium metabolism. Further studies are required to determine whether additional calcium supplementation can help prevent BMD reduction or improve BMD.

The proportion of abnormal bone mass in patients with AS was significantly correlated with sex, where it was found that female patients has significantly lower abnormal bone mass. Estrogen receptors are expressed in osteoblasts and osteoclasts. It is an important factor in the regulation of bone formation and resorption. Studies have shown that estrogen inhibits bone destruction.[16] In our study, the proportion of abnormal bone mass in female patients with

AS was significantly lower than that in male patients. As a result, young and middle-aged male patients with AS should be particularly vigilant about the occurrence of abnormal bone mass. However, it should be noted that AS is highly prevalent in male patients, so there were only 38 female patients in this study, which may have led to deviations in the results. This requires further clarification of the clinical observations and future research.

Ossification of the soft tissue around the anterior longitudinal ligament of the spine can occur in patients with AS. The most commonly used dual-energy X-ray absorption (DXA) method in the clinic is the attenuation and absorption difference between two different energy X-rays passing through the human skeleton. After computer processing, the mineral content of the skeleton can be obtained, which is the gold standard for the diagnosis of osteoporosis. The main measurement sites of DXA are the mid-axis bones, including the lumbar spine and proximal femur. The measurement area of DXA includes the
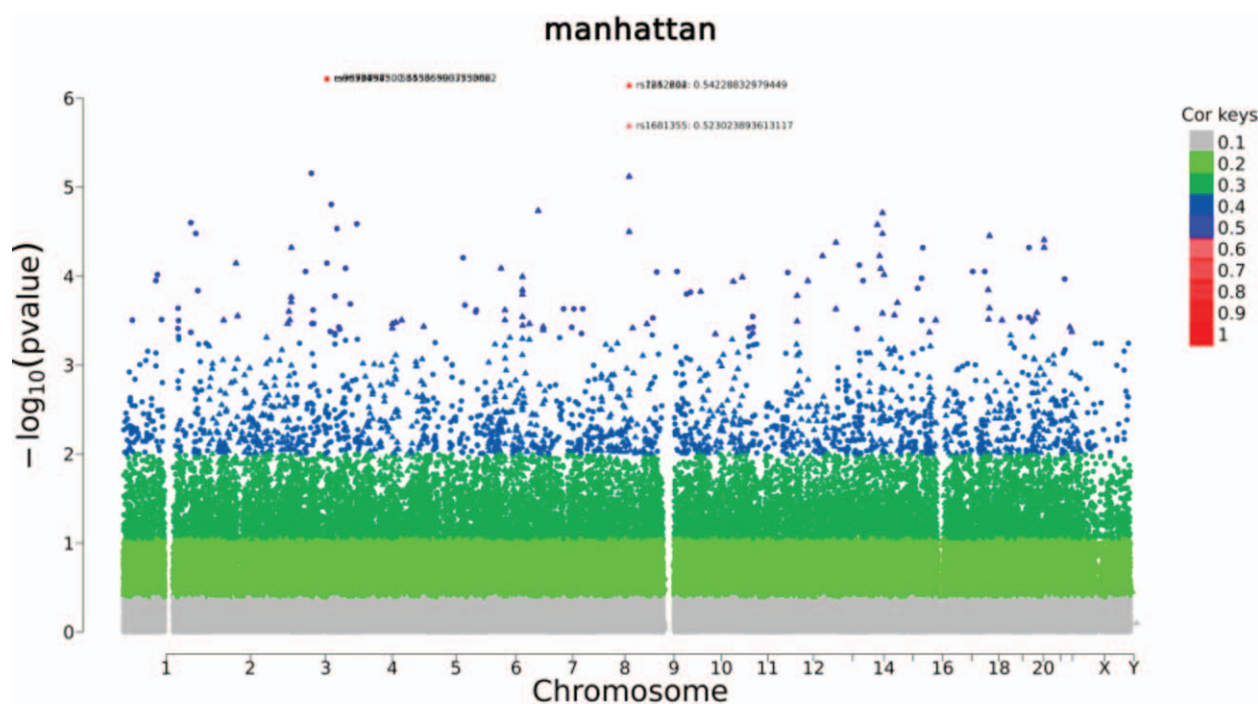
Figure 6: BMD and genotype Manhattan map of the femoral head: the abscissa represents the chromosome; the ordinate represents -log10 *P* value; and color represents correlation, with gray being no correlation, green weak, blue moderate, and red strong correlation. There are seven significant correlations between BMD and genotype in the femoral head. BMD: Bone mineral density.

vertebral body and its posterior appendages, so the measurement results are affected by degenerative changes of the lumbar spine and abdominal aortic calcification.[18] Fitzgerald also found that the BMD of the spine measured in the anterior position was significantly higher than that measured in the lateral position.[9] AS patients may have ossification of soft tissue around the anterior longitudinal ligament of the spine, which may interfere with X-ray penetration. Mehmet used DXA to measure BMD in 86 patients with AS and found that the detection rate of osteoporosis in the proximal femur and lateral lumbar spine was significantly higher than that in the lumbar spine.[19] Our study found that BMD of the femoral head decreased with the course of the disease, but BMD of the lumbar spine was not statistically correlated with the course of the disease. The ossification and degenerative changes in the soft tissue around the lumbar spine gradually increase with the course of the disease, which seriously interferes with X-ray penetration. However, the proximal femur is less affected by the aforementioned phenomenon, therefore, X-ray detection is less affected. The evaluation of BMD of the femoral head in patients with AS is better than that of the lumbar spine. However, the correct evaluation of lumbar BMD needs to be studied further.

AS is closely related with genetic factors. As early as the 1960s, it was found that AS has family clustering. The discovery of HLA-B27 in the 1970s confirmed that AS is a polygenic genetic disease. In recent years, many studies have used GWAS to analyze the difference in SNPs between patients with AS and healthy individuals, and found many susceptible SNP loci.[20] However, the relationship between SNP polymorphisms and BMD in patients with AS is not yet fully understood. This study suggested that lumbar spine and femoral head BMD are related to multiple SNP loci and that RALYL and ZPLD1 genes are related to femoral head BMD. According to the literature, the RALYL gene is located at 8q21.2, encoding a zona pellucida-like protein domain (Q8TCW7-ZPLD1_HUMAN). The ZPLD1 gene is located at 3q12.3, encoding an RNA-binding protein (Q86SE5-RALYL_HUMAN), but the related pathways are not clear. Indeed, no related diseases have been reported.[21,22] These results suggest that genetic factors are one of the causes of BMD reduction in AS patients, but due to the small number of cases included in this study, this still needs to be verified by increasing the number of samples.

In summary, the proportion of abnormal bone mass in AS patients is higher than that in healthy people of the same age. The factors related to BMD in AS patients are gender, CRP, and blood calcium. Whether controlling inflammation and supplementing calcium can prevent abnormal bone mass remains to be further studied. BMD of the femoral neck in AS patients decreases with the course of the disease, but BMD in the lumbar spine is not related to the course of the disease. This suggests that femoral neck BMD may be more helpful in the diagnosis of bone mass abnormalities in patients with AS. GWAS analysis showed that BMD in AS patients was associated with a number of SNP loci, indicating that genetic factors were also involved in the occurrence of BMD reduction in AS patients.

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