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Characterization of gut microbiota in the Uyghur osteopenia population

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The objectives of this study were to investigate the composition of gut microbiota and its relationship with bone loss in the Uyghur osteopenia population, identify potential disease-related taxa and collect information for the prevention and treatment of osteopenia in different people by regulating gut microbiota. We selected Uyghur residents, measured their heel BMD, collected faeces and general information, grouped them by BMD level, obtained faecal 16S rRNA sequences, and compared and analysed the differences between the groups. This study showed that the numbers of OTUs and species in the gut microbiota in the osteopenia group were higher than those in the control. At the phylum level, Erysipelotrichia was more abundant in the osteopenia group. At the genus level, Phascolarctobacterium was less abundant, and Ruminiclostridium_5 was more abundant in the osteopenia group compared to the control. Phascolarctobacterium and Z-score were positively correlated, and Ruminiclostridium_5 was negatively correlated with T and Z score. The different composition of the gut microbiota in Uyghur osteopenia patients and controls found in this study fills a knowledge gap in this ethnic group. The relationship between Uyghur osteopenia and BMD-associated bacterial genera deserves further exploration.

Keywords Uyghur, Bone mineral density, Osteopenia, Gut microbiota

Osteopenia is a decrease in bone strength and an abnormal but not yet osteoporotic bone density[1]. Osteopenia is the early stage of osteoporosis. After the adult skeleton reaches its maximum bone mass level, bone mass begins to decrease with age, initially manifesting itself as a loss of bone mass, which, without intervention, will gradually develop into osteoporosis[2]. A recent study showed a global prevalence of 40.4% for osteopenia and 19.7% for osteoporosis[3]. Epidemiological surveys in China show that the population over 60 years of age has exceeded 250 million, accounting for 18.1% of the total population, and the prevalence of osteopenia and osteoporosis peaks at 50–60 years of age, at rates of 46.4% and 19.2%, respectively[4]. Due to the high prevalence of osteopenia and osteoporosis, as well as the ultimate clinical features of osteopenia and osteoporosis being fragility fractures, there is an increased risk of fracture at almost all skeletal sites[5], leading to a major reduction in the quality of life of the patient and a significant economic burden on the individual and the community[6]. Therefore, more research is needed to prevent bone loss and the development of osteoporosis.

The human microbiome is a complex ecosystem inhabited by hundreds of species of bacteria, viruses, fungi, and phages that continue to shape the host's internal environment and constantly influence its function, health, and disease[7]. A growing body of research suggests that gut microbiota can have an impact on bone mass regulation, osteopenia, and osteoporosis[8,9]. Several experimental animal studies have demonstrated that certain genus-level bacteria of the gut microbiota can have beneficial effects on osteoporosis[9]. Probiotics can be ingested to maintain bone health for the host by decreasing intestinal permeability, altering microbial composition, and boosting immune system competence[10]. Probiotics, of which Lactobacillus has a favourable effect on bone metabolism[11], can prevent long-term bone loss[12]. However, some researchers have found conflicting associations between probiotics and bone health, with only a tiny percentage of the gut microbiota acting as probiotics, so more research is needed to determine the association between other gut microbiota and bone loss[13].

Multiple previous cross-sectional studies that reported an association between the gut microbiota and bone density or osteoporosis showed inconsistent results[14–17]. The composition of the gut microbiota is influenced by different factors, such as race[18], environment[19] and diet[20]. Of all the influential factors, ethnicity and diet are considered the main factors that have a significant impact on the balance of the gut microbiota. The Uyghurs, an ethnic group in northern China, originated in the mid-sixth century AD from two powerful tribes located in

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the Gaoches[21]. Xinjiang is located in the hinterland of Asia and Europe, within the traffic hub of the "Silk Road" and has long been integrating and accumulating a variety of civilisations, providing an open environment and space for the spread and development of Islam in Xinjiang. In today's Xinjiang, Uyghurs still practice Islam and maintain a traditional lifestyle and diet (halal diet), favouring mutton, beef and pasta, but with a low intake of vegetables, fruits and legumes. Therefore, this Uyghur diet, which is high-carbon, high-fat and low-vitamin, may be one of the reasons for the differences in gut microbiota between Uyghurs and the rest of the population. Some studies have found that different diets are directly correlated with different gut microbiota compositions; for example, those who prefer protein and animal fats are rich in Bacteroides, while those who prefer high carbohydrate diets are rich in Prevotella[22]. Previous studies have been limited to Han Chinese older adults[23] and Xi'an[14], Latin American[16], and European populations[15]. Consequently, little is known about the structure of the gut microbiota of Uyghurs and how their microbial communities are affected by such dietary changes, and one study found that the prevalence of osteoporosis in older adults in Xinjiang (64.5%) was significantly higher than the average prevalence in older adults in China (32%)[24]. The present study found that the prevalence of osteopenia among Uyghurs is 61.2%, which may be the result of the combination of genetic, dietary and environmental factors. A study found that there are significant differences in the structural proportions of gut microbiota between healthy Uyghurs and other ethnic groups (e.g., Han Chinese and Tibetans), and there are also differences in the structural composition of gut microbiota in patients with diseases[25,26]. Therefore, it is necessary to study the species diversity and community composition of gut microbiota in the Uyghur population to explore the association between gut microbiota and osteoporosis and to provide a theoretical basis for the prevention, diagnosis and treatment of osteoporosis in the Uyghur population.

Methods

Participant enrolment and data collection

This study was reviewed and approved by the Ethics Committee of Jinzhou Medical University, and written informed consent was obtained from all participants. Our study was conducted from July 2022 to August 2022 to collect faecal samples from different villages in Baicheng County, Aksu Region, Xinjiang Uygur Autonomous Region, China. We excluded subjects with a history of alcohol abuse; use of antibiotics or hormones within six months prior to faecal sample collection; prior hysterectomy or oophorectomy; prior partial or total colectomy; a history of hyperthyroid or hypothyroid disorders; prevalent diabetes mellitus and gastrointestinal disorders; and failed collection of faecal samples. Bone mineral density (BMD) measurements were performed using an ultrasonic bone densitometer (Achilles Express Bone Densitometer manufactured by GE Medical Systems Lunar, USA[27,28]), and the hardness index parameter was calculated based on the ultrasonic amplitude parameter and the attenuation parameter of the propagation speed of the sound waves. The index of stiffness was expressed through the T score, T score = (measured BMD − mean number of BMD in average young population)/standard deviation of the number of BMD in average young population, and BMD determination was made based on the magnitude of the T score. The BMD of the left heel bone was measured for each subject. According to the criteria recommended by the World Health Organisation[29], a T score ≥ − 1.0 is considered average bone mass, − 2.5 ≤ T score ≤ -1.0 is considered low bone mass, and a T score ≤ − 2.5 indicates osteoporosis. Low bone mass and osteopenia are both classified as osteoporosis. Finally, 27 osteopenia patients and 31 healthy controls were included in our analysis. Participants' general demographic information (sex, age, weight, and height), smoking, drinking, and dietary habits, gynaecologic information, and history of disease and medication use were collected by trained investigators prior to BMD determination. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m).

Stool sample collection and microbiota sequencing

Fresh faecal samples were preserved in tubes containing ambient preservation solution (EG-0150, No. 20210963), which can be stored at room temperature for 6 months, provided by Xiamen Treatgut Biotechnology Co., Ltd., Xiamen, China. Microbial DNA was extracted from each sample using the QIAamp Fast DNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. DNA concentration and purity were quantified using a Multiskan™ GO microplate reader, and DNA integrity was checked using agarose gel electrophoresis. PCR amplification (ABI Veriti™ 96-Well Thermal Cycler, Applied Biosystems) of the V4 region was performed using primers 515F5′-GTGCCAGCMGCCGCGGTAA-3′ and 806R5′-GGACTACNVGGGTWTCTAAT-3′. 16S library construction was performed using the Illumina library construction strategy. The fragment range and concentration of the library were determined using Q-PCR. The test libraries were sequenced using the Illumina Miniseq (Illumina Miniseq PE150). Raw bipartite sequences from sequencing were subjected to splicing and quality control using flash[30], and then chimeric sequences were filtered (Chimaera_check) to produce high-quality clean reads. All samples were pooled and merged to remove duplicates, and clustered into operational taxonomic units (OTUs) using a 97% similarity threshold. The resulting representative sequences were then annotated for species classification using UCLUST.

Bioinformatics analysis

Sample sequences from different subpopulations were randomly selected for dilution curve analysis, and visualisation curves were plotted using R 3.4.1 software. Alpha diversity indices, including the Ace, Shannon, Chao1 and Simpson indices, were calculated and plotted using R 3.4.1 software. Differences in the structural composition of the gut flora were analysed in beta diversity analysis by calculating unweighted UniFrac distances, and similarity analysis (ANOSIM), analysis of variance (ADONIS), principal coordinates analysis (PCoA) and Bray—Curtis distances were calculated and plotted using R 3.4.1 software. Linear discriminant analysis was performed using the LEfSe program to detect colonies that differed in enrichment between groups. To compare data differences

between multiple groups, the Wilcoxon rank sum test or Kruskal–Wallis test was performed. In addition, correlations between differentially abundant taxa and other metrics were analysed using Spearman correlation. In the functional prediction section, all sample sequences with good quality control were compared and normalised to the GreenGene[31] database using the PICRUSt2 program, followed by functional prediction[32] to derive differences in the corresponding KEGG[33] metabolic pathways between the two groups.

Statistical analyses

Statistical analysis was performed using SPSS 26.0 software and R software. Measurements that conformed to a normal distribution were expressed as the mean ± standard deviation, and a t test was used for comparisons between groups. The Mann–Whitney U test was used to compare the differences between groups for variables that did not conform to a normal distribution and were expressed as the median ± interquartile range. The significance of all statistical analyses was expressed as *P*, with $P < 0.05$ being considered statistically significant.

Results

General characteristics of the participants

A total of 62 faecal samples were collected from Uyghur residents, and 58 cases were included after exclusion in the osteopenia group, in which there were 14 males and 13 females, and in the control group, in which there were 15 males and 16 females. There was no statistically significant difference between the two groups in terms of age, sex, or BMI ($P > 0.05$). Detailed information is provided in supplementary Table 1. The diet of both groups was a mixed diet. The comparison of BMD between the osteopenia group and the control group was statistically significant ($P < 0.001$) (Table 1).

Increased microbiome abundance in osteopenia patients

To study the composition and function of gut microorganisms in the osteopenia group and control group, 58 faecal samples were subjected to high-throughput sequencing of the V4 region of the 16S rRNA gene. The total number of faecal bacteria was 28 phyla, 45 classes, 75 orders, 144 families, 457 genera and 474 species. The control group had fewer bacterial taxa at all levels than the osteopenia group (Supplementary Table 2).

After excluding low-quality, short, single, and ambiguous measurements, we retained 940 OTUs from 58 samples for further analysis. The number of OTUs generated per sample ranged from 89 to 376. A Venn diagram showed a total of 694 OTUs shared by both groups (Supplementary Fig. 1). Analysis of the dilution curves showed a flattening of the control and osteopenia groups, indicating that the amount of sequencing data was reasonable and that more data volume would only produce a small number of new species (OTUs). Therefore, the quality and quantity of data in this study were satisfactory, and there was no need to increase the sample size (Supplementary Fig. 2). The alpha diversity results showed no significant differences in species diversity between osteopenia and control faecal samples (Chao1 index, $P = 0.293$, Ace index, $P = 0.396$, Shannon index, $P = 0.9$, and Simpson index, $P = 0.694$) (Fig. 1A). There were significant differences in the gut microbiota composition between the groups (ANOSIM, $R = 0.096$, $P = 0.0019$; ADNOIS, $R^2 = 0.052$, $P = 0.006$; Fig. 1B,C). This finding suggested that the composition of the gut microbiota is altered in osteopenia individuals compared with healthy controls.

Alterations of microbiomes in osteopenia patients

By performing species annotation of representative OTU sequences and statistical analysis of community structure differences based on species annotation results, we found that the gut microbiota structure of osteopenia patients changed significantly at all levels. Our study further elucidates the relative abundance of microbial communities in each group at the phylum and genus levels. At the phylum level, the four most common OTUs in faecal samples were identified as Bacteroidetes, Firmicutes, Proteobacteria and Cyanobacteria (Fig. 2A). At the genus level, the dominant genera were determined to be Prevotella_9, Bacteroides, Succinivibrio, and Alloprevotella (Fig. 2B). Relative abundance of bacterial communities at different taxonomic levels, including phylum, class, order, family and genus are shown in Supplementary Fig. 3.

To further understand the microbiological composition of the faecal samples from the osteopenia and control groups, a t test for bacterial abundance was performed. At the genus level, Clostridium_sensu_stricto_1, GCA-900066575, Acidaminoccus, Erysipelotrichaceae_UCG-003, and Lachnoclostridium in osteopenia patients and

Clinical indices	Control	Osteopenia	*P*
Age (years, mean ± SD)	49.81 ± 6.84	50.19 ± 10.38	0.872
Gender			
Male (%)	15 (48.4%)	14 (51.9%)	0.792
Female (%)	16 (51.6%)	13 (48.1%)	
Dietary habit	Mixed diet	Mixed diet	
BMI (kg/m^2, mean ± SD)	22.2 ± 1.26	22.57 ± 1.27	0.274
T score	0.29 ± 0.98	− 2.32 ± 1.04	< 0.001
Z score	1.64 ± 1.00	− 0.94 ± 0.94	< 0.001

Table 1. Clinical characteristics of the participants.

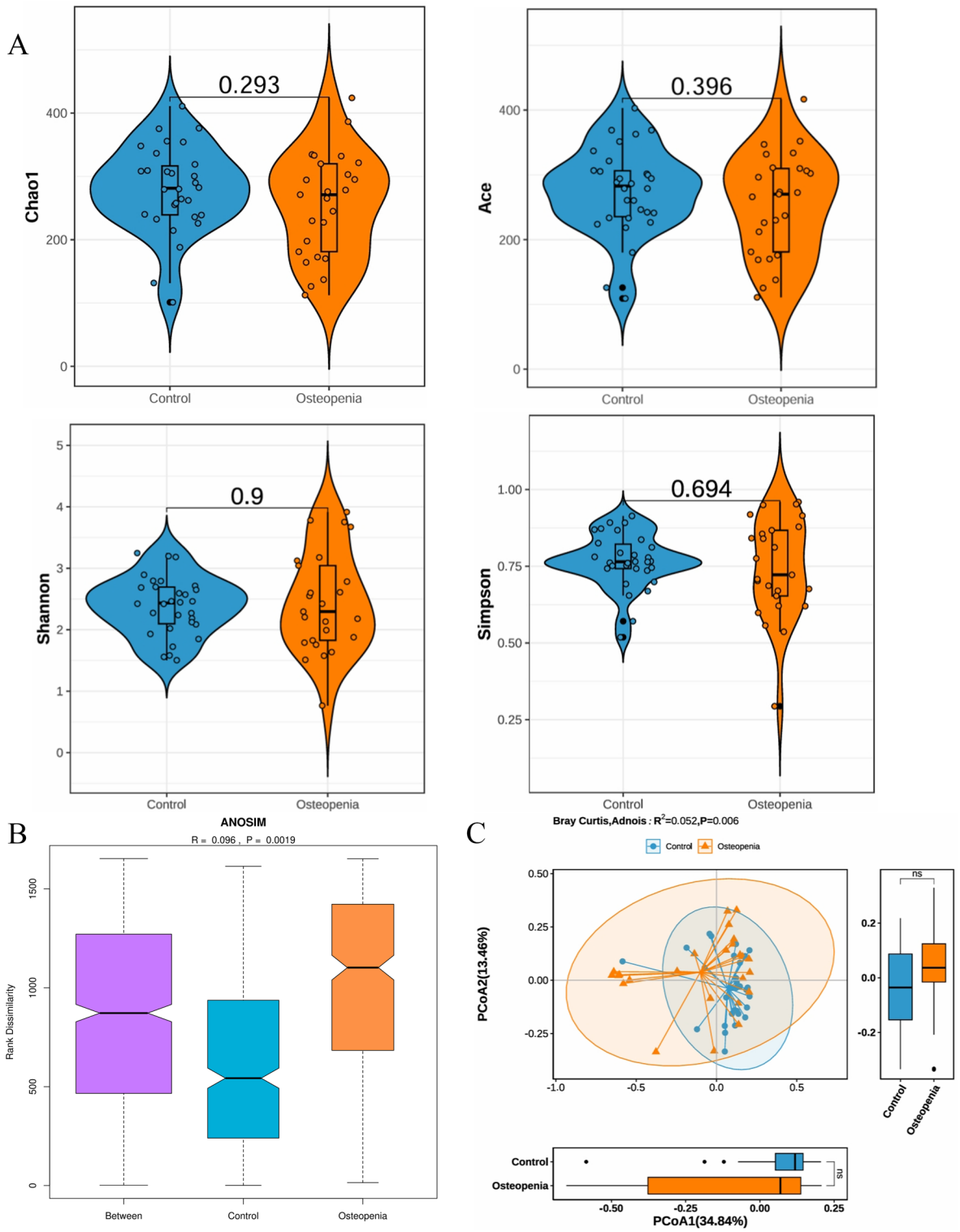

Fig. 1. Comparison of microbial diversity between the osteopenia and control groups. (**A**) Descriptions of the two groups based on Chao1, Ace, Shannon, and Simpson indices. Alpha diversity of gut microbial communities. Violin plots reflect median, dispersion, maximum, minimum, and outliers. The Wilcoxon rank sum test was used to determine *p* values. (**B**) Analysis of similarities (ANOSIM) based on the Bray–Curtis distances. (**C**) Principal coordinate analysis combined with ADNOIS (PCoA).

Ruminiclostridium_5 and Oxalobacter were significantly higher in relative abundance in the osteopenia group than in the control group ($P < 0.05$). In contrast, the relative abundances of Phascolarctobacterium, Succinivibrio,

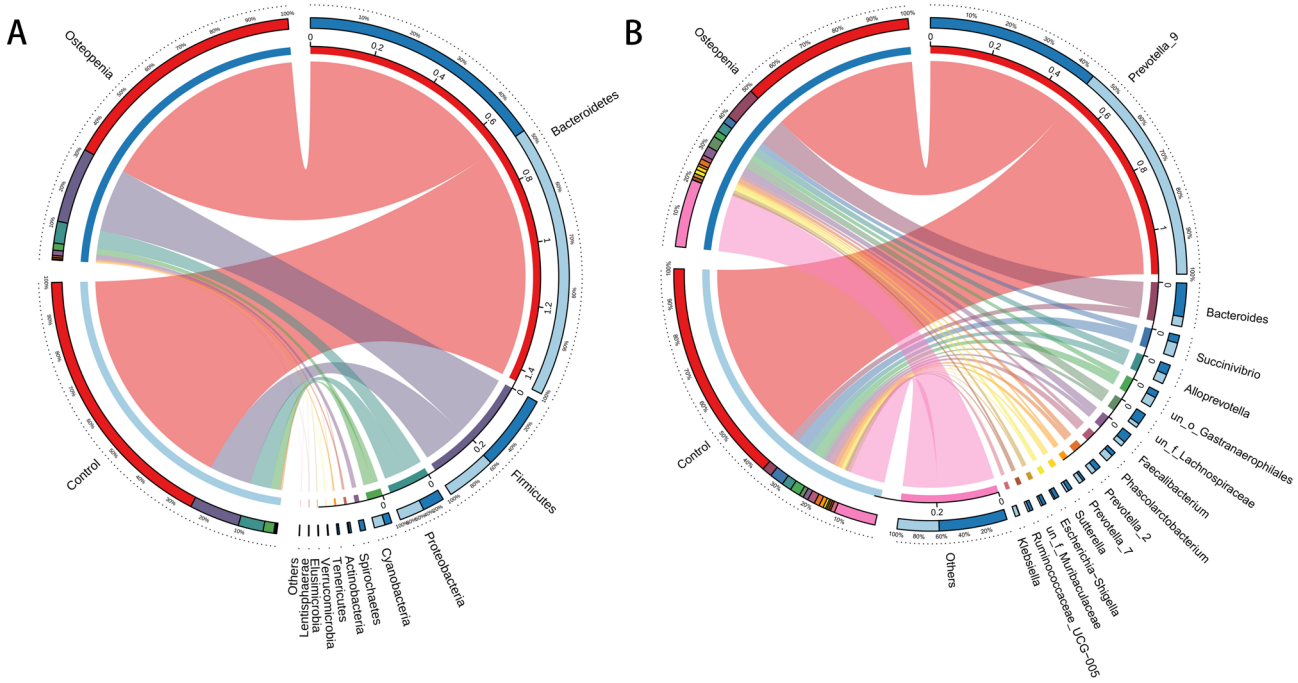

Fig. 2. Relative abundance of bacterial flora at the phylum and genus levels. (**A**) Composition of the gut microbiota at the phylum level. (**B**) Composition of the gut microbiota at the genus level.

and Sphingomonas were significantly lower in osteopenia patients than in controls ($P < 0.05$) (Fig. 3A). Among them, the abundance of Oxalobacter and Lachnoclostridium displayed a significant increase in patients with Osteopenia (Fig. 3B). It is worth noting that the relationship between genus-level bacteria and clinical diagnosis was investigated by Spearman correlation analysis. Professional society guidelines for the management of osteoporosis are based on T-scores and Z-scores, rather than on the actual BMD value[34]. Osteoporosis was defined as a bone mineral density Z-score of < -2 and osteopenia as a Z-score of between -1.0 and -2[35]. Among them, the T-score was significantly positively correlated with g_Sphingomonas, the Z-score was significantly positively correlated with g_Phascolarctobacterium, and both the T and Z score were significantly negatively correlated with Clostridium_sensu_stricto_1, g_Erysipelotrichaceae_UCG-003, and Ruminiclostridium_5 (Fig. 3C). Scatterplot of correlation between genus Horizontal Bacteria and T-scores, Z-scores (Supplementary Fig. 4). We compared the composition of the gut microbiota of the two groups by LEfSe analysis. LEfSe analysis detected 15 genera of bacteria with different abundances: in the osteopenia group, Clostridium_sensu_stricto_1, Lachnoclostridium, Acidaminococcus, Erysipelotrichaceae_UCG_003 and Fusicatenibacter showed higher enrichment; whereas in the control group, Succinivibrio, Phascolarctobacterium, Sphingomonas, and Libanicoccus had a were higher (LDA significance threshold > 1.5; Fig. 3D,E). In conclusion, we found different species in the two groups, suggesting significant differences in the composition of the gut microbiota between the osteopenia and control groups.

Functional prediction of the osteopenia-associated gut microbiota

We performed functional prediction of relevant gut microorganisms based on metabolic pathway information from the KEGG database and in conjunction with the PICRUSt2 software. Many pathways, including Primary bile acid biosynthesis and Steroid biosynthesis, Steroid hormone biosynthesis, alpha-Linolenic acid metabolism, beta-Alanine metabolism, Galactose metabolism, Other glycan degradation, RNA transport, Glycosaminoglycan degradation and Tropane, piperidine and pyridine alkaloid biosynthesis, and Insulin signaling pathway, were enriched in the osteopenia group (Fig. 4). These findings suggest that metabolic pathways related to Steroid biosynthesis and glycan degradation may be differentially regulated in the context of the pathophysiology of osteopenia.

Discussion

Although the relationship between gut microbiota and osteopenia and osteoporosis has been partially studied[15,36,37], the relationship between differences in gut microbiota and osteopenia in the Uyghur population in the Xinjiang region has not been reported. In this study, we used 16S rRNA gene sequencing to analyse representative indices of gut microbial abundance to investigate the correlation between microbial composition and the risk of osteopenia in the Uyghur population.

In this study, we found that individuals with osteopenia had significantly higher OTUs and differential strain abundance at all levels than the control group, which is consistent with the results of many previous studies and supports the notion that intestinal bacterial overgrowth leads to osteopenia[38]. The results of this study showed that there was no significant difference in species richness and diversity between the osteopenia group and the control group, which was reflected by the Chao1, Ace, Simpson, and Shannon indices ($P > 0.05$), which were the

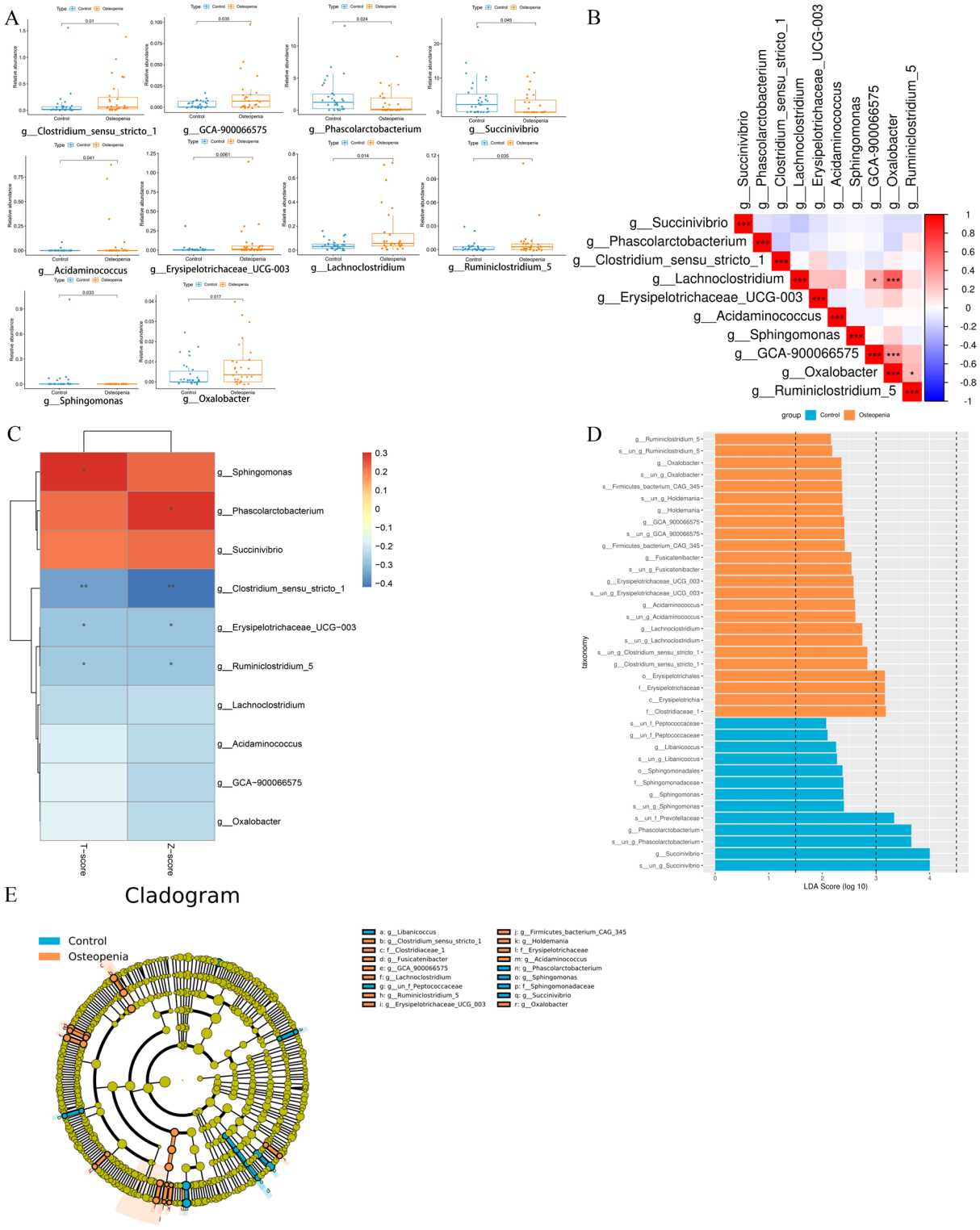

Fig. 3. Significant differences in species richness in osteopenia patients versus control stool samples. (**A**) Species differences between the two groups at the genus level. In each box plot, the differences in species abundance between groups are shown, and above the displayed results are the *P* values of the between-group significance tests for the corresponding species. Box plot of different bacterial genera in osteopenia patients versus control stool samples. (**B**) Differential species correlation heat map. Significant differences in abundance between subgroups and correlation between the top 10 species in terms of mean abundance. Red represents positive correlation, blue for negative correlation (*$P<0.05$, **$P<0.01$, ***$P<0.001$). (**C**) At the genus level, Spearman's correlation analyses were performed on the abundance and bone mineral density of two different bacterial genera to determine their correlation with T and Z scores. Spearman test, *$P<0.05$, **$P<0.01$. (**D**) The dominant gut microbiota of the two groups were distinguished based on the LDA score. The length of the bar graph represents the effect of different species (LDA score). (**E**) Taxon map generated from LEfSe and LDA scores. Bacterial taxa enriched in the osteopenia group (orange dots) and the control group (blue dots).

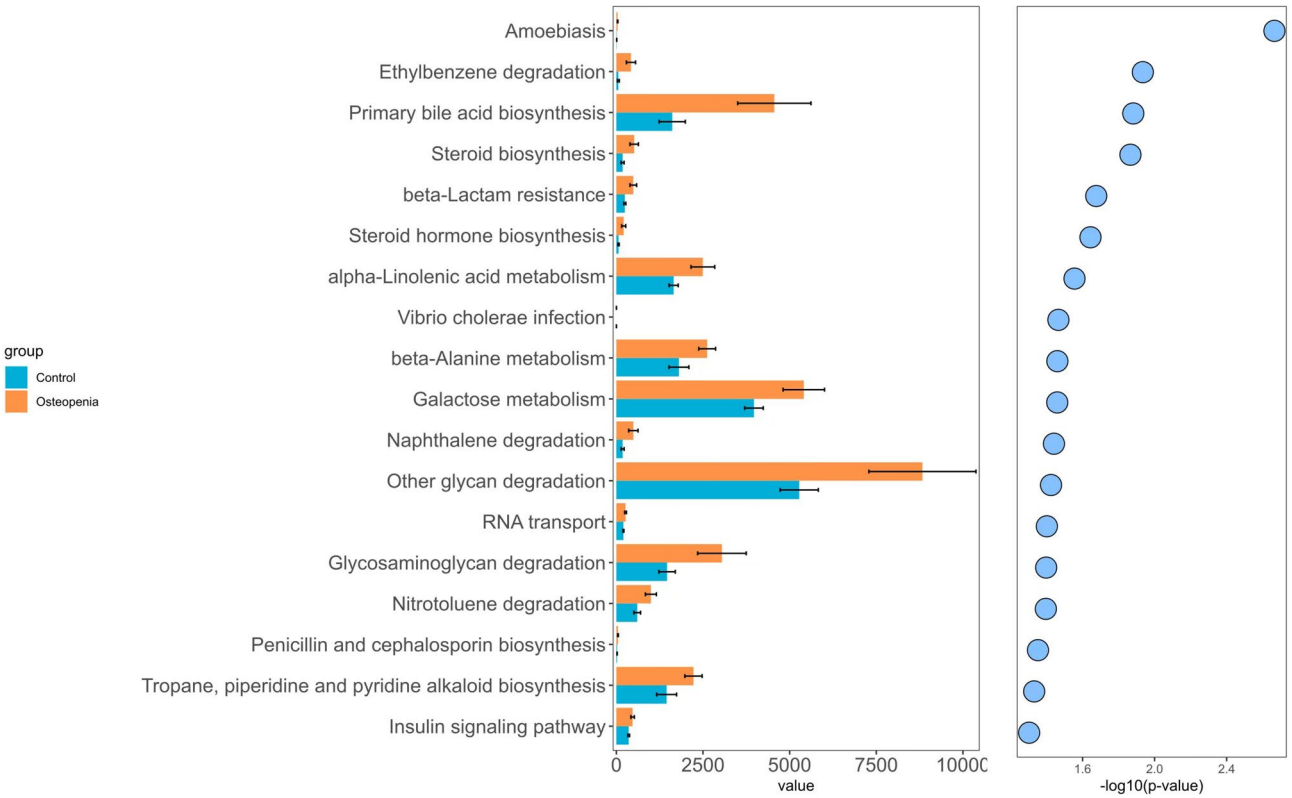

Fig. 4. Analysis of functional differences between the osteopenia and control groups. Analysis of metabolic pathways related to differential gut microbiota in osteopenia and control based on the KEGG database. KEGG, Kyoto Encyclopedia of Genes and Genomes.

same as the results of Chinese and Western studies[15,36]. The significantly higher abundance of Erysipelotrichaceae and Clostridiaceae_1 in the osteopenia group compared to the control group ($P<0.05$) provides further evidence of the differences in gut microbiota between the osteopenia group and the control group. The differential genera found in this study were not the same as previous findings[14–16,23], reflecting the variability in microbial community structure between regions and diets and highlighting the need to collect samples from populations in different geographic regions with different diets to determine the relationship between gut microbiota and osteopenia.

At the phylum level the bacterial communities of Uyghur healthy control population were mainly distributed in Bacteroidetes, Firmicutes, Proteobacteria and Cyanobacteria. Previous studies have found that the gut microbiota of the Tibetan population was distributed in Bacteroidetes, Firmicutes, Proteobacteria, and Actiriobacteria, while the Han Chinese were distributed in Firmicutes, Proteobacteria, and Actiriobacteria, Bacteroidetes distribution[25]. Our findings and those of other studies suggest that this outcome may be associated with differences in genotype and dietary structure. At the genus level, Prevotella_9 and Bacteroides predominated in the Uyghur samples, accounting for 57.13% and 6.24% of the mean total sequences of the two groups, respectively. Prevotella contains a range of carbohydrate fermenters, protein fermenters, acetate fermenters, and hydrogen-producing bacteria[39], whereas Bacteroides is primarily associated with animal proteins, metabolism of several amino acids and saturated fats[22]. A previous study on the relevance of core gut microbiological changes due to seasonal diet in Mongolians came to a similar conclusion[40], with the traditional dietary structure of Mongolians being dominated by large quantities of fried pasta, red meat, and fermented dairy products, with a lower intake of vegetables and fruits, which is similar to that of the Uyghurs. Thus, it is therefore not surprising that these two genera dominate the microbiological composition of the Uyghur gut.

There is strong evidence that altered gut microbes impair bone strength and tissue material properties[41]. Lachnoclostridium is an essential member of the gut microbial community, belonging to Lachnospiraceae, and is capable of fermenting polysaccharides to produce short-chain fatty acids (SFCA), such as butyric, propionic, and acetic acids, which have been shown to have a crucial correlation with bone mass[42,43]. Our results show that this genus was correlated with the majority of the significant differentially abundant metabolites. Yang et al.[44] and Wang et al.[45] found that the abundance of Lachnoclostridium was significantly higher in the osteopenia group than in the control group, and this finding was also verified in the present study, which further identified Lachnoclostridium as an enriched and vital community in the osteopenia group, and it was hypothesised that Lachnoclostridium could be regarded as a specific biomarker for osteopenia. To date, only one article in studies related to human faecal microbiota and osteoporosis has mentioned Phascolarctobacterium[46]. Phascolarctobacterium produces propionate through succinate fermentation[47], and propionate may play a bone-forming role indirectly by increasing the number and function of Tregs. Thus, in the present study, Phascolarctobacterium

abundance was higher in the control group than in the osteopenia group. Ling et al.[46] showed that a higher risk of osteoporosis was associated with a higher number of Phascolarctobacterium, which contradicts our findings. This inconsistency may be due to factors such as sample size, gender ratio of participants (male vs. female), number of sequence reads, and uneven coverage of microorganisms by different PCR primers. Therefore, further studies are needed to confirm how Phascolarctobacterium affects bone quality. Wang et al.[14] concluded that there was an inverse correlation between the number of bacterial taxa and BMD, which was confirmed by our results. The results of this study showed that T and Z scores were significantly and negatively correlated with the content of g_Clostridium_sensu_stricto_1, g_Erysipelotrichaceae_UCG-003 and g_Ruminantium_5. Therefore, more attention can be paid to these genera in future studies.

By means of LEfSe analyses, some taxonomic differences between osteopenia patients and controls were identified between the strata. PICRUSt2 analysis revealed metabolic pathways associated with the gut microbiota in the osteopenia and control groups, with pathways associated with Steroid biosynthesis and glycan degradation being more abundant in the osteopenia group. All steroid hormones are synthesised from cholesterol; LDL cholesterol promotes osteoclastogenesis and vice versa, while HDL cholesterol protects Osteoblasts from apoptosis[48]. Studies have shown that steroids are associated with osteoporosis[49]. Previous studies have shown that glycosaminoglycans are major organic extracellular matrix components. It regulates the attraction of skeletal precursor cells and their subsequent differentiation and gene expression, and modulates the action of proteins essential for bone regeneration[50]. Therefore, we hypothesised that over-enrichment of gut microbiota glycosaminoglycan degradation may contribute to bone mineral loss.

Our study has several strengths. First, data on the gut microbiota of Uyghur residents in Xinjiang was collected for the first time, filling a gap in research on the correlation between gut microbiota and osteopenia in Uyghurs. Second, the composition of the gut microbiota is dynamic, complex, and influenced by multiple factors. We excluded subjects who might affect the composition of the gut microbiota, such as those using antibiotics or suffering from certain diseases, before collecting samples.

We tried to perform a perfect study. However, there are still some limitations. First, this study only included Uyghur residents in Aksu. Regional differences are important factors affecting the composition and structure of gut microorganisms, and in the future, studies of Uyghur populations in more regions are needed to analyse regional differences. Second, it is difficult for us to rule out heterogeneity among different individuals, such as the potential effect of subject mental status on faeces. Third, we could only identify correlations between changes in gut microbiota in the osteopenia and control groups and could not unequivocally state that there was a causal relationship, nor could we definitively confirm that a favourable bacterium protects bones. Finally, we relied on 16S high-throughput sequencing rather than metagenomic sequencing, and 16s rRNA sequencing analyses are characterised by insufficient depth of species identification to differentiate to species or strain level. Therefore the reliability of predicted results is limited and false positives may occur.

Conclusion

We confirmed the significant enrichment of gut microbial abundance in patients with osteopenia, identified the structural composition and characteristic differences of the gut microbiota associated with average bone density and osteopenia, and identified some genera that may be associated with bone density at the genus level. Due to the ethnic and geographical characteristics of the Uyghur people, the relationship between differences in gut microbiota and osteopenia is worth further exploration, and the results of this study provide an essential reference for follow-up studies.

Data availability

Data will be made available on request. Anyone wishing to request data from this study should contact Kunchen Teng. To request data from this study, please contact the corresponding author.

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Author contributions
S.Z, N.R,T.L carried out the investigation of the Uyghur people. K.T, Q.Z, X.L, T.H designed the research and analyzed the data. K.T wrote the paper. Y.W guided the investigation, and modified and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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Competing interests
The authors declare no competing interests.

Ethics statement
Studies involving human subjects were reviewed and approved by the Ethics Committee of Jinzhou Medical University. Participants provided written informed consent to participate in the study. Research involving human research participants must have been performed in accordance with the Declaration of Helsinki.

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