Association Between Gut Microbiota and Bone Health: Potential Mechanisms and Prospective

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Context: It has been well established that the human gut microbiome plays a critical role in the regulation of important biological processes and the mechanisms underlying numerous complex diseases. Although researchers have only recently begun to study the relationship between the gut microbiota and bone metabolism, early efforts have provided increased evidence to suggest an important association.

Evidence Acquisition: In this study, we attempt to comprehensively summarize the relationship between the gut microbiota and bone metabolism by detailing the regulatory effects of the microbiome on various biological processes, including nutrient absorption and the intestinal mucosal barrier, immune system functionality, the gut–brain axis, and excretion of functional byproducts. In this review, we incorporate evidence from various types of studies, including observational, *in vitro* and *in vivo* animal experiments, as well as small efficacy clinic trails.

Evidence Synthesis: We review the various potential mechanisms of influence for the gut microbiota on the regulation of bone metabolism and discuss the importance of further examining the potential effects of the gut microbiota on the risk of osteoporosis in humans. Furthermore, we outline some useful tools/approaches for metagenomics research and present some prominent examples of metagenomics association studies in humans.

Conclusion: Current research efforts, although limited, clearly indicate that the gut microbiota may be implicated in bone metabolism, and therefore, further exploration of this relationship is a promising area of focus in bone health and osteoporosis research. Although most existing studies investigate this relationship using animal models, human studies are both needed and on the horizon. (***J Clin Endocrinol Metab* 102: 3635–3646, 2017**)

The human body is colonized with rich and diverse microbial communities consisting of bacteria, viruses, fungi, and protozoa, all of which taken together compose the human microbiome (1). The majority of these microorganisms reside in the gastrointestinal tract (gut microbiota), typically lining the mucosal surfaces of the host. These microbes begin to interact with the human body beginning from the earliest stages of life, as it is believed the human fetus may be exposed to the maternal microbiome during gestation and development (2). After birth, the gut microbiota quickly colonize in the digestive tract, and the microbiome is established within the first few years of life (1, 3). Although the composition of an individual's gut microbiota usually remains relatively stable in adulthood (1), it may be altered by various factors, including host genetics (4), diet (5), age (6), geography (6), host immune status (7), travel (8), and use of certain medications (9).

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 25 February 2017. Accepted 18 July 2017.
First Published Online 26 July 2017

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Abbreviations: 5-HT, serotonin; BMD, bone mineral density; IL, interleukin; LPS, lipopolysaccharide; MGWAS, metagenome-wide association study; OTU, operational taxonomic unit; rRNA, ribosomal RNA; TNF, tumor necrosis factor.

It is estimated that >1000 different microbial species exist in the human gut (10). The combination of the unique genomes corresponding to each of these species contributes to the gut metagenome, which is estimated to contain >450 times the number of genes as the human genome (11). Previous association studies have shown that the gut microbiome is involved in the regulation of a wide variety of biological processes, including gut physiology (12), nutrient production and absorption (13), host growth (1), energy balancing (14), metabolic functions (15), immune-system functions (16), brain–behavior systems (17), and inflammatory processes (12). Additionally, differences in the composition of the gut microbiota have been found to be associated with the pathogenesis of several complex human diseases, including obesity (18), irritable bowel syndrome (19), type 1 (20) and type 2 diabetes (21), colorectal cancer (22), Parkinson's disease (23), transient ischemic attack (24), and rheumatoid arthritis (25).

Although the number of studies assessing the impact of the microbiota composition on bone metabolism is still very limited, the current findings suggest a potentially intriguing and complex relationship that warrants further examination to determine the specific mechanisms by which the microbiome may modulate bone physiology. In this review, we comprehensively summarize the existing evidence of the connections between the gut microbiota and bone processes and discuss the potential pathways of functional influence. Whereas some aspects of the microbiota–bone relationship have previously been reviewed (26), we also contribute some useful bioinformatics methodology and approaches for metagenomics research and provide perspectives for future explorations of the role of the gut microbiota in the etiology of osteoporosis.

Evidence of the Association Between Gut Microbiota and Bone

Intestinal bacterial overgrowth and bone loss

The earliest evidence of a relationship between the gut microbiota and bone metabolism reported that bone mineral density (BMD) was associated with intestinal bacterial overgrowth. In a prospective cohort study, Di Stefano *et al.* (27) found that bone loss at the site of the lumbar spine and femoral neck was associated with intestinal bacterial overgrowth, indicating overgrowth of the gut microbiota may be an important risk factor in osteopenia/osteoporosis. These findings were further validated by Stotzer *et al.* (28), who observed that individuals with intestinal bacterial overgrowth had significantly lower BMD in the lumbar spine and the femoral neck. In contrast to these findings, a separate study in a small elderly cohort (~80 years old) found no differences in femoral BMD between intestinal bacterial overgrowth subjects and controls (29). However, we hypothesize that the negative results may be attributed to the overwhelming effects of other bone-related factors (*e.g.*, extremely low physical activity) and comorbidities that are common to elderly populations.

We note that intestinal bacterial overgrowth has also been shown to be associated with malabsorption, which can influence the metabolism of essential elements for bone processes such as calcium, carbohydrates, vitamin B, and vitamin K (30). Although malabsorption is known to be an important risk factor for bone-related diseases, the effect of bacterial overgrowth on bone loss is not solely accounted for by this deficiency. In the Stotzer *et al.* study (28), serum-ionized calcium and 1,25-dihydroxyvitamin D3 levels in the bacterial overgrowth individuals were measured to be at normal levels, suggesting that the observed osteoporosis/osteopenia is most likely mediated by several different mechanisms.

Experiments with germfree animals

Germfree mice, born and raised under sterile conditions and therefore largely free of microbiota, are useful tools to study the effects of the gut microbial communities on host physiology. Sjogren *et al.* (31) reported that 7-week-old female germfree mice had increased BMD and a reduced number of osteoclasts compared with conventionally raised mice. More importantly, colonization of the germfree mice with a normal gut microbiota reduced bone mass, suggesting that the absence of gut microbiota may be responsible for the elevated BMD in the germfree mice. In contrast, Schwarzer *et al.* (32) showed that 8-week-old male germfree mice had significantly decreased bone growth characteristics, including femur length, cortical thickness, and cortical/trabecular bone fraction of the femur compared with wild-type mice that had normal gut colonization.

We hypothesize that the conflicting findings from these studies may be attributed to the different genetic profiles of the C57BL/6J mice used in Sjogren *et al.* (31) and the BALB/c mice used in Schwarzer *et al.* (32). It has been well documented that the immune reactions of these two mouse strains to gut bacteria are very different. For example, innate secretory immunoglobulin A, a core fundamental molecule for intestinal immune homeostasis, has been shown to be elevated in BALB/c mice compared with C57BL6/J mice in both feces and serum samples (33). In the same study, it was shown that, when challenged by *Typhimurium aroA*, the level of *Salmonella*-specific IgA in the feces was similar for both strains, although the level in the serum was much less in BALB/c mice (33). Collectively, these differences in immune responses may result in differences in the levels of cytokines critical for bone metabolism such as tumor necrosis factor

(TNF)-α and interleukin (IL)-6, potentially leading to differences in BMD.

Evidence from prebiotics, probiotics, and antibiotics studies

Prebiotics are nondigestible food ingredients that benefit host health by modification of the composition and activities of the gut microbiota (34). Essentially, prebiotics include certain types of plant fiber that reside inside the gastrointestinal tract and provide nourishment for the healthy bacteria of the gut. They are mainly known for their role in digestive processes; however, common prebiotics such as galactooligosaccharide, inulin, and resistant starch have also been shown to promote mineral (calcium, magnesium, and zinc) absorption (35, 36), a process that has an important impact on the regulation of BMD and the prevention of bone loss (37).

In contrast, probiotics are living microorganisms that have important positive health effects on the host, particularly by acting on the digestive system (38). The effects of several important probiotics, mainly *Lactobacillus* and *Bifidobacteria*, on the regulation of BMD have been studied in both animal models and humans. Treating mice with the probiotic *Lactobacillus reuteri* was shown to significantly decrease osteoclastogenesis and bone resorption, preventing bone loss in a mouse model (39). Similar results have also been observed for other *Lactobacillus* strains, such as *Lactobacillus rhamnosus* and *Lactobacillus paracasei*, among others (40–42).

Narva *et al.* (42) identified *Lactobacillus helveticus* fermented milk to have an acute positive effect on calcium metabolism. Although milk has long been known to contain nutritional elements that are beneficial for bone health, there have not been any current studies exploring whether milk consumption benefits bone metabolism by altering the composition of the gut microbiota. However, it has been shown that some types of gut bacteria may aid in the breakdown of proteins contained in milk to biologically active peptides (43), suggesting that the gut bacteria may regulate the beneficial effects from milk consumption on bone metabolism. Taken together, all the evidence suggests that improved bone metabolism may be included among the many protective health benefits associated with probiotics.

It is also well known that antibiotic treatments have the ability to perturb the composition of the gut microbiota. Cho *et al.* (9) treated mice with four types of antibiotic regimens (penicillin, vancomycin, penicillin plus vancomycin, and chlortetracycline) and demonstrated that altering the composition of the microbiome by antibiotic treatment can significantly affect bone growth during early life development. Several additional antibiotics studies have supported these findings, indicating that the gut microbiota may impact bone growth through changes in metabolic hormones as well as the regulation of hepatic gene expression (44, 45).

Potential Mechanisms for Gut Microbiota Affecting Bone Metabolism

There are several potential mechanisms by which the gut microbiota may influence biological processes important for human health, and in the subsequent sections we aim to outline the current findings as they relate to bone metabolism.

Influence on nutrient absorption and the intestinal mucosal barrier

Among the extensive variety of bacterial species in the gut microbiota, many can influence the processes of nutrient absorption. For instance, an elevated concentration of the probiotics *L. reuteri* and *Bifidobacterium longum* in the gut may increase BMD by promoting mineral (calcium, magnesium, and phosphate) absorption (46). It has also been shown that the composition of the gut microbiota can influence the pH level of the gut (47), an important factor for nutrient absorption, especially calcium (48). Other studies have shown that the gut microbiota aid in the breakdown of macromolecules to smaller components that can be more easily absorbed, an important feature for both bone health and human metabolism more generally (49). Moreover, these microorganisms play an essential role in the synthesis of vitamin B and vitamin K as well as the metabolism of bile acids (50). It is well known that vitamins B and K are critical for the regulation of bone health (51, 52), and that various bile acids may play key roles in the control of calcium absorption. For example, it has been shown that ursodeoxycholic acid promotes calcium absorption, whereas deoxycholic acid inhibits calcium absorption (53).

Nutrient absorption may also be influenced by host diet, which can in turn impact the composition of the microbial profile. The intake of carbohydrates and other nutrients provides energy for the survival of the gut bacteria; however, the composition of the diet can have important effects on the microbial community. High-calorie diets are associated with a reduction in the Bacteriodetes/Firmicutes ratio (54), which can lead to metabolic disturbance of the host. In contrast, low-calorie diets increase the concentration of harmful substances in the intestinal tract (55), which may also have negative consequences for host health. Although adequate protein intake provides necessary elements for bone growth, an excess of protein in the diet may also lead to an elevated level of toxins in the intestinal tract, such as hydrogen sulfide and methane (56). Therefore, it is crucial to

maintain a balanced diet and adequate carbohydrate/protein ratio because dietary intake can lead to meaningful alterations in the gut microbiota, thereby influencing bone metabolic processes.

The relationship between the gut microbiota and the intestinal mucosal barrier is quite complex, as they are known to codevelop and work together to engage foreign pathogens (57). Hamilton *et al.* (58) demonstrated that a change in the composition of the gut microbiota induced an increase in intestinal permeability, which could result in metabolic disorders. The dysfunction of the intestinal mucosal barrier may lead to an increase in serum levels of lipopolysaccharide (LPS), which could in turn increase membrane permeability, resulting in metabolic endotoxemia (59). Early studies have suggested that LPS promotes the survival of osteoclasts *in vitro* (60, 61); however, most studies have used much higher dosages of LPS than what is necessary to induce metabolic endotoxemia, and therefore it is unclear whether endotoxemia would influence bone mass *in vivo*.

Influence on immune system

In order for the immune system to adequately function, the human body must be able to distinguish between the molecules that are normally present in the host environment and the foreign microorganisms that are not. Because the gut microbiota are acquired from the environment, they may elicit immune responses at either the local site of the gut or systemically throughout the body. Therefore, the presence of certain species may lead to an increase in a number of cytokines, including many that are associated with bone metabolism, such as TNF-α (31). In support of these findings, Sjogren *et al.* (31) found that germfree mice had reduced expression of proinflammatory cytokines TNF-α and IL-6. TNF-α is known to stimulate the receptor activator for nuclear factor κB ligand signaling pathway, which may promote bone loss (62), as well as suppress the differentiation of mesenchymal stem cells into osteoblasts, inhibiting bone formation (63).

It has been noted that there is an interesting relationship between bone loss and bariatric surgeries (64). The mechanism of the bone loss induced by bariatric surgeries is not fully understood, although it is believed to include malabsorption and certain immune system factors along with multiple other unknown components (65). In one recent study, it was reported that gut microbiota and proinflammatory cytokines (including TNF-α and IL-6) were altered after sleeve gastrectomy (66). In particular, after treatment by sleeve gastrectomy, the levels of TNF-α and IL-6 were reduced (66). However, bone loss is known to be associated with increased levels of TNF-α and IL-6 rather than decreased levels. Therefore, although it is worth noting, these findings are inconsistent, and it is unclear exactly how bariatric surgeries may impact the microbiome relevant to bone.

Influence on gut–brain axis

In recent years, it has been discovered that the gut microbiota may have important effects on the nervous system through regulation of the synthesis of hormones and neurotransmitters such as serotonin (5-HT) (17). The 5-HT signal transduction system is regarded as an important factor for the regulation of bone development and maintenance. Bliziotes *et al.* (67) reported that both osteoblast and osteocyte cells contain 5-HT receptors, and that increased 5-HT levels are associated with decreased bone mass in mice. In accordance with this discovery, another study found that decreasing the 5-HT levels with a synthesized molecular inhibitor was able to prevent ovariectomized-induced bone loss in mice (68). Additionally, Sjogren *et al.* (31) showed that germfree mice had decreased 5-HT levels and increased trabecular bone volume/tissue volume. Therefore, the evidence suggests that the gut microbiota may influence bone processes by affecting the levels of metabolic hormones within the body.

Effects by gut microbial excretion byproducts

Microbial byproducts not only help digestion and absorption of nutrients, but also have their own potential function in the regulation of BMD. For example, some short chain fatty acids produced by the gut microbiota (*e.g.*, butyrate) play an important role in bone formation and bone mineralization by influencing the Runx and osteoprotegerin signaling pathways (69, 70). In addition, butyrate reduces osteoclastogenesis by suppressing the receptor activator for nuclear factor κB ligand signaling pathway (71). It is also known that the gut microbiota may influence intestinally derived estrogen such as flavonoids and diethylstilbestrol (72, 73). The decrease of estrogen levels is a major factor contributing to postmenopausal osteoporosis risk, and therefore, the gut microbiota may influence the regulation of bone health by altering levels of nonovarian estrogens.

Additional studies have discovered that short chain fatty acids also might indirectly affect BMD by significantly influencing the function of host endocrine factors that are related to bone metabolism, such as peptide YY and glucagon-like peptide 1 (74). Peptide YY is a gastrointestinal hormone secreted from the endocrine L cells and has been shown to be negatively associated with total body and hip BMD in premenopausal women (75). Glucagon-like peptide 1, an amino acid hormone that is also secreted from the endocrine L cells, has been shown to act as a regulator of bone metabolism by altering the

balance between osteoblast and adipocyte differentiation from bone mesenchymal stem cells (76).

The overall relationship between the gut microbiota and bone metabolism is summarized in Fig. 1.

Approaches for Metagenomics Analysis

Given the importance of the gut microbiota for bone metabolism and potentially the risk of osteoporosis development, we anticipate a growing number of metagenomic studies in this field throughout the near future. In the following section, we outline some prevailing technical and analytical approaches that are appropriate for gut microbiome studies of complex diseases such as osteoporosis.

Profiling techniques: 16S ribosomal RNA gene sequencing, metagenomic shotgun sequencing, and DNA microarrays

The 16S ribosomal RNA (rRNA) gene sequencing is a highly efficient and cost-effective method for microbiota profiling (77). Bacterial DNA is extracted after sample collection, and either the variable regions or full-length (78) of the 16S rRNA gene are selected for sequencing. Bacterial identification is accomplished using sequence alignment to cluster the microbes into operational

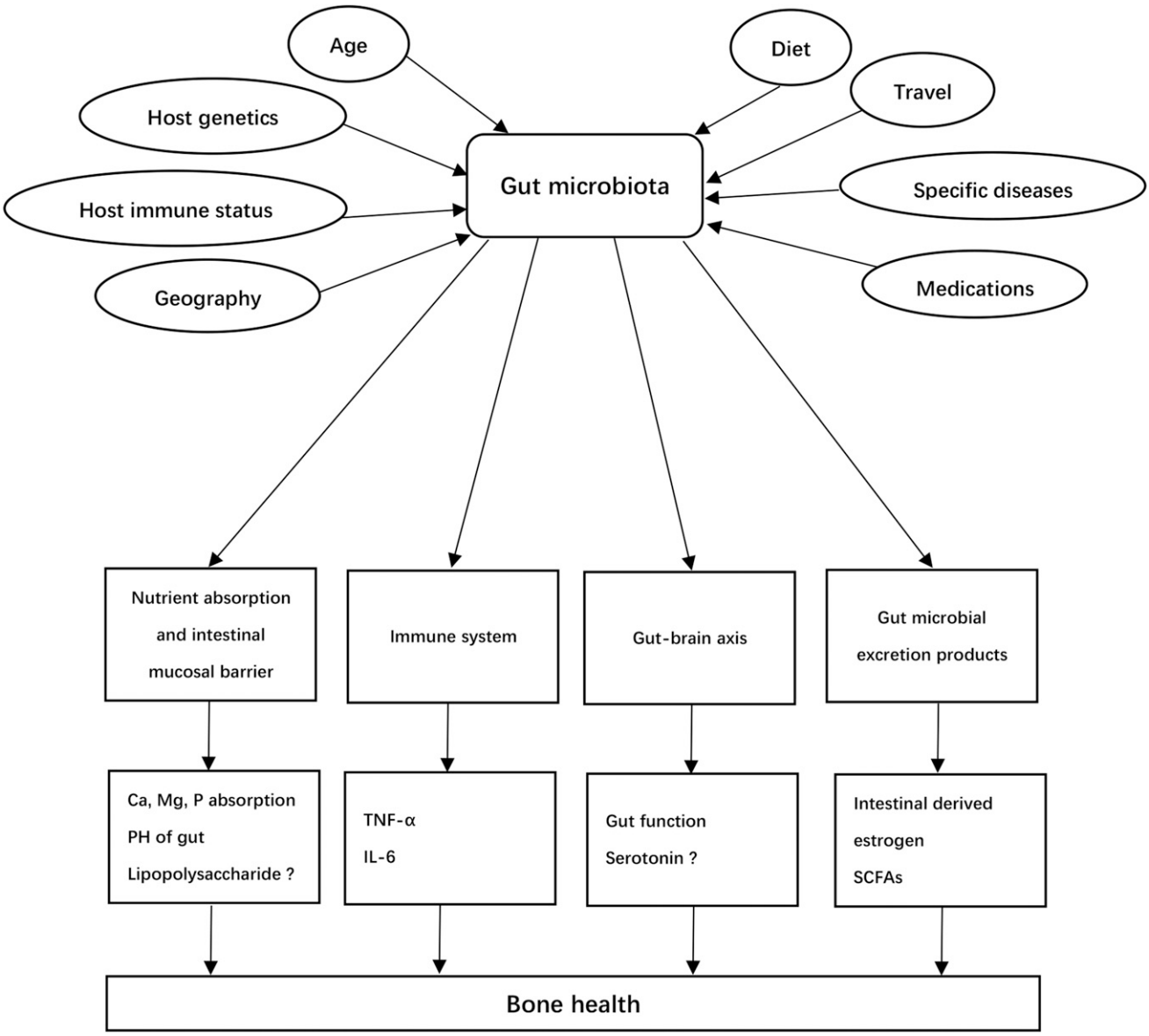

Figure 1. Potential etiology of osteoporosis attributed to gut microbiota. Gut microbiota may be altered by various factors, including host genetics, diet, age, geography, host immune status, travel, and use of certain medications. Gut microbiota play important roles on the regulation of bone via nutrition absorbing, changing the permeability of intestinal mucosal barrier, immune system, gut–brain axis, and excrete products. ?, evidence for the effects of 5-HT and LPS on bone is unconfirmed as more studies are needed for validation. PH, potential of hydrogen; SCFA, short chain fatty acid.

taxonomic units (OTUs) based on genetic similarity, which can then be incorporated into statistical association analysis. Although it has been shown that the 16S sequencing approach is fairly powerful to identify microbiota taxa (9), there are some important technical limitations, such as poor taxonomic resolution, low sensitivity making it difficult to detect the rare taxa, and it does not provide information about genomic functional information of the microbes (79).

Recent improvements in next-generation sequencing technology have stimulated the development and application of metagenomic shotgun sequencing for gut microbiome analysis (21, 22, 25). In this approach, DNA is extracted from all microbes in a community, but instead of targeting a specific marker gene for amplification, the entire nucleotide pool is separated into small fragments that are subsequently sequenced. Because this approach does not rely on the single-copy and evolutionary assumptions of marker genes, it can produce a less biased community profile than does 16S rRNA sequencing (80). In addition, metagenomic shotgun sequencing can generally provide higher-resolution descriptions of the microbial compositions, making it possible to identify particular species and even strains of microbes (81). More importantly, sequencing reads sampled from coding sequences can provide insight into the genes and biological functions encoded by specific microbes. Despite these benefits, we note that the necessary bioinformatics tools for metagenomic shotgun sequencing may be considered more challenging compared with that for the 16S rRNA sequencing data, although computational and bioinformatics tools are currently being developed (79–82).

In addition to the aforementioned sequencing-based approaches, the DNA microarray technique is also commonly used. There are many advantages to DNA microarray–based analysis, including high-throughput capabilities, cost effectiveness, as well as relative ease and quickness. DNA microarrays for the analysis of the gut microbiota, such as Human Intestinal Tract Chip (HITChip), are composed of oligonucleotide probes contained in known gene catalogs (20). Although both simple and convenient, the major disadvantage of the microarray approach is that it is based on complementary DNA hybridization, and therefore it is not possible to study uncultured/unknown strains of bacteria.

The strengths and weaknesses of the aforementioned three approaches are outlined in Table 1.

Data analysis workflow

The data analysis workflow for 16S rRNA sequencing and metagenomic shotgun sequencing data have been extensively reviewed (21, 79, 83, 84), and we summarize several commonly used computational tools for metagenomic data analysis in Table 2.

Briefly, the bacterial identification and classification of 16S rRNA sequencing data involve three main steps. The first step includes raw sequencing data processing, quality filtering, de-noising, removing artificial chimera sequences, and data normalization. The second step involves global alignment for sequence taxonomy, clustering by OTUs, and building phylogenetic trees. The final step involves statistical analyses of the OTU taxa to measure α- and β-diversity, the microbial diversity within and between samples, respectively.

Similarly, in metagenomic shotgun sequencing, the process begins with raw sequencing data processing, quality filtering, sequence alignment, removal of human sequencing reads, and *de novo* assembly of the metagenome. This is followed by taxonomic assignment,

Table 1. Strengths and Weaknesses of the Three Main Approaches in Metagenome-Wide Association Study

Features	16S rRNA Sequencing	Metagenomic Shotgun Sequencing	DNA Microarrays
Strengths	1. Identify species[a]/genus[b] of bacteria, including known and novel species[a]/genus[b] of bacteria	1. Detect very low-abundance microbes	1. High throughput
	2. Highly efficient and cost effective	2. Provide information at all taxonomic levels	2. Quickness
		3. Provide potential functional information of gut microbiota	3. Direct phylogenetic identification
			4. Cost effective
Weaknesses	1. Poor taxonomic resolution	1. Expensive	1. Can't find novel species/strains
	2. Can't detect very low-abundance microbes	2. Need complex analysis tools to generate all the data	2. Cross-hybridization and hybridization
	3. Can't provide biological functional information of gut microbiota		

[a]Full-length 16S rRNA gene sequencing can pinpoint to species.

[b]16S rRNA amplicon sequencing can pinpoint to genus, not species.

Table 2. Bioinformatics Tools for 16S rRNA Gene–Sequencing and Metagenomic Shotgun–Sequencing Studies

Tools	Main Function	Reference	Web Site/Note
DADA[a]	Denoising	107	sites.google.com.site/dadadenoiser
Denoiser[a]	Denoising	108	qimme.org
ChimeraSlayer[a]	Chimera detection	109	microbiomeutil.sourceforge.net
DECIPHER[a]	Chimera detection	110	decipher.cee.wisr.edu
UCLUST[a]	OTU clustering	111	www.drive5.com/usearch
CD-HIT-OTU[a]	OTU clustering	112	weizhing-laboratory.ucsd.edu/cd-hit/otu
Mothur[a]	ALL three steps[c]	113	Mothur.org
QIIME[a]	ALL three steps[c]	114	qimme.org
MetaIDBA[b]	Assembly	115	For short reads (75–150 bp)
MEGAN[b]	Binning	116	Compositional-based
PhyloPythiaS[b]	Binning	117	Similarity-based
MetaCluster[b]	Binning	118	Both compositional-based and similarity-based
MetaGeneAnnotator[b]	Functional annotation	119	metagene.nig.ac.jp/
METAREP[b]	Functional annotation	120	jcvi.org/metarep/

[a]Bioinformatics tools for 16S rRNA gene–sequencing studies.

[b]Bioinformatics tools for Metagenomic shotgun–sequencing studies.

[c]All three steps included in the general workflow of analysis for 16S rRNA gene–sequencing data.

which is typically accomplished by comparing metagenomic reads with a database of taxonomically informative marker genes and using sequence or phylogenetic similarity to taxonomically characterize metagenomic homologs. Finally, functional information of the microbes is provided by annotating predicted genes or sequencing reads into orthologous gene families and metabolic pathways using various databases such as the Kyoto Encyclopedia of Genes and Genomes, Clusters of Orthologous Groups (85), eggNOG (86), Pfam (87), and TIGRFAMs (88).

Although the current bioinformatics tools for metagenomic data certainly have utility, there are still several crucial limitations that leave much room for improvement. Although new approaches that can accommodate the types of analytical challenges common to metagenomic data are rapidly evolving, there is a necessity for an increased focus on methodology development.

Perspective

In this section, we offer some suggestions for research areas that may provide further insight into the relationship between the gut microbiome and bone metabolism. Although to date there are no current metagenome-wide association studies (MGWASs), multiomics, or translational studies specific to osteoporosis, there are promising examples for other complex diseases that may serve as a guide for future bone research.

Metagenomics studies for osteoporosis

With increasingly reduced costs of high-throughput sequencing, MGWASs, which aim to identify the association between the relative abundances of taxonomic units in a metagenome (*e.g.*, gut microbiome) and a phenotypic trait, are quickly becoming more popular. Recent MGWASs have not only shed insights into the pathophysiological mechanisms of a number of complex human diseases, but have also shown the effective power to distinguish between cases and healthy controls based on the composition of the gut microbiota. Qin *et al.* (21) conducted a two-stage MGWAS using metagenomic shotgun sequencing and identified several type 2 diabetes–associated biomarkers. In another study, Zhang *et al.* (25) used a metagenomic shotgun-sequencing approach to show that the composition of the gut microbiota in rheumatoid arthritis cases was significantly different from the microbial profile of healthy controls.

MGWAS for osteoporosis holds great promise in providing findings regarding the specific microbial features that are involved in the underlying biological mechanisms of osteoporosis. There is a necessity for studies in humans to further examine the impact of the gut microbiota composition on the risk of osteoporosis. We caution that the incidence and mechanisms of osteoporosis in females are largely different from that in males, and thus, sex specificity should be taken into consideration when designing future MGWASs for osteoporosis.

Multiomics studies for gut microbiota

Although metagenomic sequencing can provide information about the genetic makeup of the bacteria present in the microbiome, it cannot identify the particular genes that are actively expressed within the metagenome. Recently, there has been an increased focus to develop assays that analyze the RNA, protein expression, and small-molecule metabolites of the gut microbiota to provide useful information regarding meta-transcriptomics, meta-proteomics, and meta-metabolomics (89–91). Integrating

multiple layers of omics from the gut microbiota will provide a more comprehensive and systematic understanding of how the microbiota may affect certain aspects of host physiology such as bone metabolism (92).The simultaneous advances in multiomics studies for both humans and the gut microbiota offer opportunities for combining studies of host omics with that of the gut microbiome (93).

Several recent studies have demonstrated that the gut microbiota composition may be influenced by the host genome (94, 95), and there is also a growing appreciation for the role of epigenetic regulation of the host–microbiota interactions (96). Gut-microbial byproducts may affect the methylation status of the host genome and in turn lead to transcriptomic alterations that can modify the risk of phenotypic trait expression (97, 98). Therefore, the gut microbiota may act as an important mediator of host gene environment interactions. Further exploration through integrative multiomics and network analysis studies is needed to elucidate the full range of these interactions as they relate to bone health and other complex human diseases.

Translational potential of gut microbiota

The apparent association between the gut microbiota and bone metabolic processes suggests that the characterization and identification of important gut microbiota features may have great clinical potential. Remarkably, several recent studies (21, 22, 25, 99) have shown that distinctions in the gut microbiota composition may be used to distinguish between individuals having differential disease status with high statistical power based on the area under the curve metric (area under the receiver operating characteristic curve), a common indicator to evaluate sensitivity and specificity (Table 3). Therefore, it is conceivable that the gut microbiota may furnish effective biomarkers in the diagnosis/prognosis of bone diseases and other phenotypic traits.

Currently, the majority of therapeutic efforts targeting the gut microbiota have been focused around the preventative/protective effects provided by probiotics and prebiotics (100, 101). These types of treatments have been successfully tested in clinical interventions for dozens of human diseases, including obesity (102), ulcerative colitis (103), atopic diseases of children (104), hypercholesterolemia (105), and autism (106). Narrow-spectrum antibiotics directed toward the gut microbiota and their byproducts are believed to hold great promise as a microbiome-based therapy (100, 101). Although there has not yet been much focus to assess the success of microbiome-based therapies in the treatment of bone-related diseases, it is possible that in the future the identification of those microbiota important for the regulation of bone metabolism may serve as therapeutic targets.

Table 3. Examples of the Area Under the Curve of Using Gut Microbiota to Distinguish Patients From Healthy Samples for Complex Diseases

Disease	AUC	95% Confidence Interval
Type 2 diabetes (21)	0.81	0.76–0.85
Obesity (99)	0.78	/[a]
Colorectal carcinoma (22)	0.96	0.8788–1.00
Rheumatoid arthritis (25)	0.9396	/[a]

An AUC of 1 represents a perfect test; an AUC of 0.5 represents a meaningless test.

Abbreviation: AUC, area under the curve of receiver operating characteristic.

[a]No information could be found.

Acknowledgments

Financial Support: This work was partially supported by National Institutes of Health Grants P50AR055081, R01AR057049, R01AR059781, D43TW009107, P20GM109036, R01MH107354, R01MH104680, and R01GM109068 and the Edward G. Schlieder Endowment fund from Tulane University. Y.-C.C. was also partially supported by the National Natural Science Foundation of China (81302228), the Foundation for P Pearl River Nova program of Guangzhou (2014J2200034), and the Foundation from China Scholarship Council (20150322).

Author Contributions: All authors contributed to drafting and revision of the manuscript.

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Disclosure Summary: The authors have nothing to disclose.

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