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MEETING REPORTS

Trends in Genetics: Meeting Report from the 30th Annual Meeting of the American Society for Bone and Mineral Research

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Genetic research is one of the most important areas in bone and mineral research. At this year's ASBMR meeting, mouse genetic studies demonstrated the importance of Wnt signaling pathways in bone metabolism. Genome-wide association studies (GWAS), a newly-established and powerful approach in human genetics, have identified novel genes underlying osteoporosis. Complementary to GWAS, functional genomics has become an efficient tool to screen important bone-related genes at the expression level. Epigenetic and epigenomics studies have also sprouted in bone research to reveal new genetic mechanisms for the regulation of gene expression.

Mouse Genetics and Human Genetic Epidemiology

Recently, both canonical and non-canonical Wnt signaling pathways have been found to be very important for osteoblastogenesis, osteoclastogenesis and chondrogeneis (1-3). Mouse genetic study of important genes in Wnt signaling pathways was one of the major topics at this year's meeting. First, there were studies focusing on deleting or overexpressing genes or antagonists for Wnt signaling pathways, in order to observe bone phenotypes. For example, mice lacking the Wnt receptor Fzd9 displayed osteopenia resulting from decreased function of osteoblasts (4). Deletion of the Wnt signaling antagonist sFRP4 increased bone formation by increasing osteoblast differentiation and activity (5). Transgenic mice overexpressing the Wnt antagonist Kremen 2 in osteoblasts resulted in severely reduced bone formation and increased bone resorption (6), while deletion of Kremen 1 and Kremen 2 resulted in increased bone formation and bone mass (7). Second, studies revealed novel interactions between Wnt signaling and other signaling pathways, such as the calcium-sensing receptor (8), PTH and PTH receptor (9;10), and androgen receptor signaling pathways (11), and the impact of these interactions on bone metabolism. Third, two mouse studies demonstrated the importance of Wnt signaling for bone metabolism in two other cell types: osteocytes (12) and T cells (13).

Although the mouse is a good genetic model to investigate relevant genes for complex diseases, there are of course genetic differences between mice and humans. Therefore, it is still necessary to verify and identify causal genes directly from human samples. Genetic epidemiology is one of the most efficient ways to verify those genes identified from mouse studies and to identify novel genes for human complex diseases. Previously, genome-wide linkage and candidate gene association studies were the two major approaches for screening disease regions or genes in specific human populations. However, there are major limitations to both methods. Linkage studies can only locate genomic regions rather than specific genes. Meanwhile, candidate gene association studies can only test potential known candidate genes, which means that previous knowledge or hypotheses of the

relationship between the genes and the disease is required. However, GWAS is a promising new technology that can identify novel disease-related genes without any previous knowledge of the gene-disease relationship.

Since 2006, GWAS have been applied to genetic studies on various diseases due to the completion of the HapMap Project and the availability of high density single nucleotide polymorphism (SNP) arrays covering the human genome. At the 2007 ASBMR meeting, GWAS on bone phenotypes were initially reported. At this year's meeting, more GWAS were presented and, in particular, there was a concurrent oral session for genetic epidemiology focused on GWAS. Two groups performed GWAS and identified both novel and known genes underlying human osteoporosis using dense 500K SNP arrays (14-17). Using 1,000 unrelated homogeneous Caucasian samples, two novel genes, *ADAMTS18* and *TGFBR3*, were identified for BMD variations (14) and one novel gene, *RTP3*, for bone strength (15). In addition, three new genes, *ALDH7A1*, *UBR3*, and *PHACTR3*, were also found for osteoporotic fractures in 700 elderly Chinese Han subjects (16). Using 2073 women and 1554 men from Framingham family samples, six known and novel genes, *BMP10*, *PTPRD*, *MYC*, *SLC16A4*, *IL6*, and *PRKG1*, underlying BMD and bone geometry were identified (17).

DNA copy number variation (CNV) is another rich and important source of genetic diversity for human diseases. At this year's meeting, the first genome-wide CNV study on osteoporosis found a novel gene, *UGT2B17*, underlying various bone phenotypes in 700 elderly Chinese Han subjects (18). There were few overlapping genes between different GWAS for osteoporosis. To solve this problem, two issues need to be further emphasized. First, the study population should be homogeneous to avoid population stratifications and admixture (particularly those from different regions used in the same analyses), which may render false positive and false negative results. Second, replication of identified genes in different populations is important to further reduce the possibility of false positive results. Besides GWAS, a candidate gene association study on BMD in 1243 Hong Kong Chinese subjects found a significant association of *SOST*, an inhibitor of Wnt signaling, with BMD (19), which further supports the importance of Wnt signaling in humans.

Functional Genomics

The functional genomics approach looks at genome-wide gene expression at the mRNA and protein levels. It has been a very efficient way to screen functionally important genes and serves as a complement to genome-wide linkage and association studies at the DNA level. At the 2008 meeting, two *in vivo* gene expression profiling studies using Affymetrix HG-133A human gene expression chips on 40 Caucasian women in each study found a novel EGFR and CALM3 gene network for smoking-related osteoporosis in circulating B cells (20), and a novel CRAT and CPT2 gene network for smoking-related osteoporosis in circulating monocytes (21). These two studies indicate that it is efficient to screen functionally important genes for bone metabolism on a genome-wide basis directly from *in vivo* human cells.

Interestingly, another study using Affymetrix Rat Genome 230 2.0 gene expression arrays on RNA from rats' proximal femora also found 21 interesting candidate genes related to bone metabolism (22), indicating that the functional genomics approach is also powerful in searching functional genes for bone in mice. Moreover, a comparison study of gene expression profiles between osteocytes and osteoblasts isolated from mice identified 380 differentially expressed genes, including six genes involved in Wnt signaling (23).

In addition, functional genomics technology was applied to *in vitro* cell cultures. Expression profiles of 84 Wnt signaling pathway genes revealed important Wnt components in the response to cAMP-stimulated osteoblast maturation (24).

Comparison of expression profiles between aged and immortalized human mesenchymal stem cell cultures also discovered ~ 400 differentially expressed genes (25).

Epigenetics and Epigenomics

Besides the traditional central dogma involving DNA, mRNA and protein in molecular genetics, recently gene expression has been found to be regulated by mechanisms other than changes in the underlying DNA sequences, *e.g.*, microRNA (miRNA) regulation and DNA methylation. The term epigenetics/epigenomics refers to those non-DNA-change mechanisms. Epigenomics aims to find those changes at the genome-wide level. At this year's meeting, epigenetic and epigenomic studies were performed in bone research. Recently, miRNAs – short non-coding RNAs (~22 nucleotides) – have been found generally expressed in various cell types and to regulate gene expression by sequence-specific base paring in the 3' untranslated regions of the target mRNA, which results in mRNA degradation or inhibition of translation. The miRNA-regulating mechanisms for the expression of genes important for bone metabolism await further study.

Several studies were conducted to identify miRNAs for osteoblast differentiation (26-29). An oral presentation reported the importance of miR-29, through regulation of osteonectin expression, in osteoblast differentiation *in vitro* (26). In another study, menin protein was found to modulate osteoblast differentiation *in vitro* via BMP-2 signaling by regulating miR-26a expression (27). Furthermore, two epigenomic studies followed by functional epigenetic studies found miRNAs important for BMP-2-induced osteoblastogenesis (28;30) Both studies performed miRNA array analyses to screen differentially expressed miRNAs between BMP-2-treated and -untreated C2C12 mesenchymal cells. One study identified, among the differentially expressed miRNAs, the significance of miR-206 in osteoblastogenesis through its regulation of the Connnexin43 gene (28), while the other study revealed the roles of miR-133 and miR-135 in osteoblastogenesis through their targeting of Runx2 and Smad5 gene expression in BMP-2 osteogenic signaling (29).

DNA methylation is another type of epigenetic regulation. The methylated DNA may result in reduced gene transcription either by impeding the binding of transcription factors or by being combined with chromatin remodeling proteins and modified to form compact and silence chromatin. DNA methylation has been observed as important in carcinogenesis and stem cell differentiation. One DNA methylation profiling study reported at this year's meeting found that specific CpG loci in osteocalcin and osteopontin promoter regions in mouse bone marrow stromal cells may be involved in the regulation of gene expression (31).

Summary

Traditional mouse genetics is a robust approach for investigating the functional detail of known genes in bone and mineral research. However, GWAS and functional genomics studies are powerful methods for identifying novel genes underlying bone metabolism, particularly in humans. As newborn areas in genetics, epigenetics and epigenomics are providing new knowledge for understanding the regulation of bone-related gene expression.

Conflict of Interest: None reported.

References

1. Bodine PV, Komm BS. Wnt signaling and osteoblastogenesis. *Rev Endocr Metab Disord*. 2006 Jun;7(1-2):33-9.

2. Liu F, Kohlmeier S, Wang CY. Wnt signaling and skeletal development. *Cell Signal*. 2008 Jun;20(6):999-1009.

3. Yavropoulou MP, Yovos JG. The role of the Wnt signaling pathway in osteoblast commitment and differentiation. *Hormones (Athens)*. 2007 Oct-Dec;6(4):279-94.

4. Albers J, Gebauer M, Friedrich F, Schulze J, Priemel M, Francke U, Amling M, Schinke T. Mice lacking the Wnt receptor Frizzled-9 display osteopenia caused by decreased bone formation. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S3. [Abstract]

5. Saito H, Hinkle R, Ebert D, Blanton C, Jaiswal N, Elenich L, Cody D, Baron R, Sabatakos G. Deletion of the Wnt signaling antagonist secreted frizzled related protein 4 (sFRP4) in mice induces opposite bone formation phenotypes in trabecular and cortical bone. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S3. [Abstract]

6. Schulze J, Seitz S, Schneebauer M, Amling M, Schinke T. Transgenic over-expression of the Wnt antagonist Kremen-2 in osteoblasts leads to severe impairment of bone formation and increased bone resorption. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S2. [Abstract]

7. Saito H, Ellwanger K, Clément-Lacroix P, Hesse E, Maltry N, Niedermeyer J, Lee RW, Rawadi G, Westphal H, Niehrs C, Baron R. Deletion of the Dkk1 co-receptors Kremen 1 and Kremen 2 in mice leads to increased bone formation and bone mass. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S2. [Abstract]

8. Chang W, Tu C, Chen T, Bikle D, Shoback D. Conditional knockout of the Ca2+-sensing receptor in osteoblasts alters regulators of Wnt signaling, delays cell differentiation, and promotes apoptosis in bone. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S44. [Abstract]

9. Guo J, Liu M, Yang D, Thomas CC, Schipani E, Bringhurst FR, Kronenberg HM. Suppression of canonical wnt signaling by Dkk1 attenuates PTH-mediated peritrabecular stromal cell response and new bone formation in a model of secondary hyperparathyroidism. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S102-S103.

10. O'Brien CA, Galli C, Plotkin L, Vyas K, Cazer P, Goellner JJ, Berryhill S, Webb W, Robling A, Bouxsein M, Schipani E, Turner CH, Weinstein RS, Jilka RL, Manolagas SC, Bellido TM. PTH receptor signaling in osteocytes increases bone mass and the rate of bone remodeling through Wnt/LRP5-dependent and -independent mechanisms, respectively. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S12. [Abstract]

11. Gabet Y, Noh T, Cogan J, Tank A, Sasaki T, Criswell B, Dixon A, Tam J, Kohler T, Segev E, Kockeritz L, Woodgett J, Müller R, Chai Y, Smith E, Bab I, Frenkel B. Crosstalk between androgen receptor and Wnt signaling mediates sexual dimorphism during bone mass accrual. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S35. [Abstract]

12. Kramer I, Halleux C, Brander Weber P, Feng JQ, Boisclair J, Keller H, Kneissel M. Osteocyte-specific ablation of canonical Wnt signaling induces severe osteoporosis. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S12. [Abstract]

13. Terauchi M, Nanes MS, Weitzmann M, Zayafoon M, Weitzmann M, Lane TF, Pacifici R. T cells amplify the anabolic action of PTH through Wnt10b signaling. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S56. [Abstract]

14. Xiong D, Zhao L, Recker RR, Zmuda JM, Deng H. Genome-wide association study (GWAS) and subsequent replication studies identified ADAMTS18 and TGFBR3 as novel osteoporosis risk genes. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S27. [Abstract]

15. Zhao L, Liu X, Liu Y, Wang L, Liu Y, Yan H, Liu J, Xiong D, Pan F, Tang Z, Yang T, Chen X, Guo Y, Lei S, Recker RR, Deng H. Genome-wide association study identified RTP3 as a novel gene for bone strength. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S325. [Abstract]

16. Guo Y, Wang JT, Yang TL, Pan F, Zhang F, Zhang ZX, Liu XG, Zhou Q, Drees B, Hamilton J, Papasian CJ, Recker RR, Deng HW. Genome-wide association study identified novel susceptibility loci for osteoporosis. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S185. [Abstract]

17. Hsu YH, Demissie S, Cho K, Zhou Y, Bianchi E, Ferrari SL, Cupples LA, Karasik D, Kiel DP. Genome-wide association study of BMD and hip geometry indices. The Framingham Osteoporosis Study. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S27. [Abstract]

18. Yang TL, Lei SF, Guo Y, Pan F, Zhou Q, Zhang ZX, Dong SS, Xu XH, Liu XG, Yan H, Drees B, Hamilton J, Papasian CJ, Recker RR, Deng HW. Genome-wide copy number variation study identified a novel susceptibility gene *UGT2B17* for osteoporosis. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S476. [Abstract]

19. Huang QY, Li GY, Kung AC. The -301T/C of sclerostin(SOST) modulates bone mineral density by Wnt and estrogen signaling pathways. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S76. [Abstract]

20. Xiao P, Chen XD, Chen Y, Pan F, Jiang H, Sha BY, Recker RR, Deng HW. *In vivo* differential expression profiling study on human circulating B cells suggested a novel EGFR and CALM3 network underlying smoking and BMD. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S278-S279. [Abstract]

21. Xiao P, Chen XD, Pan F, Chen Y, Jiang H, Sha BY, Recker RR, Deng HW. *In vivo* differential expression profiling study on human circulating monocytes suggested a novel CRAT and CPT2 network underlying smoking and BMD. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S279. [Abstract]

22. Alam I, Sun Q, Liu L, Koller DL, Liu Y, Edenberg HJ, Econs MJ, Foroud T, Turner CH. Genomic expression analysis of rat chromosome 4 for skeletal traits at femoral neck. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S183. [Abstract]

23. Paic F, Wang H, Kronenberg MS, Kuo L, Shin D, Harris SE, Kalajzic I. Comparison of gene expression in osteocytes versus osteoblasts. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S387. [Abstract]

24. Kao RS, Lu W, Louie A, Nissenson RA. Expression profiles of Wnt signaling pathway components in response to cAMP stimulation at different stages of osteoblast maturation. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S383. [Abstract]

25. Benisch P, Schilling T, Klein-Hitpass L, Kassem M, Jakob F, Ebert R. Gene expression analysis of *in vitro*-aged and immortalized human mesenchymal stem cells. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S385. [Abstract]

26. Kapinas K, Kessler CB, Delany AM. Regulation of osteonectin/SPARC by microRNA-29. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S44. [Abstract]

27. Luzi E, Marini F, Tognarini I, Galli G, Falchetti A, Brandi M. Possible physiological function of menin protein as transcriptional factor that modulates microRNA expression during osteogenesis. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S382. [Abstract]

28. Inose H, Kimura A, Iwasaki M, Shinomiya K, Takeda S. The regulation of osteoblast differentiation by microRNA. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S395. [Abstract]

29. Li Z, Hassan MQ, van Wijnen AJ, Stein JL, Croce CM, Lian JB, Stein GS. A program of microRNAs control BMP2 induced bone phenotype development. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S396. [Abstract]

30. Kronenberg MS, Harrison JR, Rowe DW. Mapping the interrelationship of the adipogenic and osteogenic lineage with visual reporters. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S397. [Abstract]

31. Egusa H, Ashida S, Kobayashi M, Akashi Y, Yatani H. DNA methylation analysis of osteogenic gene promoter regions in mouse bone marrow stromal cells. *J Bone Miner Res*. 2008 Sep;23(Suppl 1):S382. [Abstract]