

SNP detection and comparative linkage mapping of 66 bone-related genes in the pig

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Abstract. Osteoporosis is a multigenic complex disorder. Though the mouse and rat are used as experimental models for human osteoporosis, the pig bone remodeling cycle is histologically more similar to human than the rat or mouse. Moreover, livestock genomics have many advantages over model organisms and human studies for complex trait dissection. Hence, in the present work 66 bone-related genes were newly genetically mapped on pig chromosomes. Com-

parative chromosomal patterns of bone-related genes in the pig, human, mouse and rat provide clues that the chromosomal organization of bone-related genes in pigs is more similar to human than that of the mouse and rat. Therefore, the pig can be considered as one of the better models for studying the molecular genetics of bone-related disorders.

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Osteoporosis is a complex bone disorder due to the effect of multiple genes and environment. Millions of humans are affected by osteoporosis. It has been estimated that the direct expenditure on osteoporosis in the U.S. is \$47 million per day (You-Qiang and Ling-Fung, 2004). Osteoporosis is characterized by decreased bone strength and an increased fracture risk. Low bone mineral density (BMD) is the strongest predictor of fracture risk. Many studies in humans and mice have shown that genes contribute 60–80% of BMD variance (Morrison et al., 1994; Klein et al., 1998; Eisman, 1999; Niu et al., 1999; Baldock and Eisman, 2004). For dis-

secting the complex traits, mice and rats have been used as model organisms (Melhus et al., 2007). However, from the genome perspective, alignment of Quantitative Trait Loci (QTL) across species especially between humans and model organisms is difficult, in part because there are significant differences between human and laboratory animals such as smaller conserved genome fragments than the resolution with which QTL are mapped. Therefore, livestock genomics may have many advantages over model organisms and human studies for complex trait dissection. Through livestock genomics genetic variation can be exploited between breeds or divergently selected lines and also within breeds and lines. The best example is the finding of the parent-of-origin QTL affecting muscularity on chromosome 2 in pigs (de Koning et al., 2007).

Among livestock species, the pig can be the best model for human complex traits including osteoporosis. The digestive physiology, kidney structure and function, pulmonary vascular bed structure, coronary artery distribution, respiratory rates, cardiovascular anatomy and general physiology have large similarities between pigs and humans. Hence the pig was considered as a potential xenograft donor

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for organ transplantation in humans (Sachs, 1994). The bone remodeling cycle is histologically similar between human and pigs (Hittmeier et al., 2006). In addition, the characteristics of porcine osteoclast formation support that the pig is an alternative mammalian model to study osteoclast differentiation and resorption activity (Sellsgalvin et al., 1996). Moreover, the occurrence of osteoporosis varies between different races and genders. Females have more occurrence than males. Similarly in pigs there are differences of leg and foot problems between breeds and sexes. Landrace has more occurrence of bone fractures than other breeds, and females have more leg problems during farrowing (Huang et al., 1995).

In pig herds the second largest cause for culling mature breeding females is feet and leg structure soundness. Unsoundness of feet and leg influences the reproductive performance and health of sows and boars (Wood, 2001). Feet and leg soundness depends on the joint angularity in legs (Draper et al., 1988), skeletal morphogenesis, bone mineral density and remodeling cycle of bones (Massaro and Rogers, 2004). These structural and functional attributes of dynamic bone growth are determined by the influence of both intrinsic (chemical) signals and extrinsic (mechanical) signals. For instance, secreted cell-cell signaling molecules WNT, hedgehog and BMP directly act at a distance and induce distinct differentiation programs during limb development (Bilezikian et al., 2002). In addition, QTLs have been found for differences in femur dimensions on pig chromosomes 2, 4, 16 and 17 and for osteochondrosis on chromosomes 5, 13 and 15 (Andersson-Eklund et al., 2000). Similarly, the QTLs related to gait and front leg pasterns were found on pig chromosomes 8 and 9 (Lee et al., 2003). It was reported by FDA (<http://www.fda.gov/womens/getthefacts/osteoporosis.html>) that people with small frames are more prone to osteoporosis. Osteoporosis is also more common in modern pig industry animals especially in the first litter gilts (<http://www.thepigsite.com/diseaseinfo/78/osteoporosis>). The incidence of feet and leg problems in pigs and their similar physiology to human bone suggest that researchers consider selecting the pig as a better model for human osteoporosis. Hence the mapping of genes related to bone physiology is an essential prerequisite to conduct further studies in pigs. Thus in the present paper a total of 66 genes (of which 46 were not previously mapped) related to bone physiology were genetically mapped in the pig.

Materials and methods

Population

The Iowa State University (ISU) Berkshire × Yorkshire (BY) resource family (n = 572) was used for gene mapping (Malek et al., 2001).

Selection of genes

Genes involved in skeletal pattern development, bone matrix biosynthesis, mineral metabolism, osteoclast and osteoblast differentiation were selected (Table 1). In addition, some genes that play a role in the BMP, IGF1, TGF β , FGF, cortisol, estradiol, WNT, calcitonin, reti-

noic acid and interleukins signaling pathways, were also selected. In total 66 genes were selected.

Genotype determination

DNA was isolated from ear tissue using the DNeasy 96 Blood and Tissue Kit (QIAGEN sciences, Maryland, USA). In order to discover single nucleotide polymorphisms (SNPs), PCR was done by standard protocols for all selected genes. The PCR products of three animals were pooled, and the pooled PCR sample was sequenced (DNA facility, Iowa State University, Ames, IA, USA). The sequence was analyzed by Sequencher 4.6 software (Gene Codes, Ann Arbor, MI, USA). Once an SNP was determined, the SNP genotyping for the entire population was done by mass array iPLEXy platform (Sequenom, Inc., CA USA). The PCR primers, SNPs and SNP accession numbers are shown in Table 2.

Gene mapping

The gene mapping was performed by linkage mapping using CRIMAP software with the genotype data obtained from the BY resource family (Malek et al., 2001). The comparative locations of these genes in the human, mouse and rat were obtained from the NCBI data base.

Results

The gene mapping locations and their linkage distances from each other can be seen in Fig. 1. The comparative locations of the mapped genes in human, mouse and rat are presented in Table 3. In total 66 bone or bone-related genes were mapped. Most of them were assigned to chromosomal regions that are homologous to human gene locations. However, the gene *ESR2* was mapped to chromosome 1. It is located on HSA14q23.2 and was supposed to be mapped on SSC7. Of these genes, 46 were newly genetically mapped in the present work. The remaining 20 genes were mapped earlier, generally by physical mapping. For instance *VDR*, *TNFI* and *COL9A1* had been assigned by physical mapping techniques such as FISH mapping and radiation hybrid mapping (Chardon et al., 1991; Pinton et al., 2000). The results presented here confirmed the location of the 20 genes. In addition, mapping results of these new 46 genes will contribute to fine mapping of QTL associated to feet and leg soundness traits.

Discussion

In this research 46 new genes and 20 previously mapped genes were mapped on all pig chromosomes except chromosome 10 and sex chromosomes (Fig. 1). The comparative analysis of the mapped genes revealed that the distribution of bone-related genes in the pig is widespread and homologous to human chromosomes as compared to mice and rats. For instance, most of the bone genes located on homologous human chromosomes 12, 4, 7, 13 and 3 are mapped on pig chromosomes 5, 8, 9, 11 and 13. These genes are distributed on many more different locations in the mouse and rat genomes (Table 3). Therefore the genetic organization of bone-related genes in pig may give more insight into understanding osteoporosis in humans.

Table 1. Selected genes and their functions in bone biology ordered by pig chromosome

Gene name	Symbol	Function in bone biology
Ectonucleotide pyrophosphatase 1	<i>NPP1</i>	Hydrolyzes pyrophosphate or phosphodiester bonds in (di)nucleotides and their derivatives to the components of bone minerals (Stefan et al., 2005).
Collagen 9A1	<i>COL9A1</i>	A bone matrix protein stabilizes the packing of the other fibrillar collagens such as types II and XI (Blaschke et al., 2000).
Melanocortin 4 receptor	<i>MC4R</i>	Inactivation of MC4R indirectly enhances the CART expression which regulates bone resorption leading to high bone density (Ahn et al., 2006).
MAD homolog 3	<i>SMAD3</i>	Enhances the levels of alkaline phosphatase, mineralization and bone matrix proteins such as type I collagen (COL 1), Osteopontin (OPN) and matrix Gla protein in MC3T3E1 osteoblastic cells (Kaji et al., 2006).
Estrogen receptor 2	<i>ESR2</i>	For estrogen's functional role, <i>ESR2</i> is a strong candidate gene for bone mineral density (BMD). A dinucleotide (CA) repeat polymorphism in the <i>ESR2</i> gene was considered to be associated with BMD (Ichikawa et al., 2005).
Pregnancy-associated plasma protein A	<i>PAPPA</i>	Promotes osteoblast proliferation by increasing IGF bioavailability due to its protease activity on IGF binding proteins 2, 4 and 5 (Qin et al., 2006).
TNF receptor associated factor 6	<i>TRAF6</i>	The strength of TRAF6 signaling determines the osteoclastogenesis (Kadono et al., 2005).
Calcitonin/calcitonin related peptide alpha	<i>CALCA</i>	Produces calcitonin (CT) and calcitonin related peptide alpha (CGRP α). CT is a hypocalcaemic hormone affecting bone resorption and CGRP is a neuropeptide regulating sympathetic and vascular tone (Huebner et al., 2008).
ADAM metalloproteinase with thrombospondin type1 motif 2	<i>ADAMTS2</i>	A procollagen proteinase which cleaves the amino propeptides during the processing of Type I and Type II procollagens to collagens (Tang, 2001).
Cartilage oligomeric protein	<i>COMP</i>	Promotes chondrocyte proliferation, and the serum level of COMP is the marker for Chondromalacia patellae (Xu et al., 2007).
Collagen 23A1	<i>COL23A1</i>	Transmembrane collagen involved in phosphate transport (Banyard et al., 2003).
Transforming growth factor beta 1	<i>TGFB1</i>	Expressed in osteoblasts and involved in the formation as well as resorption of bone. It was one of the first suggested candidate genes for osteoporosis (Langdahl et al., 2008).
Nucleotide sugar transporter	<i>NST</i>	The gene encodes endoplasmic reticulum nucleotide sugar transporter that might transport substrates needed for biosynthesis of chondroitin sulphate, a proteoglycon having a critical role in chondrogenesis and skeletal development (Hiraoka et al., 2007).
Interleukin 1 alpha	<i>IL1A</i>	IL1 α markedly stimulates bone resorption by mature osteoclasts through the activation of nuclear factor κ B (Miyazaki et al., 2000).
Matrilin 3	<i>MATN3</i>	A non collagenous extracellular matrix protein involved in modulating chondrocyte differentiation during embryonic development, in controlling bone mineral density in adulthood and preventing osteoarthritis during aging (Vander-Weyden et al., 2006).
Osteoprotegerin	<i>OPG</i>	A potent inhibitor of bone resorption. It prevents osteoclast differentiation by binding and inactivating OPG ligand (Udagawa et al., 2000).
Duffy blood group chemokine receptor	<i>DARC</i>	Regulates osteoclastogenesis by blocking the availability of chemokines such as ccl2 and ccl5 (Edderkaoui et al., 2007).
Colony stimulating factor 1	<i>CSF1</i>	Promotes osteoclastogenesis by synergistic action with RANKL (Pixley and Stanley, 2004).
Wingless related MMTV integration site 7B	<i>WNT7B</i>	Induces osteoblast differentiation in vitro via PkC δ mediated pathway (Tu et al., 2007).
Wingless related MMTV integration site 10 B	<i>WNT10B</i>	Blocks PPARG and stimulates the osteoblast pathway. High expression of this gene in bone marrow directly increases bone mass and density in experimental mice (Bennett et al., 2005).
SP7 transcription factor	<i>SP7</i>	A transcription factor required for osteoblast differentiation and bone formation (Milona et al., 2003).
Parathormone like peptide	<i>PTH1H</i>	PTH1H maintains the chondrocytes in proliferative pool. It increases chondrocyte proliferation but inhibits chondrocyte maturation in endochondral development (Provot et al., 2008).
Interferon gamma	<i>IFNG</i>	Inhibits bone resorption by inhibiting the ability of 1,25 (OH) $_2$ VitD3, PTH, and IL-1 to stimulate the formation of osteoclast-like cells (Takahashi et al., 1986).
Vitamin D receptor	<i>VDR</i>	With its ligand it is a potent regulator of osteocalcin gene expression during differentiation of primary human osteoblasts (Siggeknaw et al., 1999).
Collagen 2A1	<i>COL2A1</i>	It is the major cartilage matrix protein (Horton et al., 1992).
Matrix metalloproteinase 2	<i>MMP2</i>	One of the biomarkers for bone remodeling. Is involved in initiation of bone resorption by degrading the unmineralized osteoid layer of the bone surface to allow osteoclasts to attach to the mineralized matrix (Tournaire et al., 2007).
Fat mass and obesity associated	<i>FTO</i>	Obesity risk allele (Frayling et al., 2007).
Methylene tetrahydrofolate reductase	<i>MTHFR</i>	Mutations in MTHFR leads to high blood homocysteine levels which interfere in the cross linking of collagens in bone matrix (Miyao et al., 2000).
Cartilage matrix protein	<i>CMP</i>	An extracellular matrix protein of non-articular cartilage (Saxne and Heinegård, 1989).
Collagen IX A2	<i>COL9A2</i>	A structural component of the extracellular matrix in cartilaginous tissues (Takahashi et al., 2006).
Tumor necrosis factor-alpha	<i>TNFalpha</i>	Promotes bone resorption by osteoclastogenesis (Boyce et al., 2005).
Collagen X1 A2	<i>COL11A2</i>	Extracellular matrix protein in cartilage (Burgeson et al., 1979).

Table 1 (continued)

Gene name	Symbol	Function in bone biology
Vitamin D binding protein	<i>HDBP</i>	HDBP promotes bone resorption by inhibiting extracellular calcium sensing of osteoclasts (Adebanjo et al., 1998).
Nocturnin	<i>NOCT1</i>	A PPARG responsive 'Clock' gene. It is critical for skeletal and metabolic homeostasis (Rosen, 2007).
Fibroblast growth factor 2	<i>FGF2</i>	FGF2 reduces chondrocyte hypertrophy during chondrocyte differentiation. At low doses it promotes BMP-2 induced ectopic bone formation in mice (Nakamura et al., 2005).
Bone morphogenic protein receptor 1B	<i>BMPRI3</i>	It mediates GDF5 signaling that is essential for chondrocyte condensation, differentiation and formation of joints and digits (Demirhan et al., 2005).
Osteopontin	<i>OPN</i>	OPN is an acidic bone matrix phosphoprotein produced by osteoblasts and osteoclasts. It is required for osteoclast differentiation and bone resorption (Asou et al., 2001).
Matrix extracellular phosphoglycoprotein with ASARM motif (bone)	<i>MEPE</i>	MEPE promotes renal phosphate excretion and modulates mineralization (Rowe et al., 2004).
Calcitonin receptor	<i>CALCR</i>	It mediates the actions of calcitonin which is hypocalcaemic hormone (Purdue et al., 2002).
Collagen 1A2	<i>COL1A2</i>	Major structural protein in bone (Suuriniemi et al., 2003).
Interleukin 6	<i>IL6</i>	Stimulates osteoclast formation (Tamura et al., 1993).
Secreted friggled related protein 4	<i>SFRP4</i>	SFRP4 antagonizes WNT β -catenin signaling pathway that regulates bone mineral density (BMD) (Fujita et al., 2003).
KLOTHO	<i>KLOTHO</i>	The exact function of Klotho is yet unknown. It is thought to restrict aging and is associated with bone mineral density (Zarrabeitia et al., 2007).
Leucine rich repeats and calponin homology (CH) containing domain 1	<i>LRCH1</i>	LRCH1 is involved in chondrocyte responses to pressure through interaction with cytoskeleton (Spector et al., 2006).
Receptor activator of NF-kappa-B ligand	<i>RANKL</i>	RANKL was produced from osteoblasts and causes the differentiation of osteoclasts (Suda et al., 1999).
Vitronectin	<i>VTN</i>	A bone matrix protein (Seiffert, 1996).
Beta catenin	<i>CTNNB1</i>	A signaling molecule in WNT signaling pathway (Akiyama, 2000).
Parathormone receptor	<i>PTHr</i>	PTHr mediates the actions of PTH which promotes bone resorption (Mannstadt et al., 1999).
Peroxisome proliferator-activated receptor gamma	<i>PPARG</i>	PPARG negatively regulates osteoblast differentiation of bone marrow stromal cells and positively promotes adipogenesis resulting in bone loss (Marie and Kaabeche, 2006).
Oxytocin receptor	<i>OXTR</i>	OXTR mediates the action of oxytocin, an anabolic agent for bone turnover through PGE2 synthesis (Copland et al., 1999).
Calcium sensing receptor	<i>CASR</i>	Regulates calcium homeostasis by acting as calciostat. It senses the decrease in extracellular calcium level and effecting PTH secretion and calcium reabsorption from kidney (Pidashva et al., 2006).
Gamma glutamyl transferase 1	<i>GGT1</i>	GGT1 is a potential marker for bone resorption. It acts as a pathological bone resorbing factor by stimulating RANK ligand (Asaba et al., 2006; Niida et al., 2004).
A disintegrin and metalloproteinase domain 12	<i>ADAM12</i>	Stimulates bone growth in mice by modulating chondrocyte proliferation and maturation through mechanisms probably involving both metalloproteinase and adhesion activities (Kveiborg et al., 2006).
Fibroblast growth factor receptor 1	<i>FGFR1</i>	FGFR1 mediates the action of FGFs and upregulates Cbfa1 which causes proliferation and differentiation of osteoblast cells (Zhou et al., 2000).
Neurophilin 2	<i>NRP2</i>	Neurophilin 2 is a potent receptor for VEGF which is essential for angiogenesis, a required process for bone formation (Sena et al., 2007).
Insulin-like growth factor binding protein 2	<i>IGFBP2</i>	IGFBP2 regulates long bone growth by counteracting the stimulatory effects of co-localized IGF1 (Fisher et al., 2005).
Indian hedgehog	<i>IHH</i>	It controls chondrocyte differentiation in growth plate by regulating PTH-related peptide expression (Vortkamp et al., 1996).
Ankylosis homolog	<i>ANKH</i>	Modulates pyrophosphate transport in chondrocytes. Mutations in this gene lead to chondrocalcinosis (Netter et al., 2004).
Phosphodiesterase 4D	<i>PDE4D</i>	PDE4 reduces cAMP and promotes TNF α production thereby facilitating bone resorption (Kinoshita et al., 2000).
Secreted protein, acidic, cysteine-rich (osteonectin)	<i>SPARC</i>	It is a calcium, hydroxyapatite and collagen binding protein, implicated in cell proliferation, tissue morphogenesis, and repair and remodeling of extracellular matrix (Motamed, 1999).
Matrix metalloproteinase 9	<i>MMP9</i>	It is a key enzyme produced by osteoclasts, and it is involved in bone resorption (Lu and Rabie, 2006).
Bone morphogenic protein 7	<i>BMP7</i>	A potent osteoprogenitor (Toyran et al., 2005).
Guanine nucleotide binding protein, alpha stimulating complex locus	<i>GNAS</i>	Encodes stimulatory G protein alpha. Mutations in this gene lead to Albright hereditary osteodystrophy and progressive osseous hyperplasia (Germain-Lee et al., 2005; Chan et al., 2004).
Endothelial nitric oxide synthase 3	<i>ENOS</i>	ENOS produces nitric oxide which modulates both bone resorption and formation (Van't Hof et al., 2000).
Wingless gene 16	<i>WNT16</i>	Has a role in the induction of synovial joint development (Guo et al., 2004).

Table 2. Gene symbol, amplicon location, primers and SNP information

Gene	SSC	Amplicon location	Primers (5'-3')	Product size (bp)	SNP	SNP location at bp	SNP Accession number
<i>NPP1</i>	1	Exon 10	CAGTGCCATTTGAAGAGAGG CTGCTGACTGGTCCGTATGA	427	G/T	199	NCBI_ss 86352339
<i>COL9A1</i>	1	Intron 4	CAGTGCCATTTGAAGAGAGG CTGCTGACTGGTCCGTATGA	586	A/C	100	NCBI_ss 86352089
<i>MC4R</i>	1	Exon 1	CAGGTCAGAGGGGATCTCAA GTGCAGACTGCCAGATACA	548	A/G	283	NCBI_ss 86352451
<i>SMAD3</i>	1	Exon 1	GCCATGTCTGCCATCTCTG GGTGATGCACTTGGTGTGA	201	A/G	143	NCBI_ss 86352383
<i>ESR2</i>	1	Exon 5	AAAATACTGATACCCACCCACAT CGCCACATCAGCCCCACCAT	218	T/C	139	NCBI_ss 86352376
<i>PAPPA</i>	1	Exon 2	GTGGTCAACCTCCACGATG CAGTTCCTCGTCCCCGATCT	203	A/G	68	NCBI_ss 86352425
<i>TRAF6</i>	2	Intron 4	CCATCATCTTAGCACCCCTCA ACCACAAGAACCCTGTCTCC	501	A/T	250	NCBI_ss 86352262
<i>CALCA</i>	2	Intron 2	TCCTGGCTTTCAGCATCTT AGTGAGAAGGAAAGGTGCGCT	383	C/G	187	NCBI_ss 86352250
<i>ADAMTS2</i>	2	Exons 20-21	CATGCCACCTTCACATACG TACTTGACGAGGGCTTCTGG	1236	C/T	1154	NCBI_ss 86352454
<i>COMP</i>	2	Exon 6	CTTCCGCTGTGAGGCTTG CTCACAGCGCTTCTGTCCAG	619	A/G	434	NCBI_ss 86352315
<i>COL23A1</i>	2	Intron 2	TCCTTTCCTCCACCAAAGA ACAGTTCGGAAGCACCAT	502	A/C	309	NCBI_ss 86352457
<i>TGFBI</i>	2	Intron 9	ATGAGCGGTTGACTCTCCTG CCAGAGTGTCCAGGGTCTGT	700	C/T	144	NCBI_ss 86352468
<i>NST</i>	2	Exon 3	CTTCTAGCCTTCGCTGTGGA CGGAGGCAGAGGCAGTAGA	580	T/C	92	NCBI_ss 86352427
<i>IL1A</i>	3	3'UTR	TCCTGGGTCCATAGCTTCTC CCGCCATCCAAAGTTGT	497	C/T	145	NCBI_ss 86352273
<i>MATN3</i>	3	Intron 2	GGGTGGCAGTGGTGAACATA TCCGACTCTAACCTCCTTCC	470	A/G	434	NCBI_ss 86352214
<i>OPG</i>	4	Exon 4	CCTGTGTGAGGAGGCGTTC GGAAGTGGAGGCAGTGAGAA	419	A/G	137	NCBI_ss 86352133
<i>DARC</i>	4	Exon 3	CACTCCTGTAGCCTGCTTGAC ACCCAGCACCTTCTTCAGC	555	A/G	118	NCBI_ss 86352330
<i>CSF1</i>	4	Exon 6	CCTGATTGCAACTGCCTGTA ATCAGTGCCAGCATAGAGC	348	G/C	259	NCBI_ss 86352345
<i>WNT7B</i>	5	Intron 1	TACTGGCACTCGTTGATGC CGGGGTCTGTGTCTGATT	473	A/G	238	NCBI_ss 86352476
<i>WNT10B</i>	5	Intron 4	GGAAGCGACAATGCCAGAT ATCAGAGGTGACGGGGAAG	435	A/G	117	NCBI_ss 86352479
<i>SP7</i>	5	Exon 1	TGCGGGACTCAACAACCTCT CTGGACCTGTGGGCAGTGT	462	C/T	186	NCBI_ss 86352256
<i>PTH1H</i>	5	Intron 2	CCACCACAACCTCTGAAAACC GAGGGGGAGGAAACAATGAC	386	C/T	111	NCBI_ss 86352176
<i>IFNG</i>	5	Exon 1	CTGGGCCTGATCGACTGTAT GCCAAAGCTTTAAGGACCTC	622	G/T	553	NCBI_ss 86352489
<i>VDR</i>	5	Intron 8	ACCAGATCGTGCTGCTGAAG GGGAGACGATGCAGATGG	279	C/T	316	NCBI_ss 86352119
<i>COL2A1</i>	5	Intron 11	AGGTGAAGGTGGGAAACCAG CATCCGTGCCAGGAGTTC	600	C/T	372	NCBI_ss 86352100
<i>MMP2</i>	6	5'UTR	AAAATGCTCTTCAGGCAGGA AGGAGATGGGACTGGGAGTT	509	C/G	298	NCBI_ss 86352465
<i>FTO</i>	6	Intron 6	GTGCCCCGTGCTTTTCTCTTA TTACCGCACCTTCCATCTTC	545	A/G	143	NCBI_ss 86352280
<i>MTHFR</i>	6	Exon 2	GCTCGGACAAGGAGACCT ATCAGACGCCAGCCAGT	407	A/G	122	NCBI_ss 86352185
<i>CMP</i>	6	Intron 7	AGGACGCTCTTGTGGAGTT GCTTCTGCTTTGGAGTCAGT	496	A/G	380	NCBI_ss 86352203
<i>COL9A2</i>	6	Intron 31	GGACAGGGGAGAAGTTGGAC GCAGAGGCGTAGGCTGAG	639	C/T	105	NCBI_ss 86352243
<i>TNFa</i>	7	Intron 1	CTGGGGGAAAGAAGTGTGC AAGGGAAAACAGCCACCTG	463	G/T	131	NCBI_ss 86352149
<i>COL11A2</i>	7	Intron 60	CTTCGGGAGAGATTGAGCAG GCTGACACCCTGTATTGG	423	A/T	315	NCBI_ss 86352190
<i>WARS</i>	7	Intron 5	CCCTTGTTCCCTGGTGACT GGCCACCACCACTGATTAAC	363	C/T	214	NCBI_ss 86352417

Table 2 (continued)

Gene	SSC	Amplicon location	Primers (5'-3')	Product size (bp)	SNP	SNP location at bp	SNP Accession number
<i>HDBP</i>	8	Intron 4	GCTTGCCCATCCCTGTAAAT GTGTCAGAGGAGCTTGTCCA	548	C/T	290	NCBI_ss 86352124
<i>NOCT1</i>	8	Exon 1	TGCCCTCTGGAAGCACTC GACCTCCTCTGTTGGCTCTG	507	C/T	271	NCBI_ss 86352276
<i>FGF2</i>	8	Exon 1	TGAGTATTCGGCAACAGCAC TTGTCTGGCAGTTCCTTGTG	307	G/A	76	NCBI_ss 86352327
<i>BMPR1B</i>	8	Intron 7	CCTGCTGTGCTTACTGCTTG CCCCCTGTTGAATGTCTTCT	501	G/T	175	NCBI_ss 86352480
<i>OPN</i>	8	Promoter	GAACGCTCTGCTTCTCTTGG GTCAGTGTGCTGGAGGGAAG	412	A/G	277	NCBI_ss 86352322
<i>MEPE</i>	8	Exon 5	GGTGAAACCTCCTGTTGGAA CAGCAGTGTGGAGCTGAAA	359	G/A	310	NCBI_ss 86352394
<i>CALCR</i>	9	3'UTR	TTCTCCTGGCCTGCCTTC TCTGCCTGACACTGAACCAT	424	C/T	100	NCBI_ss 86352113
<i>COL1A2</i>	9	Exon 3	GTGGTGAAGTGGGTCTTCCA ACCAACAGGGCCAGGAATA	113	C/T	130	NCBI_ss 86352087
<i>IL6</i>	9	Intron 2	GTGCCATTCTCCACTTGT AAATCCCAACACCGAGGA	486	C/T	279	NCBI_ss 86352128
<i>SFRP4</i>	9	Intron 3	AAGGCAGAACCAGACTAAGC TACCCCAACCAAGGCATCTA	598	A/G	217	NCBI_ss 86352472
<i>KLOTHO</i>	11	Exon 4	CCACGTCAAGTCTGGATCTC CCCGATTAGCTTTTTCTC	357	C/T	145	NCBI_ss 86352189
<i>LRCH1</i>	11	Intron 2	GTGCTCGTGATTCCCATTG GCTGAAGGCTGTGGCGTAG	402	C/T	148	NCBI_ss 86352234
<i>RANKL</i>	11	Intron 5	TCCTTTGGGGTGAACCT GGATGTCTGTGGCGTTGAT	410	A/G	114	NCBI_ss 86352129
<i>VTN</i>	12	Exons 3-6	AGCGTCCAAGAAGAGCAGAG AGGGCAAAGTGTGCAAAGAC	1123	A/G	268	NCBI_ss 86352325
<i>CTNNB1</i>	13	Exons 9-10	TTCTTGGGACCCTTGTTCAG ATGAGCACGAACCAGCAAC	702	G/C	317	NCBI_ss 86352341
<i>PTHR</i>	13	Intron 12	ATTCAGGCTCAGGATGTGCT TTCAGCCTGTACCTGGGATG	350	A/G	174	NCBI_ss 86352174
<i>PPARG</i>	13	Promoter	AACCCCAAACCTCTCAGTCCA GGTGAAGGCACCAATGAGTC	570	G/A	302	NCBI_ss 86352388
<i>OXTR</i>	13	Intron 11	TTCGCTTCCACTTACCTGCT GTCCCAGACGCTCCACAT	540	G/T	161	NCBI_ss 86352270
<i>CASR</i>	13	Intron 6	CCCCTTATCTCAGCATCTCC CCACTCAAAGCAGCAGTTGG	621	C/T	197	NCBI_ss 86352179
<i>GGT1</i>	14	Exon 10	TACCGTGCAGAGCTGATTGA TGGTAGGTCAGGCCTTTCTG	700	C/G	449	NCBI_ss 86352433
<i>ADAM12</i>	14	Intron 20	TGCTGCGTCTGTGCTCTCA GCACCTCGTCCAAGTTCAG	538	C/T	151	NCBI_ss 86352230
<i>FGFR1</i>	15	Exon 13	TGTGGAGTATGCCTCCAAGG TTCAGGCACCACACCTTCT	189	G/C	129	NCBI_ss 86352342
<i>NRP2</i>	15	Intron 2	CTCTTCCAGATCCACCCTGT ATTTTAGGGGCGCTGCTCTA	429	C/G	276	NCBI_ss 86352299
<i>IGFBP2</i>	15	Intron 2	GGAACCTTGCTCACCTTGTG CATACCTGGGCTTCTCCTG	361	A/G	167	NCBI_ss 86352411
<i>IHH</i>	15	Exon 3	GAGACCGAGTGTGGCTTT CATGCCCAGTGAGTGAAG	546	G/A	499	NCBI_ss 86352349
<i>ANKH</i>	16	Intron 11	TCCTGTTCTTGGCACCTTTC GACTGGGCATCGTCTGTGT	431	C/T	52	NCBI_ss 86352216
<i>PDE4D</i>	16	Intron 2	CTATGTCCCGAACCTCCTCA TTGGTGCTTGCCTCCAGT	547	G/T	358	NCBI_ss 86352171
<i>SPARC</i>	16	3'UTR	CCTGGGGACAAGGTGCTAAT AGGGGAAGTGGTGAGGAACT	504	C/T	139	NCBI_ss 86352238
<i>MMP9</i>	17	Exon 6	TATGGCTTCTGCCCTACCC AGGAAGGTGAAGGGGAGAC	485	G/C	281	NCBI_ss 86352323
<i>BMP7</i>	17	Intron 3	CCTCCTTTCCGTCAGTGC GCAGGTTTGAAGCGGTAGG	558	A/G	438	NCBI_ss 86352286
<i>GNAS</i>	17	Intron 8	AAGCAGGCTGACTACGTG TCACCACAAGGGCTACCA	321	A/T	253	NCBI_ss 86352249
<i>ENOS</i>	18	Intron 12	GATTCTGGGGTTTCTCTTGG CCCTCACTGCCATCACTTCT	533	A/T	143	NCBI_ss 86352296
<i>WNT16</i>	18	Exons 1-2	GAGGGTCAAGAGGAGTGG GGTTTCTCTTGCACAGCTC	403	C/T	246	NCBI_ss 86352423

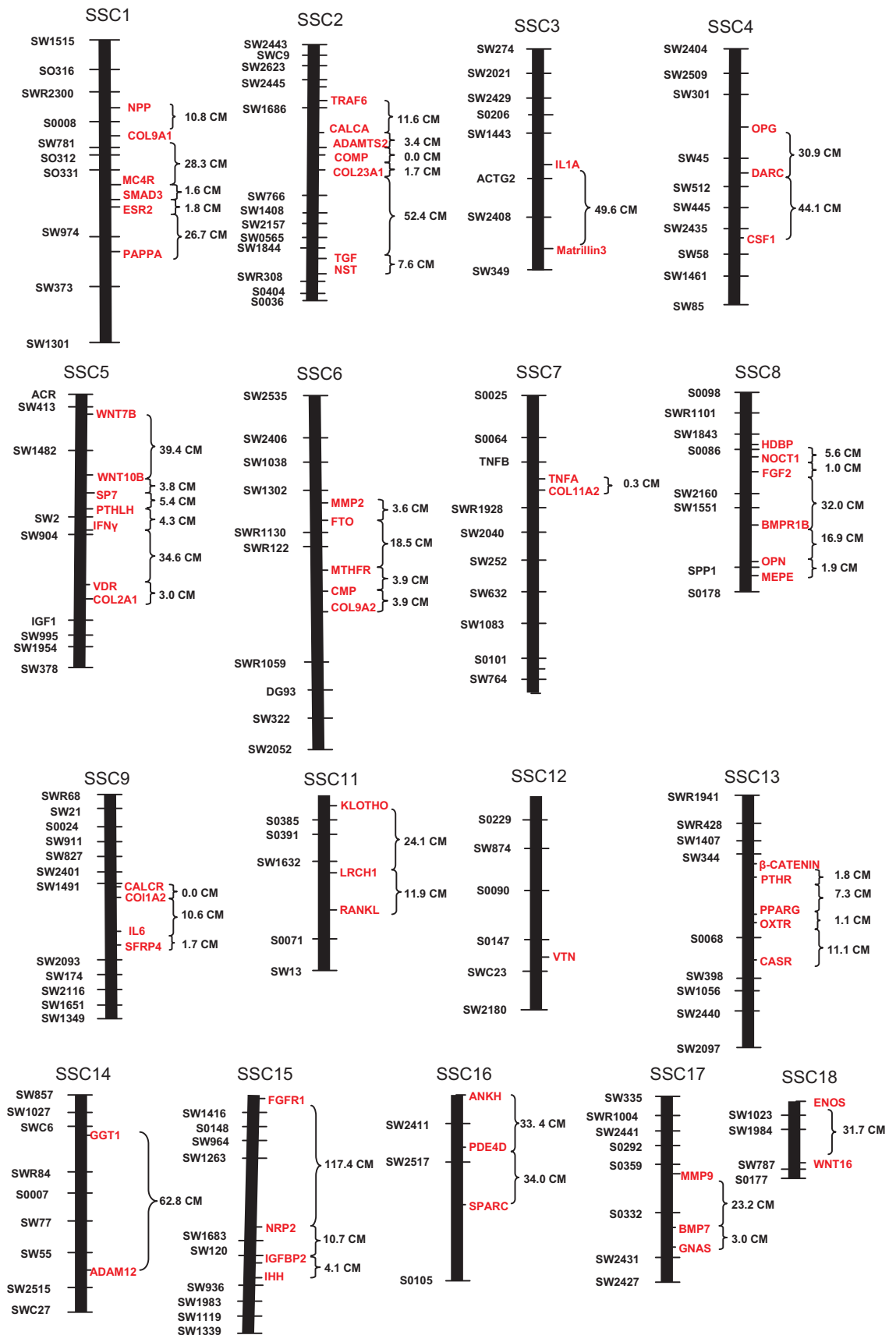


Fig. 1. Mapping of bone-related genes on pig chromosomes. The mapped genes are displayed on the right hand side of each chromosome. For clear illustration the chromosomes are not scaled to real length.

Table 3. Comparative mapping of bone-related genes in the pig, human, rat and mouse

Gene symbol	Chromosomes				Gene symbol	Chromosomes			
	Pig	Human	Mouse	Rat		Pig	Human	Mouse	Rat
<i>NPP1</i>	1	6p21.10	10	1	<i>NOCT1</i>	8	4q31.1	9	8
<i>COL9A1</i>	1	6q13.00	1	9	<i>FGF2</i>	8	4q27.0	3	2
<i>MC4R</i>	1	18q21.32	18	18	<i>BMPR1B</i>	8	4q22.3	3	2
<i>SMAD3</i>	1	15q22.33	9	8	<i>OPN</i>	8	4q22.1	5	14
<i>ESR2</i>	1	14q23.20	12	6	<i>MEPE</i>	8	4q22.1	5	14
<i>PAPPA</i>	1	9q33.10	4	5	<i>CALCR</i>	9	7q21.3	6	4
<i>TRAF6</i>	2	11p12.00	2	3	<i>COL1A2</i>	9	7q21.3	6	4
<i>CALCA</i>	2	11p15.20	7	1	<i>IL6</i>	9	7p15.3	5	4
<i>ADAMTS2</i>	2	5q35.30	11	10	<i>SFRP4</i>	9	7p14.1	13	17
<i>COMP</i>	2	19q13.11	8	16	<i>KLOTHO</i>	11	13q13.1	5	12
<i>COL23A1</i>	2	5q35.30	11	10	<i>LRCH1</i>	11	13q14.3	14	15
<i>NST</i>	2	22q13.20	18	18	<i>RANKL</i>	11	13q14.11	14	15
<i>IL1A</i>	3	2q13.00	2	3	<i>VTN</i>	12	17q11.2	11	10
<i>MATN3</i>	3	2p24.10	12	6	<i>CTNNB1</i>	13	3q22.1	9	8
<i>OPG</i>	4	8q24.12	14	7	<i>PTHrP</i>	13	3p21.31	8	8
<i>DARC</i>	4	1q23.20	1	13	<i>PPARG</i>	13	3p25.2	6	4
<i>CSF1</i>	4	1p13.30	3	18	<i>OXTR</i>	13	3p25.3	6	4
<i>WNT7B</i>	5	22q13.31	15	7	<i>CASR</i>	13	3q21.1	16	11
<i>WNT10B</i>	5	12q13.20	15	7	<i>GGT1</i>	14	22q11.23	10	20
<i>SP7</i>	5	12q13.13	15	7	<i>ADAM12</i>	14	10q26.2	7	1
<i>PTHrP</i>	5	12p11.22	6	4	<i>FGFR1</i>	15	8p12.0	8	16
<i>IFNG</i>	5	12q15.00	10	7	<i>NRP2</i>	15	2q33.3	1	9
<i>VDR</i>	5	12q13.11	15	7	<i>IGFBP2</i>	15	2q35.0	1	9
<i>COL2A1</i>	5	12q13.11	15	7	<i>IHH</i>	15	2q35.0	1	9
<i>MMP2</i>	6	16q12.20	8	19	<i>ANKH</i>	16	5p15.2	15	2
<i>FTO</i>	6	16q12.20	8	16	<i>PDE4D</i>	16	5q11.2	13	2
<i>MTHFR</i>	6	1p36.22	4	5	<i>SPARC</i>	16	5q33.1	11	10
<i>CMP</i>	6	1p35.00	4	5	<i>MMP9</i>	17	20q13.12	2	3
<i>COL9A2</i>	6	1p34.20	4	5	<i>BMP7</i>	17	20q13.31	2	3
<i>TNFalpha</i>	7	6p21.30	17	20	<i>GNAS</i>	17	20q13.32	2	3
<i>COL11A2</i>	7	6p21.32	17	20	<i>ENOS</i>	18	7q36.10	5	4
<i>HDBP</i>	8	4q13.3	14	14	<i>WNT16</i>	18	7q31.31	6	4

Specially, pig chromosome 1 harbors several genes which are located on different homologous portions of different chromosomes in human, mice and rat (Table 3). These genes are involved in neuroendocrine regulation (*MC4R*) of bone, TGF β signaling pathway (*SMAD3*) and steroid signaling (*ESR2*). As these three genes are located on a single chromosome (SSC1) with an estimated linkage distance of 3.4 cM (Fig. 1), it may be useful to study this region's effect on bone-related traits rather than focusing on multiple chromosomes in mice and the rat. Similarly, the genes β -catenin, *PTHrP*, *PPARG* and *OXTR* are located on different chromosomes of mice and rats. They were mapped closely together on pig chromosome 13 similar to human chromosome 3. The pig chromosome 13 bears QTL for osteochondrosis (Andersson-Eklund et al., 2000). These observations may suggest that the pig chromosomal organization may give better clues to understanding the complexity of human bone disorders, and hence mapping of such genes should be useful for further future studies.

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