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Feature Review

Osteoporosis and Bone Mass Disorders: From Gene Pathways to Treatments

Fernando Rivadeneira[1,*,@] and Outi Mäkitie[2,3]

Genomic technologies have evolved rapidly contributing to the understanding of diseases. Genome-wide association studies (GWAS) and whole-exome sequencing have aided the identification of the genetic determinants of monogenic and complex conditions including osteoporosis and bone mass disorders. Overlap exists between the genes implicated in monogenic and complex forms of bone mass disorders, largely explained by the clustering of genes encoding factors in signaling pathways crucial for mesenchymal cell differentiation, skeletal development, and bone remodeling and metabolism. Numerous of the remaining discovered genes merit functional follow-up studies to elucidate their role in bone biology. The insight provided by genetic studies is serving the identification of biomarkers predictive of disease, redefining disease, response to treatment, and discovery of novel drug targets for skeletal disorders.

Trends

GWAS and whole-exome sequencing studies have revolutionized the identification of genetic determinants of monogenetic and complex conditions including osteoporosis and bone mass disorders.

GWAS pinpoint factors in pathways crucial to bone biology (WNT, NOTCH, INDIAN HEDGEHOG signaling), which are currently targets of drug compounds for the treatment of osteoporosis and bone mass disorders.

Given the hypothesis-free nature of these genomic screens, functional follow-up of the numerous remaining discovered genes will be necessary to elucidate their role in bone biology.

The considerable overlap in factors and biologic pathways underlying common and monogenetic forms of osteoporosis and bone mass disorders opens new avenues for diagnosis and personalized medicine.

The emerging discovery of novel skeletal biology by genetic studies is of huge potential for the identification of novel drug targets for skeletal disorders.

Introduction

Musculoskeletal conditions are the most common causes of severe short and long-term pain and physical disability, affecting hundreds of millions of people across the world, with costs approaching 3% of gross national product globally [1] and constituting the second greatest contributor to years lived with disability worldwide [2]. One of the diseases with greatest burden is osteoporosis, affecting one in three women and one in five men globally. This debilitating condition presents with a high incidence of low-trauma hip, spine, and other fractures, leading to immobility, associated comorbidity, and early death [1]. About 43 000 deaths occur each year in Europe as a direct consequence of hip or spine fractures, where approximately 20% of senior citizens who suffer a hip fracture die within a year [3]. Those who survive the fracture are often significantly disabled and have a reduced life-expectancy [1].

In this review we provide a succinct overview of the main molecular pathways governing bone metabolism, with an overlay of the genes that underlie monogenic conditions and complex forms (Box 1) presenting with low bone mass. We evaluate the genetic determinants of some forms of monogenic skeletal disorders with abnormalities in bone matrix, mineralization, or homeostasis, together with those implicated in the pathogenesis of adult-onset osteoporosis and fracture. We place particular emphasis on GWAS findings on bone mineral density (BMD) and associated phenotypes to show that, despite incomplete scrutiny, there is an important overlap in the genes and pathways underlying both mono- and poly-/multigenic conditions. We end by discussing the implications for diagnosis and particularly treatment of skeletal conditions.

Key Aspects of Skeletal Metabolism

Integrity and Function of the Skeletal System

The primary function of the skeleton is to provide structural support for the soft tissues of the body. The skeleton also has a metabolic function to provide a mineral reservoir, primarily for

[1]Musculoskeletal Genomics, Health and Metabolism, Departments of Internal Medicine and Epidemiology, Erasmus University Medical Center, 3000CA Rotterdam, The Netherlands
[2]Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[3]Department of Molecular Medicine and Surgery, Karolinska Institutet, and Clinical Genetics, Karolinska University Hospital, 171 76 Stockholm, Sweden

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Box 1. Genetic Architecture of Monogenic and Complex Diseases, and Approaches to Identify Genes

Allele Frequency and Effect Size of Underlying Genetic Variants

The genetic architecture of genetic traits and conditions can be categorized as a function of the properties intrinsic to the underlying variants, namely the minor allele frequency (MAF) and the effect size on the outcome of study (Figure I). Typically, variants of very rare frequency (usually called mutations) underlying monogenic traits have large effects (harboring very little influence of the environment) on the outcome and usually cluster within families. The search for such rare variants has been very successfully performed by genome-wide linkage studies in pedigrees of affected individuals [4]. More recently, exome-wide sequencing studies (studying the coding variation of the genome) have proved successful in identifying several 'unsolved' monogenic conditions, and are currently the main approach used to investigate these types of traits [5,6,7]. At the other end of the spectrum, involving relatively common genetic variants (MAF >10%) with very weak (but real) effects [8] and a prominent influence of the environment, are the so-called 'complex' traits and the underlying susceptibility (risk) to multifactorial diseases. It has become evident that, for most complex traits and common diseases [9–11], the underlying genetic architecture comprises hundreds (if not thousands) of variants. From this perspective, well-powered studies incorporating several independent populations (for replication), scrutinizing a well-defined selection of polymorphisms and gene regions, while employing a robust control for multiple hypotheses testing in the analysis, is the setting suited to identify genuine genetic effects [12]. There are relatively few examples of common variants that exert large effects on complex traits (e.g., *CFH* in myopia, *APOE* in Alzheimer), and it is unlikely that others of this type remain to be identified. In addition, rare variants of small effect probably exist but are unlikely to be identified by current methods and approaches in human populations. On the other hand, less-frequent variants (in the 0.5–5% MAF spectrum) are the current objective of GWAS using increasingly larger and diverse sequenced references (1000 Genomes Project, UK10K), facilitated by the increasing performance of imputing techniques to call confidently these types of variants. One final distinction between Mendelian disorders and complex traits is that the former are usually caused by mutations that primarily affect the coding sequence, while the latter usually involve common variants that map to regulatory elements, for example DNase I hypersensitivity sites [13].

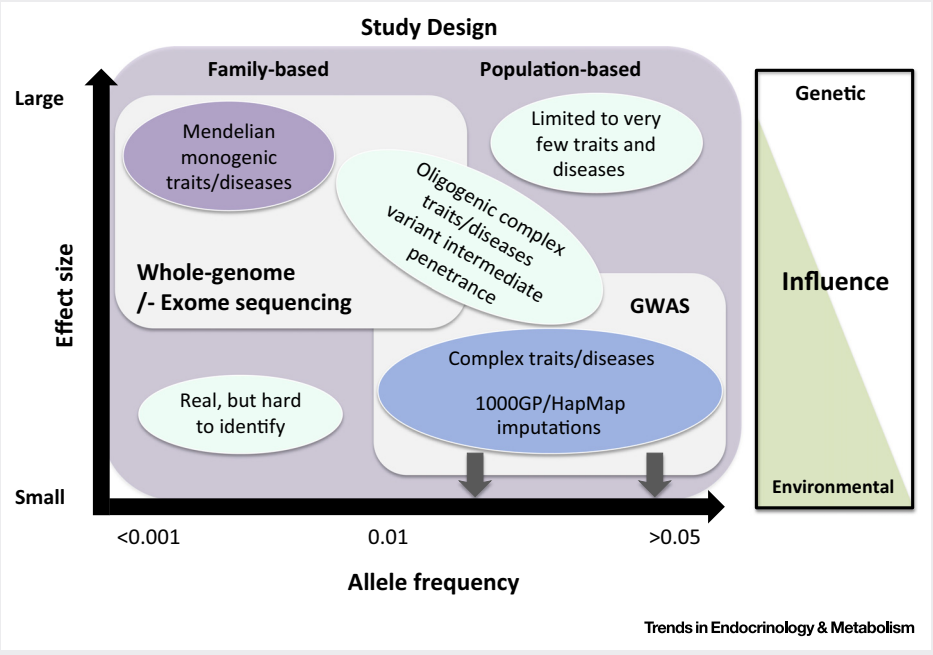

Figure I. Genetic Architecture of Traits and Diseases. The allele frequency and effect size spectrum of the underlying variants will shape the genetic architecture of a given trait or condition. Mendelian/monogenic diseases (purple balloon) were in the past mapped in familial collections employing linkage approaches; these have been recently replaced by whole-exome and whole-genome sequencing studies to identify rare variants (mutations with allele frequency <1%) exerting large effects on the phenotype. Complex traits and diseases (blue balloon) are usually common and are found through the study of large populations; genome-wide association studies employing imputation from sequenced reference sets are used to identify the typically rare (between 1% and 5%) and more common (>5%) variants with weak effects. Mendelian/monogenic traits usually have a large genetic influence with little contribution of the environment. By contrast, large environmental influences underlie the presentation of complex traits and diseases. Genes can harbor both mutations of large effect, causing Mendelian/monogenic diseases, and (low-frequency and common) polymorphisms causing complex diseases (for the overlap in such genes see, Table 4).

*Correspondence:
f.rivadeneira@erasmusmc.nl
(F. Rivadeneira).
www.glimdna.org
Twitter: @frivadei

calcium, but also for magnesium and phosphorus. These bone minerals can be mobilized to maintain systemic homeostasis of mineral metabolism, even at the expense of skeletal structural integrity. Bone also serves as a depository for cytokines and growth factors that can be released upon resorption, exerting local and systemic effects [14]. Throughout life, bones are constantly reshaped and renewed through the processes of modeling and remodeling. Modeling involves the sculpting of bone during growth in children and adolescents to ultimately achieve its proper shape during adulthood, and this process continues from birth to the mid-twenties when peak bone mass is achieved [15]. During adult life bone mass is maintained at a steady-state via constant remodeling through the processes of bone resorption and bone formation; in particular serving to repair compromised mechanical structure consequent to changes in weight-bearing and mechanical stress. After the 5th decade of life, bone resorption exceeds bone formation, leading (in postmenopausal women and the elderly) to bone loss, osteopenia, and osteoporosis [16]. In some monogenic conditions, low bone mass presents with earlier onset and greater disease severity.

Bone Cells and Low Bone Mass

From a cellular perspective, osteoclasts (derived from hematopoietic stem cells), osteoblasts (derived from bone marrow mesenchymal stem cells), and osteocytes (differentiated from osteoblasts and crucial for mechanosensing) are the key players of the bone remodeling process. Bone resorption is conducted by the osteoclasts, while bone formation is carried out by the osteoblasts [17]. Osteocytes are cells located within the bone matrix which play key roles in mechanosensing, acting as orchestrators of bone remodeling by regulating both osteoclast and osteoblast activity, while also exerting endocrine functions [18]. From this perspective, low bone mass (and risk of fracture) can be the consequence of diverse pathogenic mechanisms all of which are under genetic control and include: (i) failure to achieve optimal peak bone mass during skeletal growth, largely determined by genetic factors; (ii) increased bone resorption as a result of abnormal endocrine regulation and/or local action of cytokines or growth factors, which are also under genetic control; and (iii) compromised bone formation owing to impaired osteoblast function or insufficient stimulus, subject to both environmental and genetic influences [19]. With the exception of rare disorders affecting bone matrix structure or material properties (e.g., osteogenesis imperfecta) or vitamin D metabolism (e.g., rickets), most bone diseases presenting with low bone mass are the result of an imbalance between bone resorption and formation. Such is the case of primary osteoporosis typically occurring in postmenopausal women and elderly men usually as a consequence of estrogen deficiency, calcium deficiency, and/or processes related to aging.

Biological Pathways Underlying Rare and Common Skeletal Conditions

While the consequences of osteoporosis are well established, the specific causes of the disease remain elusive. Despite our increasing knowledge of the individual molecular mechanisms of osteoblast and osteoclast activation, how these mechanisms are orchestrated to maintain normal bone structural integrity or to cause osteoporosis remains poorly understood [20]. This is one of the motivations to turn to genetic studies – both on monogenic conditions where the underlying mutations in genes have been identified, as well as to GWAS in the field of osteoporosis – to provide insights into the genetic loci and pathways involved.

At the molecular level, diverse pathways have been identified to be of key importance in diverse aspects of bone metabolism. The regulation of bone metabolism is governed by an integrated and complex endocrine system set to maintain calcium, phosphate, and magnesium homeostasis [21]. Similarly, complex regulatory networks underlie the processes of bone modeling and remodeling. While the influence of the (micro)environment plays a substantial role, most of these regulatory processes will be under genetic control. This is the case for the calciotropic hormones exerting key metabolic roles and which include parathyroid hormone (PTH), calcitonin (CT) and

1,25-dihydroxycholecalciferol (1,25-D3). Other hormones, including insulin, cortisol, growth hormone (GH), thyroxine, epinephrine, estrogen, and testosterone, also influence the action of these calciotropic hormones and/or act directly on osteoblasts and osteoclasts during the processes of bone modeling and remodeling. Further, growth factors in the bone matrix, including insulin-like growth factors IGF-II (during fetal development) and -I (postnatally), transforming growth factor β (TGFβ), acidic and basic fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and bone morphogenetic proteins (BMPs), also exert local effects on bone formation [22]. Mesenchymal cell differentiation of the bone cell precursors is particularly governed by the action of many transcription factors (TFs) that intervene in the control and regulation of the differentiation pathways. As discussed in detail below, the main TFs involved in osteogenic differentiation are RUNX2 and osterix, together with several members of the SRY-related HMG-box (SOX) family of TFs (e.g., SOX4, SOX6, and SOX9) and the myocyte enhancer factor-2 (MEF2) family of TFs (e.g., MEF2C) [23–25].

Signaling Pathways in Bone Metabolism

Among several others, four main pathways have been shown to be crucial for bone metabolism, either acting directly on mesenchymal stem cells (regulating the differentiation and proliferation of bone cells) or by controlling the function and crosstalk between osteoblasts and osteoclasts: these include (i) WNT, (ii) NOTCH, (iii) Hedgehog, and (iv) OPG–RANK–RANKL signaling pathways. The first three pathways act through glycogen synthase kinase 3 (GSK3), a regulator of stem cell pluripotency. GSK3 is a highly conserved Ser/Thr kinase of the CMGC family of proline-directed kinases and is ubiquitously expressed in all tissues [26]. GSK3 has two isoforms encoded by separate genes located on chromosomes 19q13.2 (α) and 3q13.3 (β).

WNT Signaling

WNT signaling is crucial for bone development during embryogenesis and for bone formation, resorption, and coupling during the regeneration of postnatal bone. WNTs are secreted proteins involved in cell proliferation, differentiation, and apoptosis of bone cells. When cells are stimulated by WNTs through the membrane receptors Frizzled and low-density lipoprotein (LDL) receptor-related protein 5/6 (LRP5/6), the conformation of the multiprotein complex is disrupted. This results in inhibition of phosphorylation (blocking subsequent ubiquination) of β-catenin, thus increasing its cellular abundance and leading to its translocation to the nucleus. In the nucleus, β-catenin binds to the TFs LEF1/TCF and initiates the transcription of target genes. The WNT pathway is inhibited by the action of Dickkopf (DKK), a family of secreted proteins that bind to LRP5/6, and exert an antagonizing effect [27]. In common with other pathways, GSK3 is also key to WNT signaling [28]. GSK3 forms a multiprotein complex with APC, Axin, and other proteins that facilitates phosphorylation of β-catenin, creating a recognition site for β-catenin ubiquitination and subsequent degradation via the proteasome.

NOTCH Signaling

In the skeleton, both osteoblasts and osteoclasts require NOTCH signaling for proper differentiation and function, and the specific roles of NOTCH are dependent on the differentiation status of the cell [29]. NOTCH is a family of four transmembrane proteins (NOTCH1–4) that are expressed on the cell surface and require cell to cell contact for activation [30] through ligands binding to receptors expressed on the surface of neighboring cells. Ligands that activate NOTCH are single-pass transmembrane proteins and include Delta-like 1, 3, and 4 (Dll1, Dll3, Dll4) together with Jagged 1 and 2 (JAG1, JAG2). Ligand-mediated activation of NOTCH leads to proteolytic cleavage of the NOTCH receptor by the γ-secretase complex, resulting in the release of the NOTCH intracellular domain (NICD). Within the cell, the NICD translocates to the nucleus where it interacts with the DNA-binding protein RBPjκ/CBF1, displacing corepressors and leading to the assembly of an activator complex, allowing transcription of NOTCH target genes. GSK3 also plays key role in NOTCH activation and it is capable of binding to and phosphorylating NICD. To

date it is not clear if this phosphorylation stabilizes and reduces proteasomal degradation or if it actually destabilizes NICD and promotes its degradation [31,32]. There is also crosstalk between the NOTCH and WNT signaling pathways. NOTCH can degrade β-catenin and antagonize WNT signaling in the absence of ligand stimulation [33], although the precise mechanisms are not clear. Nevertheless, NOTCH signaling does not act in isolation, and contributes to an integrated (but complex) phenotypic response.

Indian Hedgehog (IHH) Signaling

The IHH signaling pathway is a crucial regulator of cell fate during development and for the proliferation of stem cells in adult life [34]. The importance of IHH in development is well established, the pathway being responsible for the processes of intramembranous ossification of cranial bones and endochondral ossification in other parts of the skeletal system. During endochondral ossification, chondrocytes differentiate and go through a tightly regulated developmental program of proliferation, prehypertrophy, hypertrophy, and apoptosis to be eventually replaced by osteoblasts in the ossification center [35]. Even though IHH signaling does not require β-catenin, interaction with WNT/β-catenin has been implicated in the regulation of osteoblast differentiation during endochondral bone development [36]. Indirect regulation of osteoclast activity can occur due to increased osteoblast production through increased Osterix expression in osteoblast cell lines [37]. Another form of indirect regulation of osteoblasts on osteoclasts occurs via IHH signaling, inducing osteoclast maturation and promoting bone resorption by increased RANKL expression through upregulation of PTHrP expression [38]. Similarly, IHH signaling has also been shown to induce collagen type X (Col10α1) expression, through either direct regulation of the Col10α1 promoter via Gli1 or Gli2 or indirect interaction with the Runx2/Smad pathway [39].

OPG–RANK–RANKL Signaling

The *OPG–RANK–RANKL* signaling pathway predominantly governs coupling between osteoblasts and osteoclast activity. Osteoblasts secrete the receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG). RANKL binds to its receptor activator of NF-κB (RANK) on monocytes to stimulate osteoclast differentiation in the presence of monocyte colony-stimulating factor (M-CSF). After binding, the adaptor protein TRAF6 is recruited, leading to NF-κB activation and translocation to the nucleus. Then NF-κB increases c-Fos expression, and c-Fos interacts with NFATc1 to trigger the transcription of osteoclastogenic genes [40]. OPG is a decoy receptor of RANKL and blocks osteoclast induction by competing with RANK to bind RANKL. These factors are part of the tumor necrosis factor (TNF) superfamily of ligands and receptors, and have been shown to have other functions beyond bone remodeling, including potential roles in other disease processes (i.e., vascular calcification and diabetes). Interaction with WNT signaling has been confirmed because β-catenin regulates OPG expression in osteoblasts [41], indirectly modulating bone resorption.

Together, bone mass and remodeling are determined by the combined efforts of osteoblasts and osteoclasts under the regulation of these major signaling pathways.

Human Monogenic Conditions Affecting Bone Mass and Strength

The main skeletal determinants of bone strength are mass, geometry, composition, material properties, and microstructure. From this perspective, genetic mutations affecting one or more of these factors will result in disease manifestation, despite the normal influence of environmental factors or other skeletal processes, including adaptation of the musculoskeletal system.

Several monogenic forms of osteoporosis have been described in which osteoporosis is caused by a single mutation in a gene that has a major role in the skeleton. Osteogenesis imperfecta (OI) is the most common monogenic disorder with skeletal fragility. It is usually caused by mutations

Table 1. Genes Underlying Osteogenesis Imperfecta (OI) According to 2015 Nosology[a]

Gene	Chromosomal Location	Protein	Gene MIM[b] Number	Phenotype/ OI type
COL1A1	17q21.33	Collagen type I α-1 chain	*120150	1, 2, 3, 4
COL1A2	7q21.3	Collagen type I α-2 chain	*120160	1, 2, 3, 4
CRTAP	3p22.3	Cartilage-associated protein	*605497	2, 3, 4
LEPRE1 (P3H1)	1p34.2	Prolyl 3-hydroxylase 1 (P3H1)	*610339	2, 3
PPIB	15q22.31	Cyclophilin B (CyPB)	*123841	2, 3, 4
SERPINH1	11q13.5	Heat shock protein 47 (HSP47)	*600943	3
BMP1	8p21.3	Bone morphogenetic protein 1	*112264	3
FKBP10	17q21.2	Peptidyl-prolyl *cis-trans* isomerase FKBP10	*607063	3, 4
PLOD2	3q24	Procollagen-lysine, 2-oxoglutarate	*182120	3
SERPINF1	17p13.3	Pigment-epithelium-derived factor (PEDF)	*172860	3, 4
SP7	12q13.13	Osterix	*606633	3, 4
WNT1	12q13.12	Wingless-type MMTV integration site family, AR member 1	*164820	3, 4
TMEM38B	9q31.2	Trimeric intracellular cation channel B (TRIC-B)	*611236	3
CREB3L1	11p11.2	cAMP responsive element binding protein 3-like 1	*616215	3
SEC24D	4q26	SEC24-related gene family, member D	*607186	3
IFTM5	11p15.5	Interferon-induced transmembrane protein 5	*614757	5

[a]Data from [42].
[b]MIM, Mendelian Inheritance in Man[iii]

in one of the two genes (*COL1A1*, *COL1A2*) encoding type I collagen, the major matrix protein in bone. Parallel to recent developments in genetic methodology allowing the identification of disease-causing mutations in even single families, several new types of OI with variable severity have been described (Table 1). These are usually related to defects in post-translational processing of type I collagen, resulting in severe early childhood-onset skeletal fragility and skeletal deformities, and observed only in a very small number of patients [43].

In addition to matrix proteins, skeletal regulation and homeostasis depend on several signaling pathways and TFs. Mutations in the genes encoding components of these pathways may also result in monogenic early-onset osteoporosis or phenotypes associated with skeletal fragility or increased bone loss (Table 2). One of the most extensively studied genes relevant to osteoporosis is the *LRP5* gene, encoding a coreceptor involved in the WNT signaling pathway (mentioned above). Loss-of-function mutations in *LRP5* cause the autosomal recessive osteoporosis–pseudoglioma syndrome, characterized by severe childhood-onset osteoporosis and blindness [44]. Carriers of *LRP5* mutations may have reduced bone mass or symptomatic osteoporosis. The significance of the WNT signaling pathway in the maintenance of skeletal homeostasis is further underscored by the identification of biallelic and monoallelic mutations in the gene encoding WNT1 in patients with severe OI and early-onset osteoporosis, respectively [45]. Furthermore, activating mutations in *LRP5*, and loss-of-function mutations in the gene for sclerostin, an inhibitor of the WNT pathway, result in a significant increase in bone formation and a high bone-mass phenotype in individuals harboring a mutation [46]. In addition to defective

Table 2. Monogenic Disorders with Low Bone Mass, Osteolysis, and/or Skeletal Fragility[a]

Gene	Syndrome	Location	Protein	Phenotype MIM Number
PLS3	X-linked osteoporosis	Xq23	Plastin 3	#300910
FKBP10	Congenital brittle bones with congenital joint contractures. Bruck syndrome 1	17p21.1	Peptidyl-prolyl *cis-trans* isomerase FKBP10	#259450
PLOD2	Congenital brittle bones with congenital joint contractures. Bruck syndrome 2	3q24	Procollagen-lysine, 2-oxoglutarate	#609220
LRP5	Osteoporosis pseudoglioma syndrome	11q13.2	Low-density lipoprotein receptor-related protein 5	#259770
P4HB	Cole-Carpenter dysplasia (bone fragility with craniosynostosis)	17q25.3	Prolyl 4-hydroxylase, β subunit	#112240
XYLT2	Spondyloocular syndrome	17q21.33	Xylosyltransferase II	#605822
B4GALT7	Ehlers–Danlos syndrome, progeroid form	5q35	Xylosylprotein 4-betagalactosyltransferase	#130070
GORAB	Geroderma osteodysplasticum	1q24.2	SCYL1-binding protein 1	#231070
PYCR1	Cutis laxa, autosomal recessive form, type 2B (ARCL2B)	17q25.3	Pyrroline-5-carboxylate reductase 1	#612940
ATP6V0A2	Cutis laxa, autosomal recessive form, type 2A (ARCL2A) (Wrinkly skin syndrome)	12q24.31	ATPase, H^+ transporting, lysosomal, V0 subunit A2	#278250, #219200
IFIH1	Singleton–Merten syndrome 1	2q24.2	Interferon induced with helicase C domain 1	#182250
RANK (TNFRSF11A)	Familial expansile osteolysis	18q22.1	Tumor necrosis factor receptor superfamily, member 11a	#174810
LMNA	Mandibuloacral dysplasia type A; progeria, Hutchinson–Gilford type	1q22	Lamin A/C	#248370, #176670
ZMPSTE24	Mandibuloacral dysplasia type B	1p34.2	Zinc metalloproteinase	#608612
MMP2	Torg–Winchester syndrome	16q12.2	Matrix metalloproteinase 2	#259600
NOTCH2	Hajdu–Cheney syndrome	1p12–p11	Notch (*Drosophila*) homolog 2	#102500
MAFB	Multicentric carpotarsal osteolysis syndrome	20q12	v-Maf avian musculoaponeurotic fibrosarcoma oncogene homolog B	#166300
RIN3	Paget disease of bone 3; PDB3	6p21.3	RAS and RAB interactor 3	#167250
RUNX2	Cleidocranial dysplasia	6p21.1	Runt-related transcription factor 2	#119600
TNFRSF11B	Paget disease of bone 5, juvenile-onset	8q24.12	Tumor necrosis factor receptor superfamily, member 11b	#239000
ANO5	Gnathodiaphyseal dysplasia (osteopenia with radiolucent lesions of the mandible)	11p14.3	Anoctamin-5	#166260

[a]Data from [42].

WNT signaling, mutations in the genes relevant to NOTCH signaling or the RANK/RANKL pathway underlie some rare monogenic skeletal disorders with low bone mass (Table 2). More recently, mutations in the plastin 3 (*PLS3*) gene were shown to underlie an X-linked form of childhood-onset primary osteoporosis affecting mainly males [47]. The function of PLS3 in bone

Table 3. Monogenic Disorders Presenting with Abnormal Skeletal Mineralization[a]

Gene	Syndrome	Chromosomal Location	Protein	Phenotype MIM Number
ALPL	Hypophosphatasia, perinatal lethal, infantile, juvenile and adult forms; odontohypophosphatasia	1p36.12	Alkaline phosphatase, tissue nonspecific (TNSALP)	#241500 #146300
PHEX	Hypophosphatemic rickets, X-linked dominant	Xp22.11	X-linked hypophosphatemia membrane protease	#307800
FGF23	Hypophosphatemic rickets, autosomal dominant	12p13.32	Fibroblast growth factor 23	#193100
DMP1	Hypophosphatemic rickets, autosomal recessive, type 1 (ARHR1)	4q22.1	Dentin matrix acidic phosphoprotein 1	#241520
ENPP1	Hypophosphatemic rickets, autosomal recessive, type 2 (ARHR2)	6q23.2	Ectonucleotide pyrophosphatase/ phosphodiesterase 1	#613312
ClCN5	Hypophosphatemic rickets with hypercalciuria, X-linked recessive	Xp11.23	Chloride channel 5	#300554
SLC34A3	Hypophosphatemic rickets with hypercalciuria, autosomal recessive (HHRH)	9q34.3	Sodium/phosphate cotransporter	#241530
CASR	Neonatal hyperparathyroidism, severe form; familial hypocalciuric hypercalcemia with transient neonatal hyperparathyroidism	3q13.3–q21.1	Calcium-sensing receptor	#239200 #145980
ANKH	Calcium pyrophosphate deposition disease (familial chondrocalcinosis) type 2	5p15.2	Homolog of mouse ANK (ankylosis) gene	#118600
PTHR1	Metaphyseal dysplasia, Jansen type; Eiken dysplasia	3p21.31	Parathyroid hormone receptor 1	#156400; #600002

[a]Data from [42].

metabolism is not completely understood but it has been speculated to play a role in mechanosensing by osteocytes.

In addition to intact matrix proteins and balanced function of bone cells, skeletal integrity depends on mineral homeostasis. Defects in the regulatory pathways of calcium and phosphate homeostasis, or in the mineralization process itself, may result in variable degrees of bone fragility (Table 3). The most severe of these is hypophosphatasia, caused by mutations in the alkaline phosphatase gene, which presents with variable severity of skeletal undermineralization, and clinically ranges from near-complete skeletal undermineralization and perinatal death to only mild adult-onset osteomalacia [48].

Osteoporosis and Fracture Risk: Genetic Complex Conditions Affecting Bone Mass and Strength

From a genetic perspective the etiology of osteoporosis and fracture risk susceptibility is multifactorial, involving significant environmental influence together with genetic factors across numerous biologic processes. The components of the musculoskeletal system involved in determining the risk of fracture are diverse. As such, the musculoskeletal system can be seen as a heterogeneous set of processes that undergo fusion in a 'funnel' leading to the occurrence

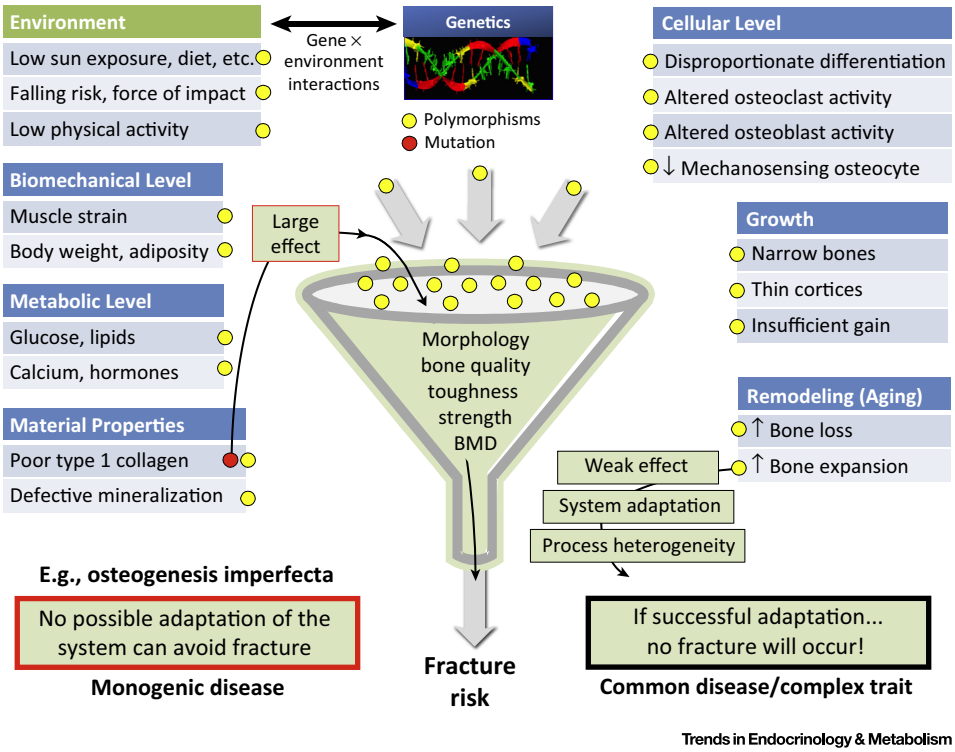

Figure 1. Representation of the Different Processes Governed by Genetic and Environmental Factors Acting on the Musculoskeletal System Which Can Lead to Increased Fracture Risk. Pathways leading to fracture can be conceived as the fusion of processes entering a 'funnel', which ultimately lead to increased fracture risk. Genetic polymorphisms (yellow dots) and mutations (red dot) have the potential to influence fracture risk through diverse mechanistic pathways involving growth, bone remodeling, and material properties, or by acting at a metabolic, biomechanical, or cellular level; either alone or through gene × environment interactions. From a genetic perspective the increased fracture risk underlying monogenic conditions (e.g., osteogenesis imperfecta) is typically consequence of the large effect of a mutation (red dot), which is sufficient to destabilize the system and lead by itself to the occurrence of fracture. By contrast, fracture risk can be conceived as a complex trait, when multiple genetic variants (yellow dots) exerting weak effects are insufficient individually to destabilize the overall integrity of the musculoskeletal system, and need to synergize to increase fracture risk. Abbreviation: GWAS, genome-wide association studies. BMD, bone mineral density.

of fracture (Figure 1). This includes the influence of genetic variants (SNPs and mutations) across each of these processes. In addition, the musculoskeletal system also adapts to subtle threats or alterations in one or more of the components, further complicating the search for fracture susceptibility genes. This mixed set of etiological pathways is the basis of its complexity, and the reason why large GWAS are needed to achieve a sufficient sample size to permit the identification of the weak genetic effects underlying osteoporosis and the risk of fracture. As in other medical fields, the field of genetics of osteoporosis has been revolutionized by the advent of the GWAS approach described in Box 2 [49–51]. Before the GWAS era, the literature about the genetics of osteoporosis and fracture had been confined to a very large number of 'genome-wide linkage' and 'candidate gene association' studies. The majority of these were inadequately powered studies that resulted in conflicting and frequently irreproducible reports [52]. One of the few exceptions was the work arising from the Genetic Markers of Osteoporosis (GENOMOS) consortium. These efforts sought large-scale evidence (n = 20 000–45 000, which by current standards is still substantial) for association of variants in six of 'the usual suspects' in genetics of osteoporosis at the time, including the *ESR1* [53], *VDR* [54], *COLIA1* [55], *TGFB1* [56], and *LRP5/6* [57] genes. Few of these polymorphisms were identified as being associated with either BMD or fracture, with variants in the *LRP5* showing the strongest and most significant

Box 2. Genetic Approaches To Identify Genes

GWAS

GWAS incorporate into their design hypothesis-free (genome-wide) screening and the power of association testing, and have revolutionized the field of complex traits, including the field of osteoporosis [58]. Most successful identifications of variants using GWAS have, with few exceptions, resulted from large collaborative efforts, as illustrated by the work of more than 100 consortia[iv]. From this perspective, GWAS have demonstrated that the number of loci identified depends on the total sample size obtained in a given meta-analysis: more samples are expected to yield more GWAS 'hits' even at the cost of more accurate phenotype definitions [59]. Sample size is still a constraint, resulting in detection of only a small fraction of all the variants expected to be associated with a complex trait. The stringent genome-wide significance (GWS) level of $P<5\times10^{-8}$ (i.e., $P=0.05$ after the Bonferroni correction for the number of independent tests for common variants) has been accepted as the current standard to exclude chance findings due to multiple testing (false positives). Despite the very limited number of loci discovered at this stringent level, the overall count of discovered loci is in the thousands, across hundreds of traits [60], thanks to the large collection of samples that are becoming increasingly available. Nevertheless, a considerable fraction of other (real) loci have not still been identified at GWS owing to lack of power (false negatives). While for most complex traits of only about 5–20% of the genetic variance has been accounted for by variants reaching GWS, recent methods quantifying the proportion of phenotypic variance tagged by all common SNPs simultaneously have demonstrated that it is much higher (33–50%) [61,62]; it has even been postulated that most of all the genetic variation of a trait can be identified through efforts combining GWAS and imputation to large sequencing reference panels [63]. Therefore, even larger sample sizes will be necessary to identify the small effects underlying the genetic architecture of complex traits [64,65].

Rare Variants, Monogenic Disorders, and Sequencing

As mentioned in Box 1, in monogenic disorders great advances have been achieved over the past 5 years with the emergence of whole-exome sequencing. Despite the hypothesis-driven approach, in other words targeting coding variation in the genome, the exome-wide search for the etiology of coding variants underlying monogenic disorders has been very fruitful. This is in line with the contention that changes in rare coding variation resulting in altered protein products are more likely to be severe, and hence result in a greater effect size and lower allele frequency of the underlying variants [66]. Advances in genetic sequencing, combined with falling costs and increased computational power, are bringing new opportunities to mine further the range of genetic variation that exists at the loci that underlie the genetic contribution to common complex diseases. Next-generation sequencing (NGS) technologies are making it feasible to scrutinize the entire spectrum of allelic variation within a population (with a MAF of <1%) and are improving our understanding of the genetic architecture of many complex diseases through analysis of structural variation, epigenomics, transcriptomics (gene expression), proteomics, and metabolomics. In contrast to GWAS, that require huge sample sizes to confer enough statistical power to observe the small effects of common genetic variants, a representative sample selection is required (i.e., based on specific phenotypic inclusion criteria), at least until NGS technologies become sufficiently affordable to permit scrutiny of very large samples.

associations, achieving $P<5\times10^{-8}$, the current standard for declaring genome-wide significance (GWS). Nevertheless, the efforts within GENOMOS were restricted by the genotyping technology, scrutinizing only small numbers of polymorphisms over months to years of work.

The advent of high-throughput genotyping technology, together with the knowledge derived from the HapMap project[i] [67], enabled the GWAS approach to be applied in the already established epidemiological and biobank infrastructure of the GENOMOS Consortium. This gave birth to the Genetic Factors of Osteoporosis (GEFOS) Consortium[ii], incorporating GWAS in the field of osteoporosis, permitting GWAS discoveries to be replicated in a large-scale DNA collection by *de novo* genotyping. This collaborative setting is the largest coalition of researchers worldwide in the field of osteoporosis genetics. GWAS meta-analyses performed by GEFOS have now led to large increases in the crude number of loci identified for more than 18 bone-related phenotypes [68–82]. The largest GWAS meta-analysis performed in the field of osteoporosis to date [81] identified 56 BMD loci, of which 14 were also associated with risk for osteoporotic fracture (including *SPTBN1*, *MEPE/SPP1*, *SLC25A13*, *MBL2/DKK1*, *LRP5*, *FAM210A*, *ZBTB40/WNT4*, *CTNNB1*, *STARD3NL*, *WNT16*, *FUBP3*, *DCDC5*, *RPS6KA5*, and *SOST/C17orf53*). GWAS on BMD measurements resulted in the highest yield in genetic discoveries so far, providing novel insights into bone biology. Such a high yield is likely to reflect the fact that BMD is a highly-heritable, quantitative, precise, and widely-available trait. Nevertheless, BMD is not the only determinant of fracture and, in fact, close to 50% of fractures occur below the BMD threshold

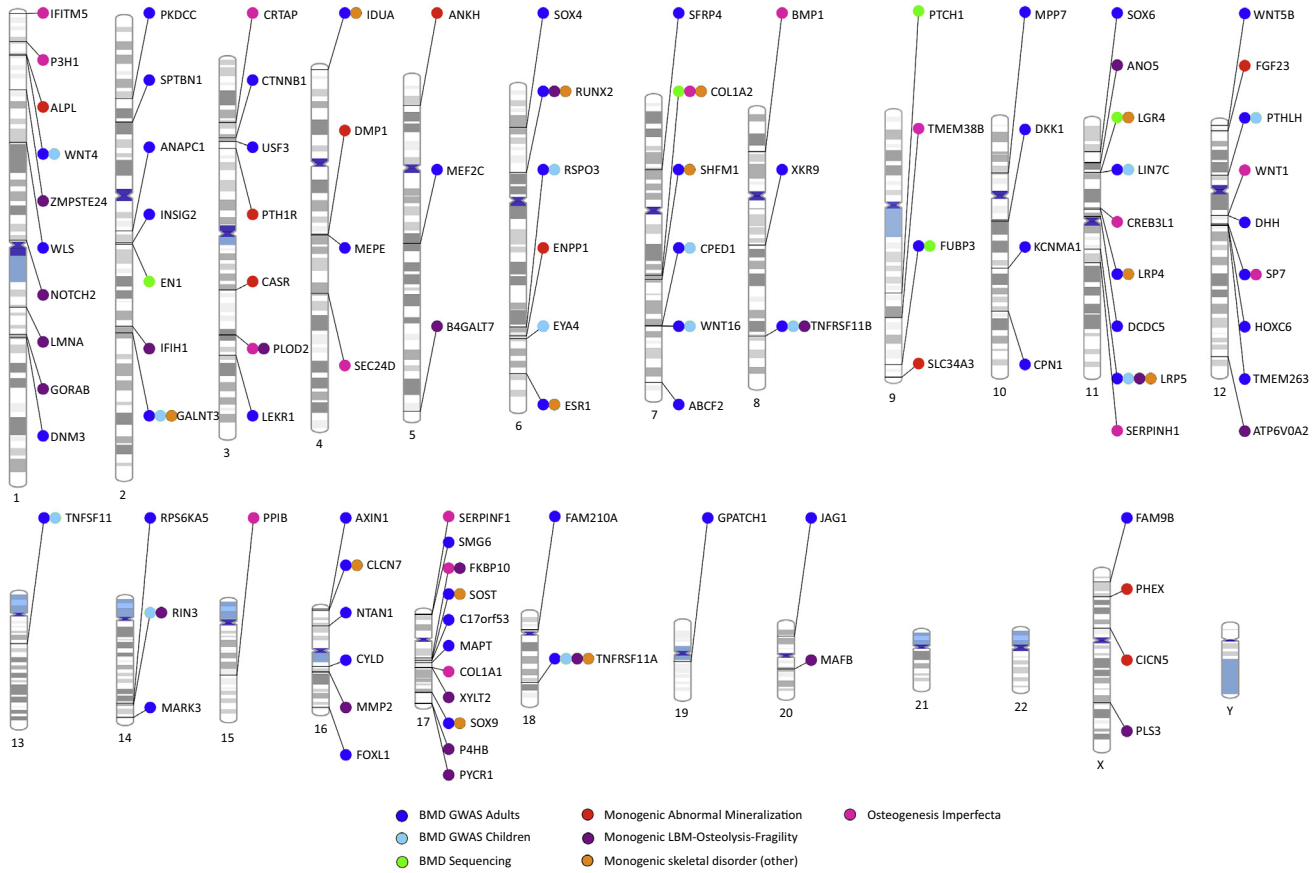

Figure 2. Gene Overlap Between Bone Mineral Density (BMD) GWAS Discovery and Monogenic Conditions Presenting with Skeletal Fragility. Chromosomal ideogram illustrating the genomic distribution and overlap of genes mapping within BMD loci identified by GWAS and those harboring mutations underlying monogenic conditions presenting with bone fragility. Genes in BMD loci comprise those discovered by GWAS in adults (dark blue), GWAS in children (light blue), and recent studies applying whole-genome sequencing (yellow). Genes underlying monogenic conditions presenting with skeletal fragility include osteogenesis imperfecta (pink), syndromes of abnormal mineralization (red), syndromes presenting with low bone mass, osteolysis, and/or fragility (purple), and any other skeletal syndrome (orange). Several genes (described in Table 4) harbor mutations leading to monogenic skeletal conditions and (common and low-frequency) polymorphisms associated with BMD (in children and/or adults). Abbreviation: The ideogram was constructed using Phenogram[v].

for osteoporosis [83]. Looking at other endophenotypes of fracture, which incorporate biological properties not captured or discernable by the BMD measurement (i.e., bone structure, geometry and extra-skeletal factors), will also be necessary to understand the intrinsic mechanistic pathways leading to bone fragility. In the meantime, we have learned a great deal about bone biology from the results of GWAS and the genes underlying monogenic disorders.

Pathway analysis of the 63 variants from the 56 BMD loci performed using text-mining connectivity (GRAIL) methods [84] illustrates how several of the genes underlying GWAS signals for BMD cluster around the main pathways discussed earlier that are known to be crucial for bone biology. In addition, several of the loci identified were found to overlap with human monogenic conditions presenting with severe skeletal abnormalities or with bone fragility (Figure 2 and Table 4). Nevertheless, in addition to the identification of factors in well-established biological pathways, the GWAS approach is a hypothesis-free approach, and is thereby frequently confronted with completely new biology. For example, in the largest GEFOS study, variants in the chromosome 18 genomic region mapping to *FAM210A* constituted the strongest genetic factor associated with fracture risk [81]. *FAM210A* was (until very recently) annotated as

Table 4. Overlap between Monogenic Skeletal Disorders and BMD GWAS-Identified Genes[a]

Gene	Locus	Protein	OMIM Number	Syndrome	Major Clinical Features	Ref.
GALNT3	2q24.3	GALNAC TRANSFERASE 3	211900	Hyperphosphatemic familial tumoral calcinosis	Hyperphosphatemia due to increased renal absorption, progressive deposition of calcium phosphate crystals in periarticular spaces, soft tissues, and sometimes bone	[85]
IDUA	4p16.3	ALPHA-L-IDURONIDASE	607014	Hurler syndrome (MPS IH)	Mucopolysaccharidoses of variable severity; coarse facies, corneal clouding, mental retardation, hernias, dysostosis multiplex, and hepatosplenomegaly	[86]
			607016	Scheie syndrome (MPS IS)		
			607015	Hurler–Scheie syndrome (MPS IH/S)		
RIN3	6p21.3	RAS AND RAB INTERACTOR 3	601530	Paget disease of bone 3; PDB3	Focal lesions of increased bone turnover affecting primarily the axial skeleton with increased osteoclastic bone resorption and disorganized bone structure	[87]
RUNX2	6p21.1	RUNT-RELATED TRANSCRIPTION FACTOR 2	119600	Cleidocranial dysplasia	Persistently open skull sutures with bulging calvaria, hypoplasia or aplasia of the clavicles, wide pubic symphysis, short middle phalanx of the fifth fingers, dental anomalies, and often vertebral malformation	[88]
			156510	Metaphyseal dysplasia with maxillary hypoplasia with or without brachydactyly	Metaphyseal flaring of long bones, enlargement of the medial halves of the clavicles, maxillary hypoplasia, variable brachydactyly, and dystrophic teeth	[89]
ESR1	6q25.1	ESTROGEN RECEPTOR 1	615363	Estrogen resistance	Tall stature, incomplete epiphyseal closure, continued linear growth into adulthood despite otherwise normal pubertal development. Low bone mineral density; osteopenia on bone biopsy	[90]
COL1A2	7q21.3	COLLAGEN, TYPE I, ALPHA-2	166210 259420	Osteogenesis imperfecta type 2, Osteogenesis imperfecta type 3	Bone fragility, severe bowing of long bones, undermineralization, and death in the perinatal period (in OI2) or severe skeletal fragility, deformities and short stature (in OI3)	[91]
SHFM1	7q21.3	26S PROTEASOME COMPLEX SUBUNIT DSS1	601285	Split hand-split foot malformation	Limb malformation involving the central rays of the autopod and presenting with syndactyly, median clefts of the hands and feet, and aplasia and/or hypoplasia of the phalanges, metacarpals, and metatarsals	[92]
TNFRSF11B	8q24.12	OSTEOPROTEGERIN, OPG	239000	Juvenile Paget disease	Short stature, progressive long bone deformities, fractures, vertebral collapse, skull enlargement, and hyperostosis with progressive deafness	[93]
LRP4	11p11.2	LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 4	212780	Cenani–Lenz syndactyly syndrome	Syndactyly resembling Apert syndrome, severe shortening of the ulna and radius with fusion, fusion of the metacarpals, and disorganized phalangeal development	[94]
			614305	Sclerosteosis	Severe sclerosing bone dysplasia characterized by progressive skeletal overgrowth	[95]

Table 4. (continued)

Gene	Locus	Protein	OMIM Number	Syndrome	Major Clinical Features	Ref.
LGR4	11p14.1	G PROTEIN-COUPLED RECEPTOR 48	615311	Susceptibility to low bone mineral density	Low bone mineral density and osteoporotic fractures	[96]
LRP5	11q13.2	LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 5	259770	Osteoporosis-pseudoglioma syndrome	Severe osteogenesis-imperfecta-like skeletal fragility with skeletal deformities, spinal compression fractures, and early-onset blindness	[44]
			144750	Hyperostosis	Increased bone density, endosteal sclerosis of the diaphyses of long bones (including metacarpals and metatarsals), osteosclerosis of the pelvis, endosteal sclerosis of the calvaria, osteosclerosis, and hyperostosis of the mandible	[46]
SP7	12q13.13	TRANSCRIPTION FACTOR SP7	613849	Osteogenesis imperfecta type XII	Recurrent fractures, mild bone deformations, generalized osteoporosis, delayed tooth eruption, no dentinogenesis imperfecta, normal hearing, and white sclerae	[97]
CLCN7	16p13.3	CHLORIDE CHANNEL 7	166600	Osteopetrosis, autosomal dominant 2	Osteosclerosis, predominantly involving the spine, the pelvis, and the skull base; fragility of bones and dental abscesses	[98]
			611490	Osteopetrosis, autosomal recessive 4	Infantile malignant osteopetrosis, anemia, reticulocytosis, hepatomegaly, and optic atrophy	[99]
SOST	17q21.31	SCLEROSTIN	269500	Sclerosteosis	Severe sclerosing bone dysplasia with progressive skeletal overgrowth	[100]
			239100	Van Buchem disease	Increased thickness of bones, enlarged jawbone, enlargement of the skull, ribs, diaphysis of long bones, resulting in increased cortical bone density. Increased thickness of the skull leads to facial nerve palsy, hearing loss, and visual problems	[101]
SOX9	17q24.3	SRY-BOX 9	114290	Campomelic dysplasia	Congenital bowing and angulation of long bones, especially the tibias, with other skeletal deformities; often genital defects	[102]
TNFRSF11A	18q21.33	RECEPTOR ACTIVATOR OF NF-KAPPA-B; RANK	174810	Familial expansile osteolysis	Increased bone remodeling with osteolytic lesions mainly affecting the appendicular skeleton; medullary and cortical expansion of the bone without sclerosis, leading to deformities and pathologic fracture	[103]
			612301	Osteopetrosis	Severe osteoclast-poor osteopetrosis	[104]
			602080	Paget disease of bone-2	Focal abnormalities of increased bone turnover affecting one or more sites throughout the skeleton, primarily the axial skeleton	[93]

[a]For details see [71,74,75,81,82,105,106].

an 'open reading frame' factor of which nothing was known about its potential involvement in bone biology. At least 30 of the 56 BMD loci contain genes underlying the GWAS signals of which nothing is known regarding their potential roles in bone biology [81].

The greatest new insight into bone biology is undoubtedly illustrated by the discovery of *WNT16* using the GWAS approach. After the initial identification of *WNT16* as a BMD locus [81], several other GWAS in premenopausal women [107], wrist BMD [68], total body regional BMD [105], skull BMD in children and adults [79], cortical thickness from pQCT of the tibia [68], and quantitative ultrasound of the heel [108] all confirmed *WNT16* as a crucial molecule for bone biology. The regulatory mechanisms of WNT16 have been recently elucidated by a functional study in murine models [109] showing that *Wnt16* knockout mice have reduced cortical bone thickness and increased cortical bone porosity (but not trabecular bone mass), which leads to spontaneous non-vertebral fractures in these mice. Most interestingly, this demonstrates a dissociation between the regulation of cortical and trabecular bone homeostasis: in other words, WNT16 acts directly on osteoclasts by inhibiting osteoclastogenesis via a non-canonical Wnt pathway, while also exerting an indirect effect by increasing OPG expression in osteoblasts, the latter effect operating both via canonical and non-canonical WNT pathway activation. Further, a subsequent study recently showed how overexpression of WNT16 predominantly increases trabecular bone mass, suggesting that WNT16-targeted therapies might be useful for treatment of postmenopausal trabecular bone loss [110].

Age, Sex, and Ethnicity in the Context of Genetic Factors

As described above, BMD reflects a combination of physiological processes across the life course [111,112]. As such, it is expected that genetic variants related to BMD could display age-dependent effects – that is, some associated variants may exert a greater influence on developmental processes taking place during childhood and adolescence than on those taking place during adulthood. BMD measured in early life periods may be less influenced by the cumulative effect of non-genetic (i.e., environmental or lifestyle) factors. The first robust evidence that genetic variants may display age-dependent effects on BMD encompassed ~2200 6-year-old children from the Generation R Study [113], and an additional five cohorts that represented distinct age groups ranging from 10 to 75 years (n = 11 052) [79]. While variants in the 7q31.31 *WNT16* locus were associated with whole-body BMD, variants mapping to the neighboring *CPED1* gene presented with a larger effect on skull BMD in children, as compared to the effects observed in older adult individuals [79]. The role of *CPED1* in bone biology remains to be elucidated. While some BMD-associated loci can present age-dependent effects, most of the associations are observed throughout life, likely indicating that their role in peak bone mass acquisition during early life will still be evident in adults as a main source of BMD variation [114]. Alternatively, this may also indicate that these loci continue to regulate bone metabolism throughout life in the forms of bone modeling (continued expansion via periosteal apposition), a consequence of adaptive changes in bone shape and size mostly in response to mechanical loading.

Despite the pronounced sexual dimorphism of bone, there is little evidence that genetic effects arise from loci outside the sex chromosomes. Of the 56 BMD loci identified by the GEFOS Consortium [81], only variants mapping to the Xp22.31 *FAM9B/KAL1* locus showed significant sex heterogeneity, and these were shown to influence testosterone levels in a GWAS meta-analysis [76]. Consistent with this, genome-wide sex-interaction meta-analyses confined to the autosomes failed to identify any significant interactions with BMD [80].

Racial differences in BMD are well documented and partially explain differences in osteoporosis and fracture risk across populations. Individuals of sub-Saharan African ancestry tend to have higher BMD levels and lower fracture risk than other populations [115,116], even before achieving peak bone mass [117–121]. Two recent cohort studies of children of multiethnic

background demonstrated that the frequencies of BMD-increasing alleles, discovered in adult European populations, are systematically elevated in individuals of sub-Saharan African ancestry, consistent with their higher BMD levels [119]. The inclusion of ethnic groups other than European as well as admixed populations in GWAS studies is rapidly rising, following the pressing need to extrapolate findings to non-European populations, fine-map existing BMD loci, discover new associations, and increase statistical power. Very recently, a GWAS study drawn in 20 162 Icelandic individuals, with replication in 10 091 subjects from two studies of European background and other two of East Asian descent [122], identified variants mapping to a new locus harboring the *PTCH1* gene, the receptor for the three hedgehog morphogens (SHH, IHH, and DHH).

Lessons From Rare Variants

Less-common variants are predicted to exhibit stronger effect sizes than common variants (Box 1), consistent with the view that functional allelic variants are subject to purifying selection pressure [5,66]. Recent efforts in the field of osteoporosis employing whole-genome sequencing (WGS) followed by imputation to larger populations have proved successful and constitute pioneering work in the field of complex traits [122,96,123]. A mutation (C376T) within the leucine-rich repeat-containing G protein-coupled receptor 4 (*LGR4*) gene was identified in a sample of Icelandic individuals [96] comprising 4931 individuals with low BMD and 69 034 controls. The T allele was shown to have a large effect on (decreasing) BMD and was associated with increased risk for osteoporotic fractures, among other disease outcomes (e.g., cancer and cardiovascular disease). This is a rare nonsense mutation which terminates LGR4 and completely disrupts the function of the protein, a respondin involved in WNT signaling. In a subsequent effort, two rare mutations in *COL1A2* (minor allele frequency, MAF = 0.1%) were identified through WGS and were shown to be associated with low BMD in 2894 cases and 206 875 controls derived from the Icelandic population without signs of osteogenesis imperfecta [106]. An effort using the UK10K/1000GP sequencing reference to impute less-frequent variants across 53 236 individuals from 27 population-based cohorts of European ancestry identified a novel less-frequent (MAF = 1.7%) non-coding variant near the engrailed-1 (*EN1)* gene, exerting large effects on BMD and fracture [123], with an effect size about fourfold greater than observed for any previously reported common variant. While the exact role of EN1 in human bone metabolism remains to be elucidated, En1 has been shown to interact with Wnt factors in the regulation of limb patterning in mice [124].

From Bench to Bedside

The studies discussed above illustrate how the application of new technologies (i.e., NGS) will yield additional insight into the genetic architecture of bone diseases. Most promising is the potential to translate these discoveries into palpable clinical applications. Impressively, several of the pathways identified by GEFOS GWAS efforts correspond to pathways being targeted for the treatment of osteoporosis [81]. In fact, most of the factors and pathways critical to skeletal biology are well characterized when examining the connectivity between all the genes from GWAS-identified loci and those underlying monogenetic conditions presenting with bone fragility or high bone mass (Figure 3). In addition, nearly all current osteoporosis agents, either in clinical use or in advanced clinical trials, target pathways identified by BMD-associated genes that are key to bone metabolism [125]. This is the case for the OPG–RANK–RANKL pathway that plays a key role on bone resorption though regulation of osteoclast fate and activation. All three genes of the OPG (*TNFRSF11B*), RANK (*TNFRSF11A*) and RANKL (*TNFSF11*) pathway harbor variants identified by GWAS which are associated with BMD [81]. Denosumab (commercial name Prolia) is a human monoclonal antibody against RANKL, which serves as a decoy that inhibits bone resorption by reducing the formation, activation, and survival of osteoclasts [126]. Even more interesting is the identification of several factors that may constitute targets for true bone-building drugs. Such anabolic treatments have the potential to exceed the benefits, and possibly overcome some side-effects, of compounds targeting only the inhibition of bone resorption.

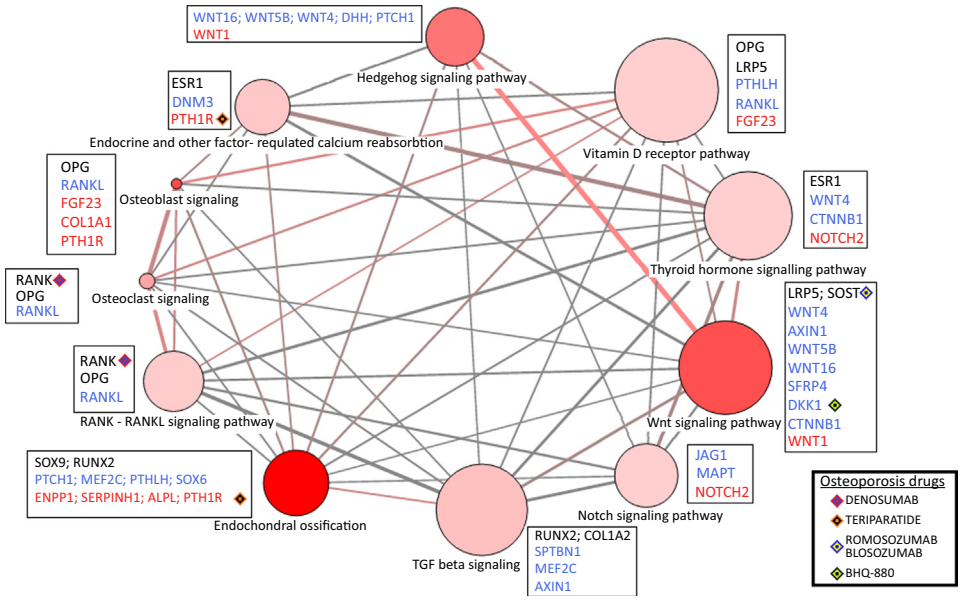

Figure 3. Prioritized Pathway Connectivity between Nodes Harboring Genes from Bone Mineral Density (BMD) Loci Identified by Genome-wide Association Studies (GWAS) and Those Genes Underlying Monogenic Conditions Affecting Bone Mass or Characterized by Skeletal Fragility. Circles represent nodes and their size reflect the number of genes ranging between 14 (small circle), 74 (medium-sized circles) up to 398 (largest circle) genes. Dark red and light red clusters have significant enrichment at $P<10^{-10}$ and $P<10^{-5}$ levels, respectively. Thicker connection lines represent larger number of shared genes across nodes, while colored lines (dark and light red) represent higher number of input genes driving the connection between nodes. Input genes include those identified by BMD GWAS (blue), monogenic conditions (red) or both (black). The nodes with more representative enrichment include the WNT, Hedgehog, and Endochondral ossification signaling pathways. Prominent gene sharing is observed between multiple nodes; being particularly high between WNT and Hedgehog; and also between RANK–RANKL and Osteoclast/Osteoblast signaling. The pathway diagram was constructed using the ConsensusPathDB (CPDB) web-based software[vi].

PTH 1–34 (teriparatide) is currently the only available bone-building compound used for the treatment of osteoporosis, and this molecule not only stimulates bone formation but also bone resorption in a dose-dependent manner. PTH binds to the PTH/PTHrP type 1 receptor to activate distinct signaling pathways, including the canonical WNT signaling pathway in osteocytes, to regulate bone remodeling [127]. Variants in PTHRP have also been identified by GWAS of BMD [81]. PTHrP 1–36 (abaloparatide) binds to the PTH/PTHrP 1 receptor to increase bone formation and bone resorption [128]. Nevertheless, to achieve an optimal therapeutic outcome, bone formation and bone resorption should be modulated in different directions, as demonstrated by simultaneous treatment with teriparatide and denosumab, which increases BMD at all skeletal sites considerably more than either monotherapy alone [129]. Denosumab treatment likely inhibits teriparatide-stimulated RANKL production, reducing bone resorption to exert a stimulatory effect only on bone formation. This is one of the appealing factors of potential drug targets within the WNT signaling pathway. Variants in the *SOST* (sclerostin), *DKK1*, *LRP5*, *LRP4*, *AXIN1*, and *CTNNB1* (β-catenin) genes have been identified by GWAS to be associated with both BMD and fracture risk [81]. Sclerostin (which is produced by osteocytes) inhibits the proliferation, differentiation, and survival of osteoblasts, leading to reduced bone formation. Sclerostin also stimulates (in neighboring osteocytes) the production of RANKL, leading to bone resorption. In osteoblasts, sclerostin binds to LRP5/6 and inhibits canonical (β-catenin-dependent) activation of the WNT signaling pathway, an action facilitated by LRP4 [130]. The role of sclerostin in bone metabolism was identified in studies of patients with sclerosteosis and van Buchem disease, two rare sclerosing bone dysplasias with very similar phenotypes and high

bone mass [101,100]. This gave rise to the development of anti-sclerostin antibodies, named romosozumab and blosozumab (which are soon expected to on the market) [131–134]. Recent results from Phase I human studies show a dose-dependent effect on increased bone formation and also, importantly, decreased bone resorption [133,134].

Although significant progress has been made in the therapeutic reduction of vertebral fracture risk, non-vertebral fracture risk has only been marginally improved by currently available treatments, defining an unmet medical need. Cortical bone, which comprises 80% of the skeleton, is a major determinant of bone strength and therefore of fracture susceptibility. Most osteoporotic fractures occur at non-vertebral sites and are the consequence of cortical bone fragility [135]. Currently used anti-resorptive drugs reduce the risk of vertebral fractures by up to 70%, whereas the risks for non-vertebral fractures and hip fractures, which are strongly associated with cortical bone mass, are only reduced by 20% and 40%, respectively [136]. This suggests that trabecular and cortical bone might respond differently to signals involved in the regulation of skeletal homeostasis, as recently illustrated by the mechanisms underlying the GWAS-identified *WNT16* [109,110], and this opens a broad array of opportunities to develop new compounds targeting this molecule.

Together, these examples provide a proof of concept of the great potential of incorporating genetic information into the search for suitable drug targets, notably because many new loci are identified that point to potential new biology in or outside the known pathways. This potential has recently been highlighted by a study demonstrating how successful drug mechanisms are predicted by known genetic associations (i.e., the protein product modulated to elicit a clinical response), and how that prediction may change across the drug development pipeline, from preclinical and clinical phases to launched drugs [137]. In fact, this study also showed that the highest degree of genetic support for drug-target indications was related to the musculoskeletal (BMD), metabolic, and blood categories. In this context, drug mechanisms with genetic support succeed twice as often as those without it (from Phase I to approval), and this is the case for osteoporosis drugs, as illustrated in the same paper.

Concluding Remarks and Future Perspectives

Insights arising from genetic investigations can, in the long term, lead to new molecular definitions of bone disease, potentially adding to or replacing the current clinical definitions. However, the translation of these discoveries continues to be hampered by the difficulty of pinpointing the actual genes underlying the GWAS signals, and genetic analyses must therefore be combined with functional studies. Understanding the underlying mechanisms at the cell, tissue, (model) organism, and population levels will be crucial to bridge the gap between concept and clinical application, and to incorporate molecular definitions of disease into medical practice, thereby bringing the field realistically close to personalized medicine.

Outstanding Questions

Many monogenetic conditions affecting bone mass remain unsolved. Are some of the genes underlying these conditions the same as those identified by GWAS of osteoporosis traits? Given the overlap between genes and pathways identified by GWAS and those underlying the occurrence of monogenetic diseases, this is likely and warrants further investigation.

Are the remaining genes identified to underlie monogenetic conditions in families also expected to harbor variants playing a role in osteoporosis trait variation at the population level?

What is the actual function of the large fraction of genes underlying the GWAS signals for which little or nothing is known about their role in bone biology? Functional scrutiny through bioinformatics and wet-lab approximations will be necessary to determine their roles.

What is the influence of the structural configuration of DNA on the regulatory function of genes (i.e., chromatin folding) underlying the GWAS signals?

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Resources

[i] http://hapmap.ncbi.nlm.nih.gov
[ii] www.gefos.org
[iii] www.omim.org
[iv] www.wikigenes.org/e/art/e/185.html
[v] http://visualization.ritchielab.psu.edu/phenograms/plot
[vi] http://cpdb.molgen.mpg.de/CPDB

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