Review Article

The genetic architecture of osteoporosis and fracture risk

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ABSTRACT

Osteoporosis and fracture risk are common complex diseases, caused by an interaction of numerous disease susceptibility genes and environmental factors. With the advances in genomic technologies, large-scale genome-wide association studies (GWAS) have been performed which have broadened our understanding of the genetic architecture and biological mechanisms of complex disease. Currently, more than ~90 loci have been found associated with DXA derived bone mineral density (BMD), over ~500 loci with heel estimated BMD and several others with other less widely available bone parameters such as bone geometry, shape, and microarchitecture. Notably, several of the pathways identified by the GWAS efforts correspond to pathways that are currently targeted for the treatment of osteoporosis. Overall, tremendous progress in the field of the genetics of osteoporosis has been achieved with the discovery of WNT16, EN1, DAAM2, and GPC6 among others. Assessment of the function and biological mechanisms of the remaining genes may further untangle the complex genetic landscape of osteoporosis and fracture risk. With this review we aimed to provide a general overview of the existing GWAS studies on osteoporosis traits and fracture risk.

1. Introduction

Osteoporosis is a progressive silent disease with devastating clinical and economic consequences. Approximately 1/3 of postmenopausal women suffer osteoporosis worldwide and at least half of these will experience a fragility fracture during their lifetime. Fragility fractures are often associated with increased morbidity and mortality, dramatically decreasing quality of life [1–3]. As population age, the prevalence of osteoporosis and its sequelae will increase substantially, becoming one of the largest global healthcare burdens.

Osteoporosis and fracture risk are determined by a complex interplay of genetic and environmental factors. Positive family history of osteoporosis is an important risk factor for fracture, which underscores the pivotal relationship between an individual’s genetic makeup and disease susceptibility. Many monogenic forms of bone fragility have been identified, which are caused by a single mutation in a gene that has a major role in skeletal biology such as observed in X-linked osteoporosis, osteogenesis imperfecta and Paget disease among many others [4]. However, these monogenic mutations explain a very small fraction of the variation in bone mineral density (BMD) and osteoporosis risk in the general population. Just as in other complex diseases, advances in high-throughput genomic technologies, increasing insight on how genetic variation is organized in the genome (i.e., HapMap project) and the availability of large biobank studies have led to the advent of genome-wide association studies (GWAS) in the osteoporosis field. Given the enormous progress in the genomics of osteoporosis, the aim of this review is to provide a general overview of the existing GWAS studies on osteoporosis and fracture risk.

2. Heritability of bone properties

In order to evaluate the genetic architecture of any trait it is important first to establish if that trait is heritable. DXA derived BMD, used for the diagnosis of osteoporosis, is a highly heritable trait (h² = 50–80%) and an excellent biomarker capturing intrinsic properties of bone biology that have led to the identification of hundreds of associated loci [5]. Twin and family studies have also shown that other bone parameters like geometry (h² = 30–70%) [6], bone ultrasound measures (h² = 40–50%) [5] and high resolution peripheral quantitative computed tomography (HR-pQCT) measures of bone microarchitecture (h² = 20–80%) [7] are also highly heritable. Such other determinants of bone strength like geometry, cortical thickness and porosity, trabecular bone morphology and intrinsic properties (quality) of bone tissue contribute to the genetic predisposition to fragility fractures but have been less well-studied due to limitations in sample size. Large samples are needed since all these bone parameters are expected to be highly polygenic (i.e., determined by many variants with small effects).

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3. Fracture risk: an extremely complex trait

Numerous genetic and environmental factors, individually or combined, can cause fracture. These risk factors can act through one or multiple systems, triggering different processes across hormonal/metabolic, biomechanical and material property pathways, which eventually in combination will lead to fracture. Importantly, certain non-genetic factors can exert their influences during different life stages where distinct processes have greater influence like bone accrual in children or bone loss with aging. Genes can act alone or in combination with environmental factors (gene x environment interactions) to have an effect on fracture risk (Fig. 1). Sometimes it may be difficult to distinguish fractures with high environmental influence from fractures having considerable influence from genetic variants. In addition, the musculoskeletal system undergoes adaptation, in principle directed to repair frequent and repetitive micro-damage and to preserve bone-strength to the needs set by strain and stresses. This adaption can also occur in presence of genetic susceptibility to fracture, making the search for fracture genes even more difficult.

Phenotype definition is also an important determinant of the success of GWAS, frequently confronted with a trade-off between accurate trait definition and sample size. Ideally, one would choose to study fractures with a strong hereditary component i.e., low-trauma fractures occurring after falls from a standing height or less which are the consequence of osteoporosis (BMD T-score < −2.5). Nevertheless, it is well established that the majority of fractures occur above the T-Score osteoporosis threshold [8] in individuals with osteopenia (low BMD with −2.5 < T-score < −1.0). Moreover, low-trauma fractures may also occur in individuals with normal BMD (T-score > −1.0), who are for example carriers of rare mutations which affect distinct bone properties such as in the monogenic forms of hypophosphatemic rickets or osteogenesis imperfecta. These fractures occur beyond what is expected from a low BMD level and are typically attributed to impaired “bone quality” – an ambiguous term for bone properties influencing mechanical performance but not well-characterized by BMD. The mechanistic pathways affecting bone quality have been set to arise from three distinct tissue material properties that describe mechanical failure of bone: including tissue strength, fracture toughness and fatigue strength [9]. Lacking to date adequate, affordable and wide-spread methods to assess bone quality, it is no wonder that the majority (if not all) current GWAS have focused on BMD-based and fracture risk traits. Interestingly, as described below all fracture risk loci identified to GWAS are BMD loci, further supporting the mediating role of BMD on fracture. Overall, the GWAS field has prioritized sample size (high powered setting) over the use of accurate phenotype definition as the most cost-effective strategy to reduce the noise introduced by phenotypic heterogeneity and identify real genetic signals.

4. Genetic studies of bone mineral density

The early genetic studies in the osteoporosis field were confined to linkage and candidate gene association studies, which have been particularly focused on BMD. However, these studies have turned out to be typically underpowered leading to negative or irreproducible findings. With the advent of GWAS a new era in bone genomics has begun. The findings from the first GWA study from the Framingham study were initially underwhelming as a result of poor marker density (100,000 SNPs) and inadequate statistical power (N_{total} = 1141) [10]. Thanks to the advances in genomic technologies and statistical genetic methodologies it became clear that the success of GWAS depends on sample
size, effect size of causal genetic variants, the frequency of those variants and the LD between observed genotyped variants and the unknown causal variants [11]. This knowledge has yielded a dramatic change shaping the success of subsequent GWAS, where with the rise of sample size the loci associated with BMD started to increase dramatically.

In early 2008 two GWAS simultaneously identified five common variants associated with BMD variation in the general population. Richards et al. [12] identified two variants associated with lumbar spine and femoral neck BMD mapping to LRPS and TNFRSF11B (OPG) in 8557 individuals. The LRPS variants were also associated with osteoporotic fractures, reproducing the findings of the largest candidate gene study drawn by the GENOMOS consortium [13]. Almost simultaneously, Styrkársdóttir et al. [14] identified in addition to TNFRSF11B (OPG) variants mapping to ESR1, ZBTB40 and the major histocompatibility complex (MHC) loci in 13,786 Icelandic individuals, associated with BMD and osteoporotic fractures. Subsequent work by the same group with expanded sample size (Ntotal = 15,375) identified additional novel variants mapping to/near TNFRSF11A (RANK), SOST, MARK3, and SP7 (osterix) [15]. The latter gene (SP7) was also identified in the first BMD GWAS of children [16] (Ntotal = 5275). Medina-Gomez et al. [17] identified variants in WNT16 associated with skull and total body BMD in children, accompanied by an effort identifying variants in the same locus associated with pQCT and wrist fracture outcomes [18]. The largest yield in discoveries has been facilitated by the rise of collaborative networks giving way to large-scale GWAS meta-analyses that identified novel bone regions and pathways. The first meta-analysis of the GEFOS consortium (Ntotal = 19,195) identified 13 novel loci associated with BMD [19] followed by a second GEFOS meta-analysis (Ndiscovery = 32,961) which replicated the majority of known BMD loci and identified additional 32 novel loci (Ntotal = 83,894) [20].

Fourteen of the BMD-associated loci were also associated with osteoporotic fractures, with those mapping to FAM210A, SLC25A13, LRPS, MEPE, SP7TN1 and DKK1 showing strongest association. This study was also the first to identify sex-specific effects by examining the X chromosome. The variant (rs5934507), associated with BMD mapping to Xp22.31, has been previously associated with male serum testosterone levels [21]. The latter study was followed by additional large meta-analysis by Zheng et al. [22] (described below) which have provided evidence that low-frequency non-coding variants have large effects on BMD and fracture. Next, in 2018 Medina et al. [23] in a meta-analysis of 30 GWAs (Ntotal = 66,628) identified 80 loci associated with total body BMD, of which 36 had not been previously identified. Moreover, in the age-stratified analyses only two loci displayed evidence for age-specific effects, including variants in ESR1 and in close proximity to RANKL. These findings suggest that most of the genes identified throughout the life-course, exert an effect on peak BMD acquisition and this effect can still be observed decades later [23].

Three recent studies have used bone mineral density estimated from heel ultrasound (eBMD), in the UK Biobank Study. In the first effort (Ntotal = 142,487) Kemp et al. [24] identified 203 loci associated with eBMD and in the following effort (Ntotal = 426,924) this number was increased to 518 (301 novel) [25]. In the latter UK biobank setting, Kim identified 613 novel loci using less stringent conditional analysis [77]. These studies highlight the value of expanding the sample size for GWAS and the amazing opportunities to unravel novel biology provided by the approach. To date, >20 GWAS have been published for different bone parameters from which three are large meta-analysis and three are based on the UKBiobank study (Table 1).

The majority of the GWA studies have scrutinized common variants (MAF > 5%). All these efforts have identified variants together explaining 10–20% of the variance in bone phenotypes. It is well established that less-common (rare) variants can have bigger effects than those from common ones. An alternative approach is to focus on individuals with extremely low or high BMD in order to identify rare variants with relatively large effect. In line with this contention, whole-genome sequencing (WGS) efforts have been also successful in mapping rare variants associated with different monogenic conditions. Using WGS and imputation to larger population sets, a rare novel variant (MAF = 0.17%) was associated with low BMD (4931 low BMD cases and 69,034 controls) and fracture risk as a result of rare nonsense mutation within LGR4 (c.376C > T) [26]. A few years later, also using WGS and imputation the same group discovered two rare mutations in COL1A2 associated with low BMD (2984 cases and 206,675 contorts) in participants without signs of osteogenesis imperfecta. In 2018 Duncan et al. [27] performed the most comprehensive extreme phenotype study in 240 individuals from UK with extreme high BMD (Z-scores ≥ +3.2) and 1955 women with high (N = 1055) or low (N = 900) BMD. The analyses yielded two novel loci mapping near NFR5 (rs9292469; MAF = 0.33%) associated with lumbar spine BMD and SPON1 (rs2697825; MAF = 0.17%) associated with total hip BMD. Finally, Zheng et al. [22] using an extremely powerful WGS design identified novel rare variants associated with BMD variation in the general population. The rare noncoding variant mapped to EN1 and showed large effects on BMD (Ntotal = 53,236, effect size = +0.20 standard deviations (SD)) and fracture risk (Ntotal = 508,253, OR = 0.85).

5. Genetic studies of fracture risk

Fracture is the most important clinical outcome of osteoporosis. In the past, most of the genes shown to be associated with fracture risk have been discovered by testing known GWAS BMD loci for association with fracture as described above. To date two GWAS have been performed using vertebral fractures as an endpoint. In the first meta-analysis one locus on chromosome 16q24 (rs11645938) was associated with the risk of radiographic vertebral fractures, which failed to replicate across 5720 cases and 21,791 controls [28]. A recent meta-analysis reported a locus mapping on chromosome 2q13 to be significantly associated with clinical vertebral fractures [29]. The first GWAS study on non-vertebral osteoporotic fractures (N = 700) was performed in elderly Chinese individuals and identified one fracture-associated locus within the ALDH7A1 gene [30]. However, this gene failed to replicate in any of the larger European meta-analyses. In 2018, Trajanoska et al. [31] conducted the largest GWAS on osteoporotic fractures to date comprising 37,857 cases and 227,116 non-cases with replication in up to 300,000 individuals (147,200 cases). Altogether, the effort identified 15 fracture loci with modest effects. Interestingly, all identified loci were known BMD loci. Overall, the effect of these SNPs on fracture was smaller than the effect on BMD (Fig. 2). Thus, the genetics of any-type of fractures in the general population is mediated through the genetic influence on BMD. This is well characterized by the genetic correlations of fracture risk with BMD. Further, among 15 tested clinical factors (including vitamin D levels and milk calcium intake) only BMD had a major causal effect on fracture [31].

6. Genetic studies of other bone parameters

Studies have been performed on other bone parameters. Loci mapping near RANK/OPG have been associated with cortical volumetric BMD [32,33]. While genetic variants in the FNM2/GREM2 locus were associated with trabecular volumetric BMD and fracture risk [33]. Moreover, five loci have been reported to be associated with lumbar spine volumetric BMD (Ntotal = 15,275) mapping near WNT4 and ZBTB40, TNFRSF11B, AKAP11, and TNFSF11; from which two loci (5p13 and 1p36.12) were associated with vertebral fractures [34]. Several GWA studies have identified RAP1A, TBCLD8, and OSBP1L1 to be associated with hip structure analysis (HSA) parameters [35]. Finally, Baird et al. [36] identified nine loci associated with hip shape. Seven SNPs were within 200 kb of genes involved in endochondral bone formation, namely SOX9, PTHP, RUNX1, NKKX3-2, FGFR4, DICER1, and HHIP [36].
Table 1
GWASs for different bone parameters and osteoporotic fractures.

<table>
<thead>
<tr>
<th>Study</th>
<th>Trait</th>
<th>Sample size</th>
<th>Total number of GWS loci</th>
<th>Total number of GWS novel loci</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richards et al. (2008)</td>
<td>Lumbar spine and femoral neck BMD</td>
<td>8557</td>
<td>2</td>
<td>2</td>
<td>TNFRSF11B, LRP5</td>
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<tr>
<td>Styrkarsdottir et al. (2008)</td>
<td>Lumbar spine and femoral neck BMD</td>
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<td>4</td>
<td>3</td>
<td>RANKL, OPG, ESR1, 2B7B40, MHC</td>
</tr>
<tr>
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<td>Lumbar spine and femoral neck BMD</td>
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<td>7</td>
<td>4</td>
<td>SORT, MARK3, SP7, TNFRSF11A</td>
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<tr>
<td>Timpson et al. (2009)</td>
<td>Lumbar spine and femoral neck BMD</td>
<td>5275</td>
<td>1</td>
<td>1</td>
<td>SP6</td>
</tr>
<tr>
<td>Xiong et al. (2009)</td>
<td>Lumbar spine and femoral neck BMD</td>
<td>9109</td>
<td>2</td>
<td>2</td>
<td>ADAMTS18, TGFBR3</td>
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<tr>
<td>Rivadeneira et al. (2009)</td>
<td>Lumbar spine and femoral neck BMD</td>
<td>19,195</td>
<td>20</td>
<td>13</td>
<td>GPR177, SPTBN1, CTNNB1, MEPE, MER2, STARD3NL, FLM2280L, LRP4, ARHGAP1, F2, DC2CS, SOX6, FOXI1, HDAC3, CRHR1</td>
</tr>
<tr>
<td>Zhao LJ et al. (2009)</td>
<td>Femoral neck bone geometry</td>
<td>5676</td>
<td>1</td>
<td>1</td>
<td>RTP3</td>
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<tr>
<td>Guo et al. (2010)</td>
<td>Hip BMD and osteoporotic fractures</td>
<td>11,568</td>
<td>1</td>
<td>1</td>
<td>ALDH7A1</td>
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<td>Kung et al. (2010)</td>
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<td>1</td>
<td>JAG1</td>
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<tr>
<td>Hsu et al. (2010)</td>
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<td>11,290</td>
<td>4</td>
<td>0</td>
<td></td>
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<tr>
<td>Koller et al. (2010)</td>
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<td>2190</td>
<td>0</td>
<td>0</td>
<td></td>
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<tr>
<td>Paternoster L et al. (2010)</td>
<td>Cortical vBMD</td>
<td>5789</td>
<td>1</td>
<td>0</td>
<td>RANKL</td>
</tr>
<tr>
<td>Duncan et al. (2011)</td>
<td>Extreme high or low hip BMD</td>
<td>21,798</td>
<td>2</td>
<td>2</td>
<td>GALNT5, RSP03</td>
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<tr>
<td>Estrada et al. (2012)</td>
<td>Lumbar spine and femoral neck BMD</td>
<td>83,894</td>
<td>56</td>
<td>32</td>
<td>*DNM3, ANAPC1, LEKR1, IDUA, WNT16, FUBP3, MPP7, MBL2/DKK1, AXIN1, SORCS9, FAM98/ KAL1</td>
</tr>
<tr>
<td>Zheng et al. (2012)</td>
<td>Cortical bone thickness, forearm BMD and</td>
<td>5878</td>
<td>1</td>
<td>0</td>
<td>WNT16</td>
</tr>
<tr>
<td></td>
<td>fracture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medina-Gomez et al. (2012)</td>
<td>Total Body BMD</td>
<td>13,712</td>
<td>1</td>
<td>0</td>
<td>WNT16</td>
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<tr>
<td>Paternoster L et al. (2013)</td>
<td>Cortical and trabecular vBMD</td>
<td>6930</td>
<td>5</td>
<td>1</td>
<td>RANK1, OPG, ESRR, LOC28575S, FNM2/CREM2</td>
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<tr>
<td>Zheng et al. (2013)</td>
<td>Lumbar spine, hip and femoral neck BMD</td>
<td>27,061</td>
<td>15</td>
<td>2</td>
<td>SMOCL, CLDN14</td>
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<tr>
<td>Moayeri A et al. (2014)</td>
<td>BUA, VOS, BMD</td>
<td>15,514</td>
<td>9</td>
<td>1</td>
<td>TMEM135</td>
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<tr>
<td>Oei et al. (2014)</td>
<td>Radiological vertebral fractures</td>
<td>2995</td>
<td>1</td>
<td>1</td>
<td>16q24</td>
</tr>
<tr>
<td>Zheng et al. (2015)</td>
<td>Lumbar spine, femoral neck and forearm BMD</td>
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<td>1</td>
<td>EN1</td>
</tr>
<tr>
<td>Pei et al. (2016)</td>
<td>Hip trochanter and intertrochanteric BMD</td>
<td>9174</td>
<td>3</td>
<td>1</td>
<td>RP11-384F7.1</td>
</tr>
<tr>
<td>Mullin et al. (2017)</td>
<td>BUA and VOS</td>
<td>16,627</td>
<td>8</td>
<td>3</td>
<td>PIPJR3B, LOC387810, SEPT5</td>
</tr>
<tr>
<td>Kemp et al. (2017)</td>
<td>heel BMD</td>
<td>142,487</td>
<td>203</td>
<td>153</td>
<td>*G0P0</td>
</tr>
<tr>
<td>Medina-Gomez et al. (2018)</td>
<td>Total Body BMD</td>
<td>66,628</td>
<td>80</td>
<td>36</td>
<td>*REB1, CSF1, SLCA1, PLC1, AQP1, SMO99, TOM112, ADAM4S, ETS2</td>
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<tr>
<td>Duncan et al. (2018)</td>
<td>Extreme high or low BMD</td>
<td>2196</td>
<td>4</td>
<td>2</td>
<td>NPRS, SPON1</td>
</tr>
<tr>
<td>Kim (2018)</td>
<td>heel BMD</td>
<td>394,929</td>
<td>899</td>
<td>613</td>
<td>*WNT1, RSP03, ESR, SPTBN1</td>
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<tr>
<td>Morris et al. (2018)</td>
<td>heel BMD</td>
<td>426,824</td>
<td>518</td>
<td>301</td>
<td>*DAAM2</td>
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<td>Nerea et al. (2018)</td>
<td>Clinical vertebral fractures</td>
<td>10,683</td>
<td>1</td>
<td>1</td>
<td>2q13</td>
</tr>
<tr>
<td>Trajanoska et al. (2018)</td>
<td>Any type of fractures</td>
<td>562,258</td>
<td>15</td>
<td>9**</td>
<td>SPTBN1, CTNNB1, RSPO3, ESR, WNT16, STARD3NL, GRB10, FUBP3, MBL2/DKK1, LR5, SOST, FAM98A, ETS2</td>
</tr>
</tbody>
</table>

BMD = Bone mineral density, BUA broadband ultrasound attenuation, VOS = velocity of sound, DXA = Dual energy x ray absorptiometry, v = volumetric. *due to the large number of genes the functional validated genes or a smaller curated list is presented. ** Novel in relation with fracture all of the loci are known BMD loci. † closest genes.
7. Variance explained in bone traits by bone-associated variants

Harnessing the information from GWAS can help improve risk prediction of a particular disease; ultimately, this information can then be used for the prevention, diagnosis, prognosis, and treatment of a particular disease [37]. However, clinically-relevant prediction was not achieved by earlier genomic studies in the osteoporosis field; i.e., genetic risk scores did not substantially increase osteoporosis or fracture risk discrimination above the use of traditional clinical risk factors, probably as a result of low variance explained by the genetic variants [20]. The discovery of new loci using more powerful settings have led to substantial leaps in the variance explained (Fig. 3) which will substantially improve risk prediction models in the osteoporosis field. So far, common to less-frequent genetic variants explain around 20% of the BMD variation, which is up to two-fold higher of what can be achieved through the use of other traditional risk factors for osteoporosis such as age or weight (8–9%) [38]. Moreover, the combination of genetic factors, height, weight, age, and sex explains around 25% of the variance in eBMD [77]. This implies that the use of genetic markers has started to materialize in the clinical setting.

8. Biological pathways underlying common bone conditions

Impressively, a large proportion of the genes discovered by GWAS are located in well-known bone-active pathways. Overall, there are five main pathways crucial for bone metabolism i) Mesenchymal cell differentiation ii) WNT, iii) NOTCH, iv) Hedgehog and v) OPG-RANK-RANKL signalling pathways.

8.1. Mesenchymal cell differentiation pathways

Mesenchymal stem cells (MSC) have the capacity to differentiate into several cell lineages including osteogenic and chondrogenic. Numerous transcription factors (TFs) such as RUNX2, Osterix (Ox), SOX9, can induce the osteogenic differentiation of MSC [39–41]. These factors have been also identified in various BMD GWAS. RUNX2 is involved in both chondrocyte osteoblast differentiation [42] and is crucial for the early stages of osteoblast development whereas the Ox effects are more pronounced in the later stages (i.e. pre-osteoblast differentiation into functional osteoblast) [43].

8.2. WNT signalling

WNT signalling plays a pivotal role in bone development during embryogenesis and bone formation, resorption and accrual during postnatal growth. WNTs are proteins secreted from the cells and regulate the proliferation, differentiation, and apoptosis of bone cells [44]. There are several pathways that can be activated by Wnt proteins
among which the Wnt/β-catenin (canonical) pathway is the most important one for bone biology. This pathway is activated when WNTs proteins bind to the Frizzled membrane receptors and the low-density lipoprotein (LDL) receptor-related protein 5/6 (LRP5/6). Thereupon the stabilized β-catenin is translocated to the nucleus where it binds to the TFs LEF1/TCF and initiates the transcription of the target genes. Many genes within the Wnt pathway have been identified by GWAS: LRP5, WNT16, AXIN1, CTNNB1, DKK1, WLS, LPR4, MEF2C, RSPO3, SERP4, SNT16, SOST, WNT4, WNT5B, and EN1. LRP5 (encoding LDL receptor-related protein 5) is one of the first genes discovered in the Wnt signalling since it acts as a co-receptor that binds Wnt proteins with Frizzled-receptors. Functional studies have shown that LRP5 can lead to low bone mass (loss-of-function) and high bone mass (gain-of-function mutation) [49]. LRP5 can be inhibited by several factors such as sclerostin and the dickkopf (DKK) proteins, thus, inhibit the formation of new bone. WNT16 biology has been confirmed by many GWAS studies in relation with different bone parameters such as areal BMD, ultrasound BMD, cortical thickness and fracture, both in adults and children [17,18,20,24,31] and in perimenopausal women [32]. Fracture, both in adults and children [17,18,20,24,31] and in perimenopausal women [32].

8.3. NOTCH signalling

In the skeleton, both osteoblasts and osteoclasts require NOTCH signalling for proper differentiation and function, and the specific roles of NOTCH are dependent on the differentiation status of the cell [57]. 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 [58] through several ligands (Dll1, Dll3, Dll4, JAG1, JAG2) binding to receptors expressed on the surface of neighboring cells. The ligand binding induces proteolytic cleavage and releases the NOTCH intracellular domain (NICD), which enters the cell nucleus and the transcription of NOTCH targeted genes starts. There is also crosstalk between the NOTCH and WNT signalling pathways which precise mechanisms are still not clear. Several BMD genes have been related to this pathway such as JAG1, MAPT, and NOTCH2.

8.4. Indian Hedgehog (IHH) signalling

The IHH signalling pathway consists of essential signalling molecules crucial for intra-membranous 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, hypertrophy, and apoptosis to be eventually replaced by osteoblasts in the ossification centres [59]. IHH signalling may also regulate osteoblast differentiation during endochondral bone development in interaction with WNT/β-catenin [60]. Within this cross-pathway signalling there are also several GWAS-identified genes such as WNT1, WNT4, WNT5B, WNT16, DHH, and PTC1. The latter gene encodes the patched −1 receptor (Ptc1) which is essential for many developmental processes such as osteoblastogenesis and chondrocyte differentiation [61]. Patched1 haploinsufficiency (Ptc1+/−) is characterized by increased adult bone mass in mice; while in culture cells exhibited accelerated osteoblast differentiation [62].

8.5. OPG–RANK–RANKL signalling

The OPG–RANK–RANKL signalling pathway predominantly regulates the coupling between osteoblasts and osteoclast activity [63]. Osteoblasts secrete the receptor activator of NF-κB ligand (RANKL) which binds to its receptor activator of NF-κB (RANK) on monocytes resulting in osteoclast differentiation and activation the presence of monocytic colony-stimulating factor (M-CSF). OPG also secreted by the osteoblast is a decoy receptor of RANKL and blocks osteoclast induction...
by competing with RANK to bind RANKL. These factors are part of the tumour necrosis factor (TNF) superfamily of ligands and receptors and have been shown to have other functions beyond bone remodelling, including potential roles in other disease processes (i.e., vascular calcification, diabetes and cancer). TNFRSF11 (RANKL), TNFRSF11A (RANK) and TNFSF11B (OPG) were also one of the first genes associated with osteoporosis found and replicated by several studies. Functional studies have shown that in RANK−/− mice the generation of osteoclasts from their myeloid progenitors is blocked resulting in absence of bone resorption and severe osteoporosis [64]. Although this pathway has not been associated with fracture risk it holds an important role in increasing BMD and decreasing fracture risk as due to its antiresorptive effects discusses below.

9. The fulfilled and unfulfilled promises of genomics

The ultimate goal of osteoporosis genomics after GWAS is to perform functional validation, that will allow translating the discoveries into clinical practice. It has been shown that genetic information may significantly improve the search of drug targets [65] and may increase the success rate of preclinical and clinical trials. Nowadays, most of the osteoporosis agents in use (or undergoing trials) target pathways related to the discovered BMD genes. Denosumab is a human monoclonal antibody which binds to RANKL and inhibits bone resorption by preventing RANKL from activating RANK, its receptor on the osteoclast surface. Such mimicking of the decoy action of OPG reduces the formation, activation, and survival of osteoclasts [66]; This RANKL inhibitor is approved for use in postmenopausal women with risk of osteoporosis. Since its approval, it has shown sustained efficacy in increasing BMD and decreasing vertebral fracture risk [67]. Anabolic agents hold promising potential, constituting bone-building drugs with proven success, such as PTH 1–34 (teriparatide) and PTHrP 1–36 (abaloparatide), which stimulate osteoblasts to make new bone. GWAS have identified genes in the PTH pathway including PTHLH [20]. Teriparatide, is the first anabolic agent approved for the treatment of osteoporosis. In clinical trials to date, abaloparatide has shown promising results in a reduction of new-onset vertebral (approximately 86% reduction) and nonvertebral fractures (approximately 43% reduction) [68]. Finally, several GWAS on BMD and fracture risk have identified variants in several genes (SOST-sclerostin, DKK1, LRPs, LRP4, AXIN1, and CTNNB1-β-catenin) in pathways with anabolic potential [20]. Sclerostin (which is produced by osteocytes) inhibits the proliferation, differentiation, and survival of osteoclasts, leading to reduced bone formation. Sclerostin also stimulates (in neighboring osteocytes) the production of RANKL, leading to bone resorption. Anti-sclerostin antibodies, Romosozumab and Blosozumab, have been developed to counteract the sclerostin effects and have undergone phase II clinical trials [69]. Only Romosozumab was followed to phase III where it showed a 73% reduction in vertebral fracture risk and a 36% reduction in clinical fractures at 1 year (reviewed by Reid et al) [70]. Overall the current agents have been mainly successful in reducing the risk of vertebral fractures (up to 70%), whereas reduction rates for non-vertebral fractures and hip fractures were of smaller magnitude (20% and 40%, respectively) [71]; indicating that trabecular and cortical bone might be regulated by different biological pathways or respond differently to signals involved in the regulation of skeletal homeostasis, as recently shown by the WNT16 contrasting mechanisms of action [52,72].

9.1. Identification of causal genes for bone traits

It is a common approach to annotate the closest gene to a SNP with the lowest p-value as the most likely causal gene. However, it is important to note that the physical distance of a variant to a gene is not substantive evidence of causality [73]. Multitude of statistical and functional fine-mapping methods have been developed in order to prioritize causal variants (reviewed by Spain et al) [73]. These methods are essential for highly polygenic traits such as bone density and can drastically improve the mapping of associated loci to their causal genes. For example, in the latest and largest GWAS to date in the field of osteoporosis, Morris et al. [25] have used UK Biobank data to build a robust analysis pipeline to prioritize eBMD-associated SNPs by combining several distinctive approaches. Firstly, two statistical fine-mapping methods were used to refine associated SNPs at each locus. These methods identify SNPs based on their conditional independence (GCTA-COJO) and posterior probability (log10 Bayes factor > 3) for causality (FINEMAP); yielding two conditionally independent SNPs and five SNP loci with a log10 Bayes factor > 3 per locus. Second, all fine-mapped SNPs were then tested for enrichment for missense protein coding SNPs, DHs (DNase I hypersensitive sites) from primary osteoblasts, and ATAC-seq (Assay for Transposable-Accessible Chromatin using sequencing) peaks from SaOS-2 osteosarcoma cells. Notably, the fine-mapped SNPs showed strong enrichment for both missense variants in protein-coding regions and osteoblast open chromatin sites. Finally, a list of positive control genes identified through pharmacotherapy or Mendelian disease which are critical for bone biology were curated. Six distinctive genomic features that link a SNP to a gene such as cell-specific 3-dimensional (3D) contact domains, cell-specific open chromatin states, physical proximity, and the presence of associated coding variation were used to construct Target Gene sets which were tested for enrichment for positive control bone genes. All six methods for linking fine-mapped SNPs to Target Genes yielded strong enrichment for positive control genes known to be central to bone biology [25]. Notably, Target Genes closest to fine-mapped SNPs in osteoblast-derived ATAC-seq peaks were seen to be ~60-fold enriched for positive control genes. This is quite an insightful finding, as transcription factor binding occurs mainly in regions of open chromatin (ATAC-seq peaks), thus, this implies that the mapped Target Genes may have substantial impact on bone biology.

10. Post-GWAS analyses and concluding remarks

In the fast-moving world of genomics, including the field of osteoporosis, hundreds of genetic markers have been identified as associated with complex traits. As discussed above the GWAS findings have aided the discovery of several novel osteoporosis drug targets. However, there is still an overwhelming amount of significant associations, for which the underlying biological mechanisms remain unknown; as the functional characterization of the discovered genetic variants have lagged far behind [74]. In order to understand the functional consequences of these loci future post-GWAS methods should be focused on bridging the gap between disease-associated loci and underlying disease biology [74]. An essential step in the translation studies, from gene discovery to biological mechanisms, is the identification of causal variants and genes. It is well known that the most strongly associated variants with a specific trait or diseases are likely to be in linkage disequilibrium (LD) with the causal variant, rather than have a biological function themselves [73]. In the last few years large numbers of studies have been devoted to pinpoint causal variants using both statistical evidence (e.g. reference panels, targeted resequencing, Bayesian methods, machine learning methods) from large association data sets and functional annotations of genetic variants (e.g. enrichment analysis, pathway prioritization, DNase sensitivity) [73,75]. Moreover, the emergence of expression quantitative trait loci (eQTLs), which characterize associations between genetic variants and gene expression at the cellular level, have provided a better biological context in disease studies [76]. Although a widely used approach, eQTL data from primary bone cells is limited. Establishing bone specific eQTLs may significantly improve the search of causal variants and provide valuable target genes. Finally, the identification of causal variants can further facilitate the interpretation of the GWAS findings and opens opportunities for more detailed downstream functional investigations such as in human and/or animal
cell and tissues models. GWAS have provided us with a comprehensive understanding of the genetic architecture of osteoporosis and fracture risk. Moreover, key bone genes and pathways have been identified which have prompted novel drug targets and treatments. In the decade to come, the advances of GWAS and post-GWAS techniques and methods will enable fruitful incorporation of genetics in clinical practice that will ensure better disease prediction and risk stratification, leading to overall improvement in disease prevention or intervention.

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References

Fig. 2 was modified from Estrada et al.


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