The case for genome-wide association studies of bone acquisition in paediatric and adolescent populations

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Abstract:

Peak bone mass, the maximum amount of bone accrued at the end of the growth period, is an important predictor of future risk of osteoporosis and fracture. Hence, the contribution of genetic factors influencing bone accrual is of considerable interest to the osteoporosis research community. In this article, we review evidence that genetic factors play an important role in bone growth, describe the genetic loci implicated so far, and briefly discuss lessons learned from the application of genome-wide association studies. Moreover, we attempt to make the case for genetic investigations of bone mineral density in paediatric and young adult populations, describing their potential to increase our knowledge of the process of bone metabolism throughout the life course, and in turn, identify novel targets for the pharmacological treatment of osteoporosis.

Introduction:

Peak bone mass is defined as the maximum amount of bone accrued throughout the life-course [1]. It accounts for more than half of the variability in bone mineral density (BMD) in the elderly and as such, represents an important predictor of future risk of osteoporosis and fracture [2]. It has been estimated that a 10% increase in peak bone mass could decrease subsequent fracture risk in postmenopausal women by up to 50% [3]. Therefore, optimizing peak bone mass represents a promising intervention strategy for preventing osteoporosis. Epidemiological studies have identified numerous environmental factors (e.g. physical activity, nutrition and lifestyle behaviours) that modulate bone acquisition [4]. In addition, they have demonstrated that intervention strategies targeting these modifiable risk factors result in gains in peak bone mass that persist into later life [4]. However, individuals at high risk of osteoporosis are often only identified after they present with low trauma fracture, minimizing the impact of the above-mentioned interventions. Furthermore, the majority of pharmacological treatments for osteoporosis function as anti-resorptives that halt further bone loss, but fail to fully restore bone quantity and quality. Only one osteoanabolic drug (i.e. Teriparatide) is presently FDA approved; however, it is far from ideal as it is expensive and requires daily administration via injection to ensure adequate bone formation [5]. For these reasons, there is considerable scope for identifying novel anabolic pathways that could in principle be targeted by new pharmacotherapies.

Genetic studies, and in particular genome-wide association studies (GWAS), offer one means to discover biological mechanisms relevant to osteoporosis pathophysiology. For example: the GEnetic Factors for Osteoporosis (GEFOS) consortium recently performed a GWAS of adult BMD that encompassed up to 84,000 adults; and detected 56 loci associated with this trait, including several regions containing genes (or their pathways) targeted by existing

pharmacotherapies [6]. Despite this success, only ~5.8% of the estimated heritability of adult BMD has been accounted for [6], suggesting that many more genetic variants remain to be discovered, thereby creating further opportunities to identify novel drug targets. It is conceivable that larger GWAS of adult and elderly individuals would provide such an opportunity. However, a complementary strategy would be to perform GWAS of BMD in cohorts of children and/or young adults. This strategy may prove valuable in finding additional loci, mainly due to increased power to target specific loci regulating bone acquisition and peak bone mass attainment, whose effects can be masked in elderly populations due to the accumulation of differing environmental influences over many years [7-9]. In addition to identifying a complementary set of variants, studies involving younger individuals may also provide a better understanding of the genetic architecture underlying variation in BMD across the life course [10,11].

In this review, we attempt to make the case for genetic investigations of BMD in paediatric and young adult populations. In so doing, we summarize the current knowledge of the genetic architecture of BMD in young individuals. We discuss lessons learned through the study of BMD in these populations, including the discovery of BMD-associated variants that display marked age heterogeneity and the detection of loci that are preferentially associated with BMD at different skeletal sites. Furthermore, we provide an outline of the GWAS results of bone-related phenotypes of paediatric and young adult populations. Finally, we discuss the success of these endeavours in identifying molecular mechanisms that influence bone growth and bone mineral acquisition and highlight some of the emerging genetic methodologies and resources that may improve our understanding of bone accrual and osteoporosis pathophysiology.

Genetic architecture of pediatric and adolescent BMD

Twin and family studies indicate that BMD is a highly heritable trait, with estimates ranging between 50 - 85% [12-14]. Even though these estimates can vary due to the analytical model used, the skeletal site measured and the population under study, some evidence suggests that heritability of BMD may be greater at younger ages [15]. Although the genetic architecture underlying normal variation in paediatric and adolescent BMD is still subject of study, there is now considerable empirical evidence to support the idea that a substantial proportion of the heritability of peak bone mass attainment is present in the form of many variants of small (yet real) effect scattered across the genome. Specifically, results of a new statistical methodology known as GREML (i.e. genetic restricted maximum likelihood [16]) have indicated that between one third to one half of the variance in paediatric BMD is tagged by common single nucleotide polymorphisms (SNPs) that are present on commercially available genome-wide genotyping arrays (i.e. termed the SNP heritability) [10].

In 2009, the first BMD GWAS involving a pediatric population was reported. Although underpowered, it implicated Osterix (*SP7*), an osteoblast transcription factor, in the variability of total-body (less head) derived BMD (TB-BMD) measures in ten-year-old children [17]. Suggestive associations between variants in *SP7* and BMD had previously been observed in a larger meta-analysis of adults [18] and later confirmed in a subsequent GWAS study of adult BMD by the GEFOS consortium [6], implying that GWAS of pediatric BMD might be an alternative method of identifying BMD related loci. Subsequently, up to 15 different loci have been robustly associated with BMD in children and young adults (**Figure 1**). As BMD at any age is considered to be a function of the peak bone mass accrued, it is not surprising that the majority of these loci are associated with BMD in adults [6,19,20]. Remarkably, adult GWAS studies were performed on sample sizes that were at least twice the size of GWAS encompassing younger individuals who had not yet attained peak bone mass.

Age-dependent effects

Bone mineral density reflects a combination of physiological processes across the life course including: i) the acquisition of bone mass from early childhood to mid-adulthood, mediated mainly by the process of bone modelling, ii) the subsequent maintenance of bone mass from mid to late adulthood, via bone remodelling, and iii) the progressive loss of bone in later life, when less bone is formed than resorbed [21,22]. It is possible that genetic variants related to bone mineral density display age-dependent effects- that is, some bone associated variants might be more strongly related to developmental processes that occur in childhood and adolescence as compared to those that occur during adulthood [11]. As mentioned before, BMD GWAS of childhood and adolescent populations may offer as well a more powerful locus detection setting (as compared to adults). BMD measured early in the life-course, may be less influenced by the cumulative effect of non-genetic (i.e. environmental or lifestyle) factors. As such, when analysing paediatric and adolescent BMD gains in power may be achieved via increased effect sizes and/or a reduction in the residual variance.

Robust evidence suggesting that some genetic variants may display age-dependent effects on BMD was first reported by a study encompassed ~2,200 six-year-old children from the Generation R Study, and an additional five cohorts that represented distinct age groups, ranging from 10 – 75 years (n = 11,052) [11]. Variants mapping to *CPED1* [also known as *C7Orf58*, (7q31.31)] showed a larger effect on skull BMD in children as compared to older individuals. Although the role of *CPED1* in bone biology remains to be elucidated, efforts involving functional follow-up of the locus in animal models are underway.

To date, the largest GWAS meta-analysis of pediatric BMD was completed in 2014, and comprised ~9,395 children aged between 5 and 11 years [10]. Six adult-BMD associated loci (*WNT4*, *WNT16*, *TNFSF11*, *GALNT3*, *PTHLH* and *FUBP3*) and a novel locus (*RIN3*) were robustly associated with TB-BMD. Variants within or in the neighborhood of *RIN3* have not been implicated in adult GWAS of hip and spine BMD to date [6,19] possibly due to the existence of age-dependent effects. However, since child and adult BMD data were obtained at different skeletal sites in these studies, we cannot exclude the possibility that variants at *RIN3* operate in a site-dependent fashion. Furthermore, it should be noted that in terms of bone research, *RIN3* is not a novel locus as variants within this locus have previously been associated with Paget's disease of bone (PDB) [23], a late-onset disorder of the skeleton.

Several studies have evaluated the role of 63 genetic variants, located in 56 different loci, known to influence BMD in adults [6] using a genetic risk score (GRS) approach. These variants collectively explained ~2.5% of the TB-BMD variation in two independent, multiethnic cohorts of school age [24]. Moreover, Warrington and colleagues also investigated the association between the rate of change in TB-BMD (spanning an 8-year period ranging from 9 – 17 years of age) and the GRS. Their analysis indicated that each adult-BMD lowering allele was robustly associated with: i) a mean decrease in BMD (centered at age 13) and ii) an overall reduction in the rate of bone acquisition across childhood and adolescence. Analyses of individual loci making up the risk score, found that SNPs in 11 loci (*AXIN1*, *FUBP3*, *SPTBN1*, *RSPO3*, *ABCF2*, *WNT16*, *CPED1*, *ZBTB40*, *WNT4*, *WLS* and *RPS6KA5*) exerted detectable effects on BMD at age 13. Furthermore, five loci influenced the rate at which BMD accrued (KIAA2018, *ESR1* and *ZBTB40*) [9].

Two recent studies by Mitchell and colleagues report interactions between adult BMD associated loci and BMD/BMC Z-scores with chronological age [25] or sexual maturation [26] using a relative small sample (n ~ 800) of children and adolescents from the Bone Mineral Density in Childhood Study, which were followed up over a six-year period. In the first study different GRS were generated using adult BMD associated loci, of which three were composed of: i) all loci, ii) loci that contain genes involved in the WNT signaling pathway and iii) loci robustly associated with increased fracture risk. All three GRS showed association with lower Z-scores at hip, femur, spine and total body. Further, an interaction with chronological age was observed for the fracture GRS at all sites, being more strongly associated with increased age. In the second study, individual adult BMD-associated loci were investigated using forearm, hip, spine and total body BMD Z-scores. Evidence of an interaction with pubertal stage was detected for 23 of these loci. Interestingly, GRS-sex interactions were also observed in both studies.

Altogether, the results of these studies, suggest that whilst the effects of a number of BMD associated loci is age-dependent, the effect of the majority is detectable throughout the life-course, indicating that their role in bone growth and mineral acquisition early in the life-course contributes to the variation in adult BMD. This is plausible, considering that peak bone mass is thought to account for more than half the variability in adult bone mass [2]. Alternatively, it may also suggest that these loci continue to regulate bone acquisition throughout the life-course, perhaps a consequence of the bones continued expansion via periosteal apposition, and their ability to change shape and size in response to mechanical loading (i.e. modelling).

Skeletal site-specific effects

GWAS studies of adult BMD have reported evidence of heterogeneity, in which specific loci are more strongly associated with BMD at the femoral neck than at the lumbar spine or vice

versa [6,19]. This heterogeneity may be a consequence of a number of factors including the different types of bone measured at the sites (i.e. the proportion of cortical versus trabecular bone) or differences in biomechanical response (i.e. mechanical loading). It is possible that this form of heterogeneity is also present at other sites across the body [27-29]. Studies of paediatric BMD represent an ideal setting in which to test this hypothesis as total body DXA scans are typically used to measure BMD in children, whereas most adult studies are limited to measurement of BMD at the hip, spine and forearm. Total body DXA measurements can be partitioned into distinct skeletal sub-regions, including the skull, upper-limbs and lower-limbs. This is extremely advantageous as it enables one to investigate skeletal sites that differ in terms of their exposure to loading [i.e. skull (low), upper-limbs (intermediate) and lower-limbs (high)]. Furthermore, partitioning permits the investigation of molecular mechanisms regulating growth and development that may differ across sites. For example, the vault of the skull arises mainly through intramembranous ossification and is primarily made up of flat dermal bones that are cortical in nature [30]. In contrast, upper- and lower-limbs consist of long bones that are made up of broadly equivalent amounts of cortical and trabecular bone that collectively develop from a cartilaginous template during endochondral ossification [31].

To determine whether genetic factors contribute to the skeletal site-specific differences mentioned above, GREML analysis was used to investigate the genetic contribution to BMD measured at the skull, upper- and lower-limbs in a cohort of ~5,300 ten year old children [10]. SNP heritability estimates indicated that the common variants present on genotyping arrays, explained a larger proportion of the overall variance of BMD at the skull, when compared to BMD measured at the appendicular sites (i.e. lower- and upper-limbs) [10]. These differences possibly reflect the differential exposure of each skeletal site to varying environmental stimuli that influence BMD. Specifically, mechanical loading, as compared to appendicular sites, may

influence the skull to less of an extent. To explore this notion further, residual correlation across the different skeletal sites (i.e. the correlation between BMD measures at sites due to environmental factors and other sources of variation not tagged by SNPs on the array) was also estimated. Results suggested that whilst the environmental (and other residual) factors influencing the appendicular sites were moderately similar to each other, they appeared to be appreciably different from the factors influencing the skull. Taken together, the SNP heritability, coupled with a high residual correlation between the two appendicular sites, may reflect the greater exposure of these sites to loading and muscular stimulation, when compared to the skull. Likewise, estimates of the genetic correlations indicated that the appendicular limbs shared a more similar genetic architecture with each other than the skull [10], possibly reflecting the composition of bone at each skeletal site and/or the biological processes that govern their growth and maintenance.

To further explore the basis for the above-mentioned differences in genetic architecture, we performed GWA meta-analyses of sub-regional TB-DXA data, collectively identifying SNPs in fifteen loci that exceeded the genome-wide significance threshold at one or more skeletal sites (i.e. SNPs at *WNT4*, *GALNT3*, *CPED1*, *WNT16*, *FAM3C*, *RSPO3*, *FUBP3*, *PTHLH*, *TNFSF11*, *TNFRSF11B*, *TNFRSF11A*, *LRP5*, *LGR4*, *RIN3* and *EYA4*) [10]. A comparison of the effects of all fifteen loci across each skeletal site echoed the findings from the GREML analyses, and supported the idea that although the underlying genetic architecture influencing BMD appears to be largely similar, it varies according to skeletal site. Variants at *TNFRSF11A*, *TNFRSF11B*, *EYA4*, *RSPO3* and *LGR4* showed some evidence for site specificity, being most strongly associated with BMD at the skull, suggesting a stronger effect in the absence of habitual mechanical loading. Other patterns of site-specificity were observed which are more difficult to explain. For example, variants at *CPED1* were associated with BMD at the skull

and upper-limbs, but not with lower-limbs, whereas variants at *WNT16* were most strongly related to upper-limbs when compared to the lower-limbs and skull.

Further Phenotypic Refinement

DXA measures of BMD are only partially corrected for bone size. As a consequence, DXA derived BMD also reflects differences in bone growth and overall skeletal size, making is difficult to evaluate the independent effects of true bone density. In addition to this limitation, DXA is unable to differentiate trabecular from cortical bone and therefore fails to account for true volumetric density and other geometric and micro-architectural properties that primarily determine bone strength and quality in younger populations (i.e. periosteal expansion, cortical density and thickness, trabecular number and thickness) [32,33]. For these reasons, alternative-imaging technologies, including peripheral quantitative computer tomography (pQCT), are increasingly being used to identify novel determinants of bone strength. The primary advantage of pQCT over DXA is its ability to measure different constituents of bone mass separately [i.e. cortical and trabecular bone volumetric density (vBMD)], whilst fully adjusting for skeletal size by measuring bone slices of fixed thickness [34]. As a result, pQCT measures offer distinct advantages over DXA in terms of identifying genetic correlates of refined bone phenotypes, especially considering that the genetic underpinnings of both traits is pronounced, with larger heritability estimates reported for trabecular BMD when compared to cortical vBMD [35].

Paternoster and colleagues recently performed a GWAS of cortical and trabecular vBMD in a cohort of adolescents and young adults, with subsequent replication in elderly individuals [8,36]. Three known adult hip and spine BMD associated loci displayed associations with cortical vBMD (i.e. *TNFSF11*, *ESR1* and *TNFRSF11B*) and a further two other loci (i.e. *EYA4* and *GREM2*/*FMN2*), displayed strong associations with cortical and trabecular vBMD respectively

[8]. Subsequent analysis using high-resolution pQCT measures of bone microarchitecture of male adolescents found that the cortical vBMD association with *TNFSF11* reflected a change in cortical porosity, whereas the association of trabecular vBMD with *GREM2*/*FMN2* reflected a change in trabecular number and thickness. Interestingly, a separate GWAS combining data from 5,878 European individuals within 13 to 80 years old reported a strong association between variants in the *WNT16* locus and cortical bone thickness [37]. Altogether, these findings demonstrate how refined measures of adolescent bone traits might provide better understanding of how these loci influence bone acquisition. For example, it is likely that the *WNT16* association with cortical thickness reflects its role in bone modelling, whereas associations between *ESR1, EYA4, TNFSF11* and *TNFRSF11B* and cortical density reflect their role in bone remodelling.

Biological pathways implicated in bone growth and accrual

The primary motivation behind GWAS of pediatric and adolescent BMD is to increase our fundamental understanding of the molecular pathways that regulate bone growth and/or accrual. When viewed retrospectively, an evaluation of the collective findings reported here suggests that GWAS of pediatric and adolescent bone traits have achieved this aim with remarkable success. For example, genes in four well-known bone signaling pathways: [i.e. canonical WNT (*LRP5, RSPO3*, *LGR4*, *AXIN1*, *RSPO3*, *WNT4*, *WNT16* and *WLS*), parathyroid hormone (*PTHLH*), oestrogen (*ESR1*) and RANK/RANKL/OPG (*TNFRSF11A*, *TNFSF11* and *TNFRSF11B*)] show robust associations with paediatric and adolescent bone traits. While it is beyond the scope of this review to provide an in-depth description of each of these pathways, their role in bone homeostasis is well documented (reviewed elsewhere [38,39]).

Novel attributes of existing pathways have been uncovered as a consequence of these investigations. Most notably, it has become evident that adult-BMD associated variants located near or within *TNFRSF11A, TNFSF11* and *TNFRSF11B* influence paediatric and adolescent BMD [6,10] suggesting that bone resorption may play an important role in bone growth and accrual. Bone resorptive cells have a critical role in endochondrial bone growth [40]. It is very interesting to note, that periods of rapid growth (i.e. puberty) are associated with marked increases in markers of resorption and formation [41]. To examine this hypothesis further, the relationship between bone modelling and bone resorption was investigated in a cohort of adolescents [42]. Variants in the above-mentioned genes were associated with increased bone resorption [i.e. serum β-C-telopeptides of type I collagen (CTX)], reduced cortical thickness and cortical vBMD, and increased periosteal circumference. These relationships may imply that higher bone resorption to be permissive for greater periosteal expansion (i.e. modelling), and that this relationship reflects a compensatory mechanism that occurs during growth, whereby periosteal expansion increases in response to endosteal resorption in an effort to retain bone strength by limiting cortical thinning [43].

Furthermore, several genetic association studies mentioned in this review highlight the role of *WNT16* in bone mass acquisition. As a consequence, a number of functional studies characterizing its important role in skeletal regulation have been performed, including a study by Movérare-Skrtic and colleagues that demonstrate that *Wnt16*-deficient mice suffer from spontaneous fractures as a result of reduced cortical thickness and high cortical porosity [44]. Although no trabecular bone phenotype was evidenced in this study, the same group recently demonstrate that *Wnt16* overexpression results in increased total body BMD that is mostly attributed to increases in trabecular bone mass [45]. Notably, a further study demonstrated that

Wnt16 mediates mechanical loading-induced stimulation of periosteal bone formation via canonical Wnt signalling pathways [46].

Identification of putative anabolic drug targets

Sanseau and colleagues recently reported that the genes identified through GWA studies are likely to be amenable as targets for therapeutic intervention [47]. Therefore, the findings reported by pediatric and adolescent studies of BMD may aid the discovery of novel drug targets for bone restorative pharmacotherapies. Although we are not yet in a position to determine the implication of these recent findings (in terms of improving treatment and prevention of osteoporosis), a retrospective review of the literature illustrates the merit of this strategy at identifying clinically validated drug targets. For example, several existing drugs used to treat osteoporosis, target receptor proteins that are encoded by genes robustly associated with pediatric and adolescent BMD. These include: denosumab (TNFSF11), romosozumab and blosozumab (SOST) and several estrogen analogues (ESR1). Importantly is has recently been noted that *WNT16* may represent a novel osteoporosis target, as pharmacological overexpression of WNT16, increases trabecular bone mass [45] and its depletion has strong consequences in cortical thickness [48]. It should also be mentioned that *RIN3* could hold significant therapeutic potential, especially when considering its differential expression in osteoporotic bone [10], likely role in osteoclast function, and association with Paget's disease susceptibility [23].

Future prospects

The implementation of a recent extension of the GREML method [49], described before, suggests that almost all of the heritable variation in complex traits like body height can be explained by the aggregate additive effects of genetic variants across the genome. Thus, assuming that the

genetic architecture of paediatric and young adult BMD is similar, it should be possible, in theory, to identify the vast majority of individual genetic variants that are responsible for the variation in bone acquisition by performing a combination of GWAS using microarrays with imputation and whole genome sequencing of very large samples of children and young adults. Results following this strategy have already proved successful, as exemplified by the detection of a rare coding variant in *EN1* associated with BMD and fracture risk in adults [19]. In the following section, we highlight alternative GWAS approaches that may further our understanding of bone metabolism.

Life-course approaches

It is evident that a better understanding of the genetic complexities underpinning skeletal development, maturation and senescence can be achieved when studying BMD throughout the life-course. As a result, the GEFOS consortium recently established a new effort in which 49,300 individuals from 24 different studies with TB-DXA measurements have been collected and are presently being analyzed across (and within) three different age groups [i.e. 0 - 15 years (n = 11,200); 15 - 45 years, (n = 9,600); and > 45 years, (n = 28,500)] [50]. We expect that the results of this study will provide interesting insights into questions related to age heterogeneity.

Multivariate association methods

GWAS of pediatric and adult BMD traditionally involve univariate genetic association analysis of bone mineral density (BMD). Nevertheless, it is plausible that some genetic factors primarily influence bone growth. If genetic variants simultaneously affect bone mineral content and bone area, their effect may only be detectable in genetic association studies of bone area (BA) or bone mineral content (BMC), as BMD (the ratio of these measures) may be unaffected. Thus, a promising alternative is to analyze BMC and BA simultaneously using multivariate genetic

association methods, taking advantage of the correlation between these traits. Simulation studies and statistical theory suggest that they can be more powerful than genetic association analysis of univariate measures [51,52]. Correlation is also high across the different components of body composition (e.g., bone mass, fat mass and lean mass), and there is growing interest in their interdependence; whence this modelling could as well be applied to identify genetic variants exerting pleiotropic effects. Currently, we are developing two strategies to address the prospects mentioned above. First, a GWAS in which BMD, BMC and BA [at the skull, upper limbs and lower-limbs] in 12,713 children and adolescents from 5 cohorts [53], and second, we are evaluating total body lean mass and BMD in a bivariate GWAS approach [54].

Trans-ethnic studies

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 compared to other populations [55,56], even before achieving peak bone mass [24,57-60]. A recent multi-ethnic cohort study showed that the frequency of those alleles associated with increased BMD was systematically elevated in individuals of Sub-Saharan African ancestry consistent with their higher BMD levels [24]. 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. Pediatric BMD GWA studies have started to pursue this goal [10,11,61] and new trans-ethnic studies are on their way.

Summary and conclusions

Genomic investigations of individuals at an early age (i.e. before attainment of peak bone mass) indicate that between a third and half of variation in bone growth and mineral accretion are tagged by common genetic variants that are assayed on commercially available genotyping chips. GWAS in these populations have successfully identified variants at more than fourteen loci, some of which influence pediatric and adolescent BMD in an age-, skeletal site- and/or trait-specific manner. Disentangling these differences is providing valuable insights as to how molecular pathways influence bone growth and accrual. Genetic variants discovered so far implicate well-known bone metabolism pathways, but also point to novel genes and pathways not previously implicated in bone metabolism. Although the therapeutic significance of these findings has yet to be determined, the study of young individuals appears to be a promising strategy for the identification of novel targets for the treatment of osteoporosis. For all these reasons we suggest that GWAS investigations of pediatric and adolescent BMD have made a significant contribution to our understanding of the genetic determinants of bone acquisition and osteoporosis and represent a powerful strategy for the identification of novel genetic loci that complement genetic studies of elderly individuals.

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Conflict of interest

The authors declare no conflict of interest

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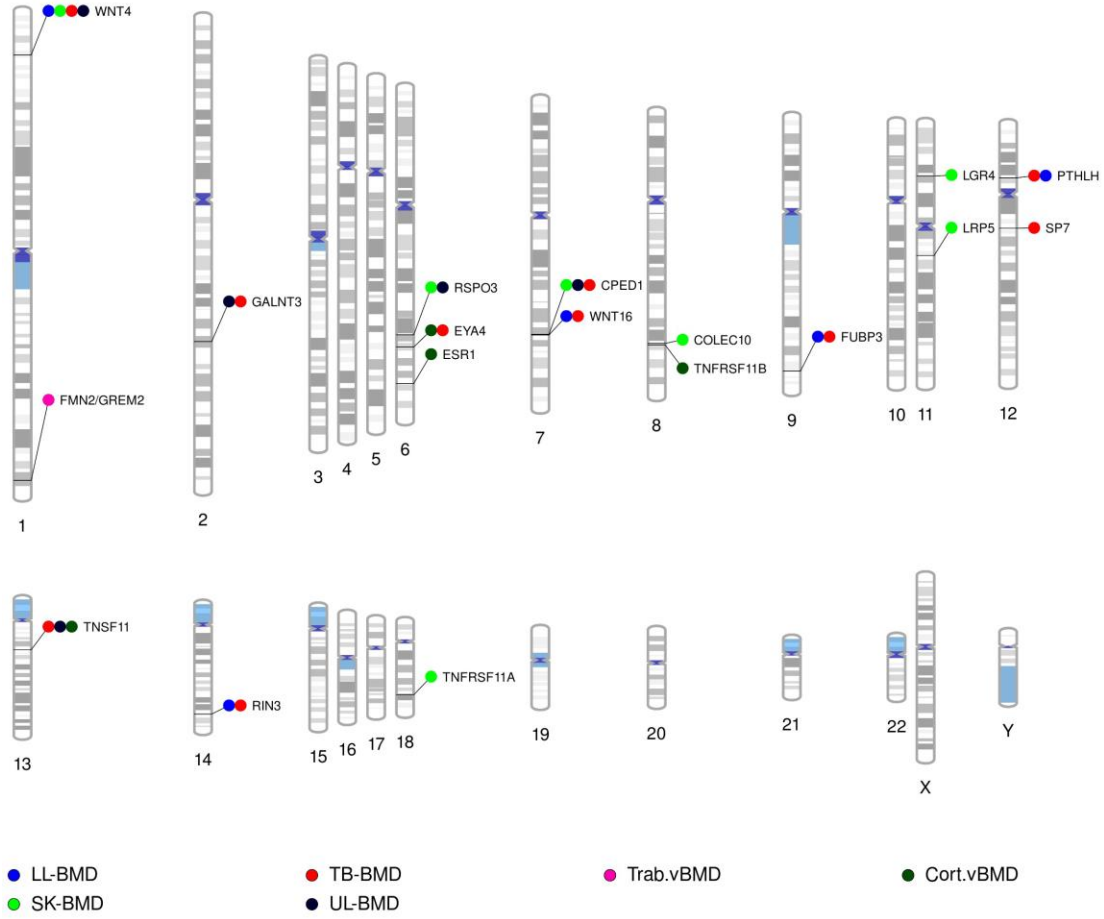
Figure Legends

Figure 1. Phenogram of all bone related loci identified by GWAS in children and/or young populations to date. Each locus is named according to either the most biologically relevant candidate gene in the region, the gene that is physically closest to the most strongly associated SNP, or in the case of intergenic regions, the cytogenic band containing the association. Note that in the vast majority of instances neither the identity of the true functional variant(s), nor the particular gene responsible for the association is known with certainty. Results from the following GWAS studies were used to generate the phenogram: Medina-Gomez *et al*. 2012 (PMID: 22792070), Kemp *et al*. 2014 (PMID: 24945404), Paternoster *et al*. 2010 (PMID: 21124946) and 2013 (PMID: 23437003). SK = skull, UL = upper limbs, LL = lower limbs, TB = total body less head, Trab = Trabecular, Cort = Cortical, BMD = Bone Mineral Density, v. = volumetric.


WNT4
FMN2/GREM2
1
GALNT3
2
RSPO3
EYA4
ESR1
3
4
5
6
CPED1
WNT16
7
COLEC10
TNFRSF11B
8
FUBP3
9
LGR4
LRP5
10
11
PTHLH
SP7
12
TNSF11
13
RIN3
14
TNFRSF11A
15
16
17
18
19
20
21
22
X
Y
LL-BMD
SK-BMD
TB-BMD
UL-BMD
Trab.vBMD
Cort.vBMD