Genetic studies of endophenotypes from spine CT scans provide novel insights into the contribution of mechanosensory pathways to vertebral fractures and spinal curvature

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Introduction

DXA-based studies have led the way in identifying genetic influences on the skeleton, exemplified by a recent genome wide association study (GWAS) followed by replication which identified allelic variation at the *EN1* locus as a novel, but low frequency determinant of bone mineral density (BMD) and fracture risk [(1)]. One of the main advantages of DXA scans is that due to their wide availability and low radiation dose, large scale GWAS meta-analyses involving many thousands of individuals are possible, providing the requisite power for studies of this type. However, an important limitation of DXA is that it conflates several different skeletal characteristics, each of which may be under separate genetic regulation, including skeletal size, cortical thickness, and volumetric bone mineral density (vBMD). It may be possible to mitigate this to some degree by use of height and weight to adjust for body size, and by comparison of GWAS outputs between different skeletal sites, according to the relative content of trabecular bone, such as hip and spine. Alternatively, specific characteristics (endophenotypes) can be measured directly using higher resolution methods such as computer tomography (CT) which, when underpinning genetic studies has, has led to important new insights compared with those obtained from DXA alone, despite the smaller size of data collections. For example, GWAS based on tibial peripheral quantitative CT (pQCT) led to the identification of the *wnt 16* locus as an important determinant of cortical thickness [(2)], as well as several new loci for trabecular vBMD [(3)].

The current issue of the *Journal* carries two papers where endophenotypes derived from spine CT scans have been used as a basis to investigate genetic influences on spinal curvature [(4)] and more specifically lumbar trabecular volumetric BMD (vBMD) and vertebral fracture [(5)]. This focus on spinal endophenotypes is important given the burden of disease associated with kyphosis and vertebral fractures, as exemplified by associations between vertebral fractures and reduced quality of life [(6)], increased risk of hospitalization [(7)], functional limitation [(8)], mortality [(7,9)], and chronic back pain [(8)]. Yau et al examined genetic influences on spinal curvature, and its potentially associated endophenotypes, as ascertained on thoracic CT scans. Though the sample size (around 2000) was insufficient to perform a GWAS, they were able to gain important insights into genetic influences by examining the heritability of spinal curvature, and its genetic correlation with other traits [(4)]. In contrast, Nielson et al, assembled over 15,000 participates, making it feasible to perform a GWAS, from which novel loci were identified which have not previously been found in GWAS of DXA-derived lumbar BMD [(5)].

Thoracic spinal curvature: heritability and genetic correlations

Yau and colleagues took advantage of the rich phenotyping typical of the Framingham Study, specifically analysing thoracic CT images from 2,063 men and women, aged 37 to 90 years, from 578 families, and measured Cobb angle between T4 and T12 on sagittal CT images as a measure of anterior curvature of the thoracic spine [(4)]. Vertebral fractures, inter-vertebral disc height narrowing and facet joint osteoarthritis (OA) were semi-quantitatively scored, and L3 BMD and T7-T8 paraspinal muscle area were also measured.

Heritability studies aim to determine the proportion of variance in a measured phenotype that is attributable to heritable genetic factors, and how much is explained by environmental influences [(10)]. The authors estimated, after accounting for age, gender and weight, that 54% of variation in thoracic spine curvature was explained by genetic factors, whereas vertebral fractures had a heritability estimate of 41%. Their finding that genetic correlations between spinal curvature and vertebral fracture were only moderate (0.39) is consistent with findings from epidemiological studies that

kyphosis is a poor marker of vertebral fracture, and is more likely to reflect associated degenerative changes [11,12]. Whereas only weak genetic correlations were observed between spinal curvature and degenerative changes, environmental correlations with lumbar facet OA were the strongest among all traits considered (0.28), suggesting shared environmental influences largely account for the relationships between spinal curvature and degenerative changes.

Of interest, moderate genetic correlations were also observed between spinal curvature and paraspinal muscle area (0.46), which itself was found to have relatively high heritability (54%). The authors speculated that larger muscle size might indicate greater muscle strength, which may in turn be expected to stabilize the spine more effectively, reducing progression in spinal curvature. It is certainly well recognised that intrinsic forces continually applied by the paraspinal musculature to the vertebral column are high [13,14]. Furthermore, a recent systematic review found some evidence that exercise programmes which target back extensor muscle strength may have modest improvements on kyphosis [15]; the finding underpins a randomised controlled trial currently underway in California which will evaluate the effectiveness of multimodal spine-strengthening exercise in older adults with hyperkyphosis [16].

In addition, increased paraspinal muscle area, and hence strength, may reduce spinal curvature by influencing the albeit lesser component explained by vertebral fracture; to what extent heritability of spinal curvature, vertebral fracture and paraspinal muscle area overlapped was not addressed directly, and may be better investigated using alternative methods to estimate genetic pleiotropy such as GCTA (genome-wide complex trait analysis) [17]. The authors allude to relationships between muscle and bone size which are well recognised, and a number of candidate genes have been proposed to exert a pleiotropic action on both these tissues [18]. Furthermore greater muscle forces applying strains on the spine might be expected to reduce vertebral fracture risk by increasing BMD, which also showed a modest genetic correlation with spinal curvature (-0.23) (see Figure 1). That said, a previous meta-analysis suggested the lumbar spine may be relatively unresponsive to mechanical inputs, based on the lack of change in BMD in response to high impact interventions in premenopausal women as compared with the hip [19]. However, the latter finding may simply reflect differences in the contribution of high impact exercise to the local strain environment according to skeletal site, rather than any inherent differences in mechano-responsiveness; in contrast to the hip, intrinsic forces exerted on the spine by paraspinal muscles may be relatively high as compared with extrinsic forces generated through exercise.

A further question is the extent to which heritability estimates for spinal curvature show any gender differences. Classically a 'Dowager's Hump' (for which the term kyphosis originates from the Greek kyphos, a hump) is observed in the older female population who are at relatively high risk of osteoporosis. Women reportedly develop kyphosis earlier in life and to a greater degree than men [20]. Conceivably, older females with spinal kyphosis may have a higher proportion of underlying vertebral fractures versus degenerative changes as compared with males, which would lead to distinct sex-specific heritability estimates and genetic correlations with other endophenotypes. Consistent with this suggestion, monozygotic twin studies have identified gender-specific differences in heritability of BMD [21]. Other important differences may also exist between genders in terms of contributors to spinal curvature heritability. For example, thoracic kyphosis has been linked to breast size [22]. Twin studies have estimated the heritability of breast size at 56%, of which approximately one third is attributable to BMI-associated genetic variation, whilst the remainder, *i.e.* 41% of total genetic variance is unique [23]. The rich Framingham phenotyping may offer further endophenotypes for future gender-specific heritability analysis, such as measured chest circumference.

Lumbar spinal volumetric BMD; novel genetic variants

Nielson and colleagues took a different approach to exploring the genetics of spinal endophenotypes [5]. They performed a classical GWAS using CT quantification of lumbar spine vBMD, with specific focus on 'pure' trabecular bone, adjusting for age, gender and weight. They were able to amass 12,287 CT images of L2 or L3 from six cohorts, including men and women aged 52 to 76 years, for their discovery cohort; findings from which they replicated in a further 2,987 individuals. From this first combined GWAS they identified six loci associated ($p<5x10^{-8}$) with vBMD and six further suggestive loci ($p<5x10^{-6}$). Of these 12 SNPs, 6 have been associated with DXA-measured lumbar spine BMD previously [24].

The authors then performed a 'look up', of these 12 SNPs in 21,701 individuals assessed for morphometric vertebral fracture, and in a further 5,893 assessed for clinical vertebral fracture. Only one locus was found to associate with morphometric vertebral fracture (*SLC1A3*) and one with clinical vertebral fracture (*ZBTB40*). Whilst the latter is well established to associate with spine and hip BMD and fracture risk [24-26], *SLC1A3* represents a novel BMD and fracture association. Minimal evidence was detected to suggest the other novel loci associated with vBMD (*FMN2/GREM2*), were also associated with vertebral fracture; this is despite the fact that the *FMN2/GREM2* locus has previously been associated with both trabecular vBMD (measured by pQCT) and fracture risk in a much smaller population [3]. Cis-expression quantitative loci (cis-eQTL) studies of human bone biopsies also identified *EPHB2* expression to be associated with allelic variation at the *ZBTB40* locus; *EPHB2* being approximately 355kb downstream. *EPHB2* is a tyrosine kinase transmembrane receptor previously implicated in bone development and fracture repair [27,28], but until now has not been identified in human genetics studies of bone traits.

SLC1A3 (solute carrier family 1 member 3), also known as *GLAST* and *EAAT1*, codes for a sodium-dependent glutamate transporter protein, which is expressed in both osteoblasts and osteocytes, and was found nearly twenty years ago to be up-regulated in mouse osteocytes by mechanical loading in *vivo* [29]. Subsequently glutamate signalling has been identified as a key signalling pathway potentially regulating bone responses to mechanical loading [30]. Of the other 5 loci Nielson et al identified as being associated with vBMD, *WNT4* is also reported to be mechanosensitive, being upregulated within the developing joint line in embryonic mice lacking skeletal muscle [31], osteoprotegerin(OPG) (coded for by *TNFRSF11B*) expression is reduced by mechanical loading in osteoblasts in mice [32], and *TNFSF11*/RANKL expression in osteoblasts is reportedly increased by unloading [33]. The suggestion that genetic variation in genes involved in skeletal responses to mechanical loading contribute to lumbar spine vBMD, complements findings presented by Yau et al implying that muscle loading, via paraspinal muscle function, contributes to spinal curvature (see Figure 1). However, a word of caution should be signalled in the role of *SLC1A3* in bone; the International Mouse Phenotype Consortium online resource (http://www.mousephenotype.org/data/experiments?geneAccession=MGI:99917) demonstrates no evidence of abnormal BMD on DXA of their *SLC1A3* knockout mice.

As the authors acknowledge, the sample size was relatively small for a GWAS, providing limited power to detect associations when applying conventional significance thresholds to account for multiple comparisons. This may explain why they only identified 9 out of 49 loci previously reported to be associated with lumbar spine BMD in large scale GWAS meta-analyses. For example, of the four loci significantly ($p<5x10^{-8}$) associated with vBMD and which had previously been identified to associate with lumbar spine BMD in GEFOS, three were in Estrada et al's top five most strongly associated BMD SNPs [24]. Theoretically, one may have expected stronger underlying associations to compensate for limited power, on the basis that spinal CT is a more specific trabecular bone phenotype compared to lumbar spine DXA. For example, as well as providing a 'pure' measurement of trabecular bone, unlike lumbar DXA scanning, CT-derived trabecular BMD is not affected by artefacts such as posterior facet joint osteoarthritis, ossification of the anterior longitudinal ligament and aortic calcification. That said,

effect sizes as reflected by beta coefficients for the four GWAS significant loci detected by Nielson et al were of comparable magnitude to those reported by Estrada et al for genetic associations with DXA lumbar spine BMD in their GWAS meta-analysis, raising the possibility that the genetic loci Nielson et al identified, do not solely associate with trabecular BMD, but may in addition reflect relationships with other structures that contribute to measured lumbar spine BMD.

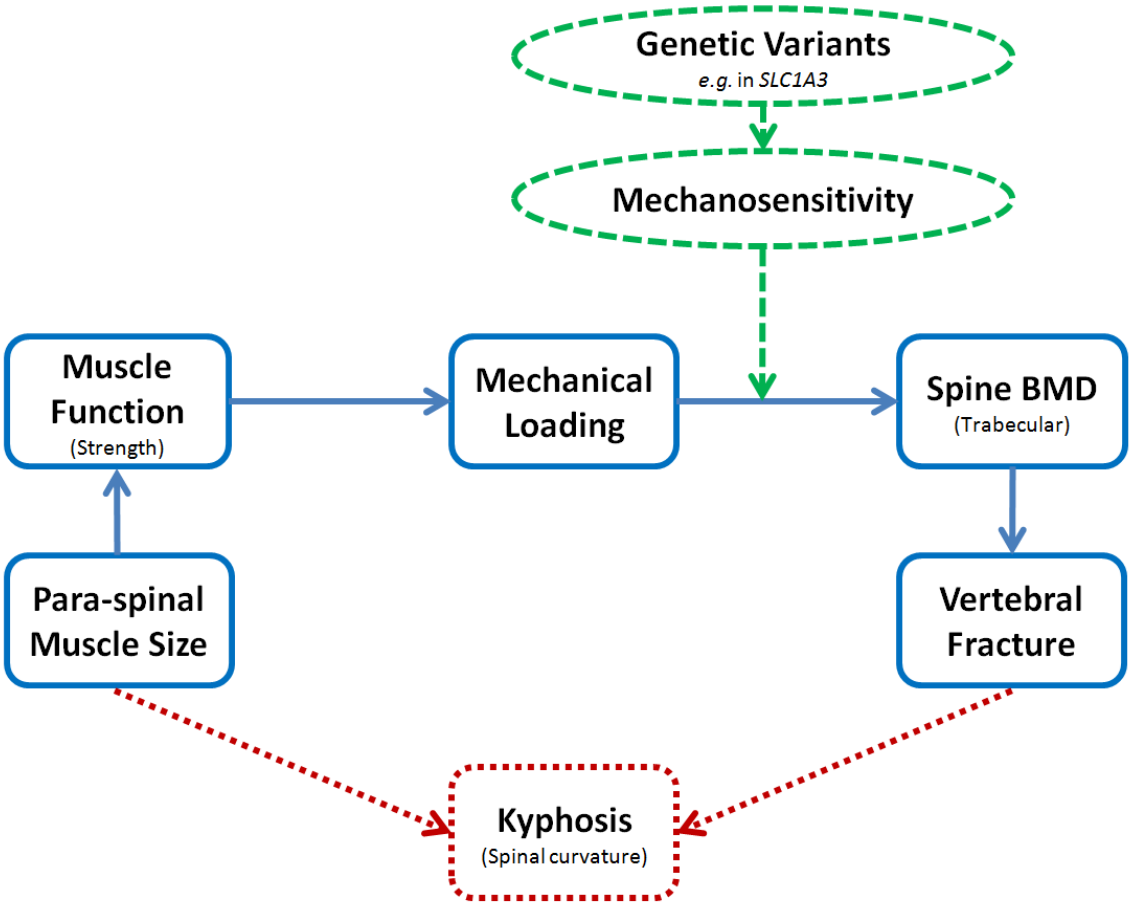
In common with earlier GWAS studies of DXA-derived BMD, Nielson et al included weight adjustment in their statistical model. Whilst this adjustment makes sense for DXA-derived measures, which are known to be related to weight, whether this also applies to CT-derived trabecular bone measures is less clear. Including unessential factors in GWAS models runs the risk of introducing spurious associations through collider bias [34]. It may be helpful to examine this question further, for example by comparing results from analyses with and without weight adjustment. Unnecessary adjustment for variables also runs the risk of attenuating real associations leading to null findings. For example, since vertebral fractures can cause height loss, it seems questionable to have included height in their genetic association models for vertebral fractures, and it may be that this additional adjustment contributed to the limited number of associations detected.

Conclusions

Taken together, these two papers demonstrate how the study of endophenotypes based on spine CT scans can provide novel insights into genetic influences on spinal fractures and deformity, helping to understand the molecular and causal pathways responsible for these pathologies, and to generate hypotheses for further study. Of particular interest, genetic influences on trabecular BMD and vertebral fracture risk identified by Nielson et al point to an important contribution of mechanosensory pathways to vertebral BMD and fracture risk. Mechanosensitive genes have previously been associated with lumbar spine BMD in GWAS (*e.g.* SOST) [24]; however, GWAS annotation has not previously highlighted the potential role of glutamate signalling pathways. Consistent with the view that mechanosensory pathways contribute to spinal BMD, Yau et al observed moderate genetic correlations between paraspinal muscle size, a possible proxy for the strength of local muscle forces, and spinal curvature. However, although paraspinal muscle size and vertebral fracture both showed genetic correlations with spinal curvature, whether these are on the same causal pathway, possibly involving mechanosensory responses, remains unclear (see Figure 1).

Given the relatively high heritability of spinal curvature observed by Yau et al, whereas only moderate genetic correlation was observed with vertebral fractures and paraspinal muscle area, it seems likely that other important genetic influences exist which are yet to be identified. Conceivably it may be possible to identify these using the GWAS approach applied by Nielsen et al. Accessing sufficient numbers of individuals with thoracic CT scans, to provide sufficient power, is likely to be challenging; these scans are relatively expensive, are associated with a not insignificant radiation dose, and are seldom collected in routine practice. However, it may be possible to derive equivalent phenotypic information from other more widely available sources such as VFA scans, which are routinely performed in patients undergoing lumbar spine DXA scans. Whereas conventional DXA-derived BMD measurements have formed the basis of our understanding of the genetics of osteoporosis and related disorders to date, it seems likely that future progress will be underpinned by the availability of distinct, more specific, phenotypes.

Figure: Hypothesized causal diagram for the role paraspinal muscles may have in determining spinal curvature



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