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MEETING REPORT

Update on the Genetic Basis of Disorders of the Musculoskeletal System (ECTS 2013)

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Meeting Report from the 50th Meeting of the European Calcified Tissue Society, Lisbon, Portugal, 18–21 May, 2013

Introduction

The 2013 European Calcified Tissue Society (ECTS) meeting in Lisbon, Portugal had several presentations in the field of genetics, extending from genome-wide association studies (GWAS) for diverse complex musculoskeletal traits and functional evaluation of variants, to the underpinning of the genetic architecture of high bone mass (HBM) syndromes. Another highlight was the first attempt to integrate high-throughput '-omics' techniques in the field of osteoporosis.

Genome-Wide Association Studies

Partners of the Genetic Factors of Osteoporosis (GEFOS) consortium presented updates on the analysis of hip, wrist and vertebral fractures, the most frequently occurring fragility fractures; together with further exploration of DXA-derived measures like regional BMD and total lean mass.

A novel hip and wrist fracture risk locus. Petterson-Kymmer *et al.*[1] examined in the discovery phase, two large hip and wrist fracture Swedish data sets (confirmed by radiographic reports) from the Umeå Fracture and Osteoporosis (UFO) cohort, with UFO-hip including 1014 cases and 862 controls, and UFO-forearm including 1060 cases and 1055 controls. Replication was performed in the Women's Health Initiative (WHI) cohort (1845 hip fractures verified by medical records and 2120 controls). One single-nucleotide polymorphism (SNP) mapping to 1q25.2 within the *SOAT1/AXDND1* locus was associated with fracture risk in the discovery (odds ratio, OR = 1.33; $P = 3.1 \times 10^{-8}$), and replicated in the WHI cohort (OR = 1.16; $P = 2.1 \times 10^{-3}$). Combining the summary statistics from both studies (3919 cases and 4037 controls) resulted in an OR = 1.24; $P = 5.6 \times 10^{-10}$. The *SOAT1* gene encodes a protein catalyzing the formation of fatty acid-cholesterol esters and implicated in the formation of beta-amyloid and atherosclerotic plaques, while *AXDND1* encodes a hypothetical protein of still unknown function. The lack of understanding of the mechanisms involved in this association is a good reflection of the hypothesis-free nature of the GWAS approach frequently unraveling new biology.

The first replicated clinical vertebral fracture risk locus. Vertebral fractures that come to medical attention with symptoms such as back pain and kyphosis are termed clinical vertebral fractures (CVF) and account for significant morbidity and mortality. Alonso *et al.*[2] presented the initial results from a GWAS involving 1634 postmenopausal women with CVF (collected by 11 centers in Europe and Australia) and 4662 regionally matched controls. Variants from nine loci were identified as associated with CVF at a suggestive level ($P < 1 \times 10^{-4}$), whereas those in one locus were on the verge of achieving genome-wide significance (lowest $P = 7.28 \times 10^{-8}$). The most significant variant of the association signal maps to chromosome 4q35 close to *SORBS2*, a gene involved in osteoclast and osteoblast activity. Expression levels of *SORBS2* in bone biopsies from a set of Norwegian women were strongly correlated with levels of *RUNX2* and other genes in the BMP pathway. These results await further replication and functional follow-up to fully characterize the observed association.

Skeletal-site specificity of the genetic regulation on BMD variation. Heritability of BMD is known to vary across skeletal sites, possibly reflecting different contributions of both environmental and genetic influences. Using an innovative statistical approach Kemp *et al.*[3] quantified the genetic correlations (shared heritability) between different skeletal sites for BMD measured at the upper limb (UL), lower limb (LL) and skull (S) obtained from total body DXA scans. The study ($n = 9395$) combined data from the Avon Longitudinal Study of Parents and their Children ($n = 5299$, mean age = 9.9 years) and the Generation R study ($n = 4096$, mean age = 6.2 years). The study showed how BMD of the extremities shared a high proportion of common genetic architecture between the lower and upper limbs ($r_g = 0.78$). The correlations of the axial skeleton (skull) were considerably less with the upper ($r_g = 0.58$) and lower ($r_g = 0.43$) limbs. Applying the GWAS approach across different skeletal sites they identified variants mapping to the *RIN3* locus (previously associated with Paget's disease) specifically associated with lower limb BMD ($P < 5 \times 10^{-8}$). Several variants in other known BMD loci showed different patterns of association across subregions. Some variants mapping to the previously identified *WNT16* locus[4] showed marked skeletal

specificity; for example, a variant associated at genome-wide significant level with skull ($P = 1.5 \times 10^{-28}$) and upper limb ($\beta = 0.19$, $P = 1.3 \times 10^{-34}$) BMD, was not associated with lower limb BMD ($P = 0.2$). Similarly, variants mapping to *CENPW* were associated with skull ($P = 3.3 \times 10^{-11}$) but less significant with upper limb ($P = 0.02$) and not associated with lower limb ($P = 0.28$). These results suggest distinct genetic influences are exerted across skeletal sites providing novel insight into the regulation of BMD variation.

Evasive genetic determinants underlying lean body mass variation. Despite being highly heritable, no replicated variants have been associated with lean mass. Kiel *et al.*[5] investigated genetic variants influencing total body and appendicular lean mass variation using a discovery set comprising 20 studies and summing to ~28 000 individuals for total body, and 38 000 subjects for appendicular mass. The study also assembled an impressive replication set including between 70 000 to 85 000 individuals of different ethnicities for total body and appendicular lean mass, respectively. Variants associated at genome-wide significant level were identified mapping in the vicinity of *IRS1* ($P = 1.5 \times 10^{-11}$), *VCAN* ($P = 1.9 \times 10^{-8}$) and *ADAMTSL3* ($P = 7.1 \times 10^{-10}$) for both studied traits. Two other genome-wide significant signals were specifically associated with total body lean mass, including a missense variant in *HSD17B11* of relative low frequency in European populations and monomorphic in other populations; and an intronic common variant within *FTO*. Although *Fto* KO mice present with reduced lean mass, little is known of the mechanisms underlying these associations. Variants in *ADAMTSL3* have been reported to influence body stature,[6] and to influence both femoral neck bone width and appendicular lean mass as identified by a bivariate analyses presented at the 2012 ASBMR meeting.[7] The authors consider that the low yield in identified variants (despite the very large sample size of the study) might be a consequence of the wide age range of the participants, obscuring differential genetic effects on muscle mass accrual or lean mass loss.

Functional evaluations of GWAS findings. Garcia-Giralt *et al.*[8] performed functional evaluations of the *RANK* and *RANKL* regions. After testing in the BARCOS cohort ($n = 1098$ postmenopausal Spanish women) 15 SNPs in *RANKL* and 18 in the *RANK* regions for association with BMD (lumbar spine and femoral neck) and fractures, the researchers focused on the distal region holding the most significantly associated SNP (rs9594738). The SNP is located in a region containing promoter histone marks. Functional experiments using luciferase gene reporter assays showed that the promoter constructs displayed inhibitory properties, which are exerted by factors contained in the region.

Genomic and transcriptomic approaches to study the high bone mass (HBM) phenotype. The same group presented work on individuals with the HBM phenotype. Sarrión *et al.*[9] established that the prevalence of HBM (defined as BMD *Z*-score > 4) in the BARCOS study was 0.7%. After mutational screening of *DKK1* and *LRP5*, one of the eleven HBM cases was identified as carrier of a rare missense change in *DKK1* (p.Y74F), but none in *LRP5*. The authors also evaluated the compound effect of common BMD-decreasing alleles identified in the normal population[10] on the set of HBM women. Just as observed in the general population,[10] the BMD *Z*-score decreased with increasing number of BMD-decreasing alleles, except in one woman with the highest allele count and highest BMD *Z*-score. Within a similar approach Gregson and colleagues[11] studied an extreme HBM population including a large set of 240 'unexplained' HBM cases (defined as L1 *Z*-score $\geqslant +3.2$ and total hip *Z*-score $\geqslant +1.2$, or total hip *Z*-score $\geqslant +3.2$ and L1 *Z*-score $\geqslant +1.2$) recruited from 15 UK centers. The researchers performed a GWAS against two existing sets of controls from the general population (1958 British Birth Cohort; $n = 5667$) and an extreme truncate selection of postmenopausal women with low BMD (*Z*-scores from -4.0 to -1.5) from Australia (Anglo-Australasian Osteoporosis Genetics Consortium; $n = 900$). Variants in two known BMD loci achieved genome-wide significance and mapped to *CTNNB1* (3p22.1; $P = 2.3 \times 10^{-8}$) and *MEF2C* (5q14.3; $P = 5 \times 10^{-8}$); whereas clear overrepresentation of association with the rest of the known BMD loci[10] was observed. Altogether, these studies suggest that the HBM phenotype is not only determined by monogenic factors, but also under polygenic control just as in the general population.

Integration of -Omics Approaches in Osteoporosis

One expected next step after GWAS is to couple genomic approaches with other levels of -omics assessments including transcriptomics, epigenomics, metabolomics and proteomics. Moayyeri *et al.*[12] have determined serum metabolomic profiles in 6055 participants (86% women) of the TwinsUK study covering 510 serum metabolites (211 unknown and 299 known molecules including those from amino-acids, lipids, carbohydrates, vitamins, peptides and xenobiotics). Applying a Mendelian randomization approach, causal associations between metabolites and bone phenotypes (hip and spine BMD) were assessed using genetic markers as instrumental variables. Epiandrosterone sulfate (encoded by *CYP3A5* on chromosome 7q22.1) and 4-androsten-3β, 17β-diol disulfate (encoded by *SULT2A1* on 19q13.33) were shown to be causally associated with femoral neck BMD, butyryl-carnitine levels (encoded by *ACADS* on 12q24.31) with total hip BMD and an unknown metabolite (encoded by *ABCC4* on chromosome 13q32.1) with lumbar spine BMD. Despite requiring independent replication, this study exemplifies how such derived knowledge of serum metabolites, as intermediary phenotypes, can help identifying causal pathways involved in the pathogenesis of osteoporosis.

Conclusion

The molecular and genetic studies presented at the 2013 ECTS Meeting have provided valuable insight in diverse areas of musculoskeletal research at the basic, translational and clinical level.

Conflict of Interest

The authors declare no conflict of interest.

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