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Periostin serum levels and gene polymorphism are associated with bone microarchitecture

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Background
We previously reported that serum periostin levels are determined by additive genetic effects. Whether serum levels and/or SNPs in the periostin gene (Postn) contribute to bone microstructure however remains unknown.
Aim
To investigate the association between periostin levels, Postn SNPs, bone-mass and bone microarchitecture in a cohort of postmenopausal women.
Methods
A total of 648 postmenopausal women from the Geneva Retirees Cohort were analyzed for six periostin SNPS (rs9547952, rs9603226, rs7322993, rs9576308, rs7338244, rs9547970). Periostin serum levels were determined by ELISA. Areal bone mineral density (aBMD) was measured by dual-energy X-ray absorptiometry at radial, lumbar and femoral sites (Discovery A, Hologic® inc, Waltham, MA, USA). Distal radius, tibia volumetric BMD and bone microstructure were measured by high-resolution peripheral quantitative computed tomography (XtremCT, Scanco Co, Bruttisellen, CH). Regression analyses were carried out to determine the association between periostin serum level and SNPs with bone traits.
Results
Mean age of the cohort was 64 ± 1.4 years. Periostin levels were positively associated with 1/3 radius aBMD (beta $= 0.10$, $P = 0.009$) and negatively with radius cortical porosity (beta $= -0.09$, $P = 0.02$). Periostin SNPs rs7322993, rs9576308, rs7338244, rs9547970 were associated with aBMD at lumbar, femoral and ultradistal radius sites. Those SNPs were also associated with radius and tibia microarchitecture (trabecular number and cortical porosity). Once adjusted for age, height, weight and years since menopause only rs7322993, rs9576308, rs9547970 remained significantly associated with both lumbar aBMD ($P = 0.02-0.04$) and radius trabecular number ($P = 0.008-0.04$). There was no association between periostin serum level and periostin SNPs.
Conclusion
Periostin levels and SNPs are significantly associated with aBMD and with radius bone microstructure. If confirmed in independent cohort, these results would contribute to understand the genetic determinants of bone fragility.

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Interaction between periostin gene (Postn) and other gene polymorphism involved in periostin expression and activity on bone microstructure in humans

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Background
Periostin is a matricellular protein involved in bone modeling and remodeling through the modulation of WNT-β catenin signaling in osteoblasts and osteocytes.
Aim
To investigate the interaction between polymorphisms of six periostin SNPs and other gene polymorphism involved in periostin expression and activity on periostin serum levels and bone microarchitecture in a cohort of postmenopausal women.
Methods
A total of 648 postmenopausal women from the Geneva Retirees Cohort (GERICO) were analyzed for six periostin SNPS (rs9547952, rs9603226, rs7322993, rs9576308, rs7338244, rs9547970) and for several SNPs in BMP2, CTNNB1, ESR 1, ESR 2, LRP5, LRP6, PTH, PTH R, SPTBN1, SOST, TGF B, TNFRSF11A and WNT 16. Periostin serum levels were determined by ELISA. Distal radius, tibia volumetric BMD and bone microstructure were measured by high-resolution peripheral quantitative computed tomography (XtremCT, Scanco Co, Bruttisellen, CH). Two ways ANOVA was carried out to assess the SNPs effects and their interaction on cortical porosity or periostin serum levels.
Results
ESR1 SNP rs851982, LRP5 SNP rs648438 and TNFRSF11A SNP rs2957137 were associated with periostin serum levels (P values range 0.03–0.0004) as well as with cortical porosity (P values range 0.04–0.005). Periostin SNP rs9547970 was also associated with cortical porosity at radius and tibia ($P = 0.04$).
Furthermore, we identified an interaction between LRP5 SNP 648438 and periostin SNP rs9547970 on radial cortical porosity ($P = 0.005$), and on periostin serum level ($P = 0.01$). In particular, lower periostin serum levels and higher cortical porosity were associated with periostin SNP 954790 GG and LRP5 SNP rs648438 CC and CT.
Conclusion
Gene polymorphism in the estrogen receptor, WNT and RANK pathway are associated with serum periostin levels and cortical microstructure. LRP5 interacts also with Postn polymorphism on bone microstructure. If confirmed in independent cohort, these data confirm the complexity of genetic determinant of bone fragility that involves periostin.

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Association among oxidative stress, Wnt signaling and trabecular bone microstructure in osteoporosis and osteoarthritis

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Experimental studies suggested that both, oxidative stress and the Wnt pathway, are important factors in the regulation of bone remodeling. Thus, low antioxidant levels and elevated markers of Wnt pathway inhibitors (sclerostin) levels are associated with a reduced bone mineral density and increased risk of osteoporotic fracture. Whether oxidative stress and the Wnt pathway are related to fracture risk is poorly understood.
M&M: Cross-sectional study in 21 subjects divided into three groups: seven osteoporotic hip fracture (age: 75 ± 5) (OP); eight osteoarthritis, undergoing hip replacement, (71 ± 4) (OA) and six OA $\leq$ 55 years old.
We carried out hip BMD (DXA-Hologic Discovery) and microstructural and biomechanical characteristics of trabecular bone (Micro-CT-Scan Sky 1172). In macerated trabecular bone, we quantified gene expression of catalase, GADD45 (oxidative stress genes), connexin 43, cyclin D1 (Wnt pathway genes), Runx2, osteoprotegerin (OPG) and sclerostin (SOST) by qPCR.
The results are analyzed statistically with the Kruskal–Wallis and Dunn's *post hoc* and correlations by Pearson coefficient (SPSS 22.0), $P \leq 0.05$.
Results
Osteoporotic subjects have an increased expression of catalase and GADD45 in trabecular bone, suggesting increased oxidative stress in these patients regardless of age and sex.
We also observed a significant increase in the expression of genes involved in the Wnt pathway, connexin 43 and cyclin D1, Runx2 and OPG, in OP group. We found no differences in the SOST gene expression.
As expected, BMD values are statistically lower in the OP subjects and they have a worse biomechanic and microstructure bone.
Conclusion
These results suggest that the trabecular bone from patients with osteoporosis have a higher activity of oxidative stress and alterations in the Wnt pathway and osteogenic genes expression.

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Genetic variants at the Wnt/β-catenin and oestrogen receptor signalling pathways are associated with low bone mineral density in dancers

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Purpose
Research suggests that dancers are at higher risk of developing low bone mineral density (BMD) compared with the general population. However, the associated factors contributing to low BMD in dancers are not fully understood. We aimed to assess the association of single-nucleotide polymorphisms (SNPs) in the Wnt/β-catenin and oestrogen receptor (ER) signalling pathways with low BMD in dancers.
Methods
A genetic association study was conducted in 151 female and male dancers and 151 controls matched for age and sex (18.2±10.8 years vs 18.2±10.7 years). Participants were stratified into different groups according to bone mass outcomes: low BMD (*Z*-score <-1.0 for adults and *Z*-score <-2.0 for adolescents) and normal BMD (*Z*-score ≥ -1.0). Eleven SNPs of the Wnt/β-catenin (*SOST*: rs851054, rs851056, rs10534024, rs4792909, rs9902563; *LRP5*: rs3736228, rs2306862, rs682429, rs491347, rs3781590, rs2508836, rs643892, rs312786) and ER (*ESR1*: rs2234693, rs9340799; *ESR2*: rs1256030, rs960070) pathways were genotyped and evaluated for association with low BMD at the forearm, lumbar spine (LS) and femoral neck (FN). A false discovery rate correction was used to claim significance ($P<0.02$).
Results
Comparing controls with normal BMD and dancers with low BMD, *ESR1* rs9340799 A allele significantly increased the odds of low BMD in dancers by 1.95-fold (95% CI=1.09–3.51, $P=0.0204$) at the forearm, 2.32-fold (95% CI=1.24–4.32, $P=0.0059$) at the LS, and 2.45-fold (95% CI=1.26–4.74, $P=0.0052$) at the FN. *LRP5* rs2508836 C allele was also associated with an increased risk of low BMD in dancers at the LS (OR=6.90, 95% CI=1.27–37.49, $P=0.009$). Haplotype analysis revealed that the blocks GCGT and GCAG at the *LRP5* gene significantly increased the odds for low BMD in dancers at the LS and forearm (OR=8.97, 95% CI=1.14–70.31, $P=0.0368$ and OR=6.43, 95% CI=1.33–31.14, $P=0.0207$).
Conclusion
Genetic variants at the Wnt/β-catenin and ER pathways are associated with low BMD in dancers.

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Search for BMD-related variants of DKK1 and SOST by resequencing in the BARCOS cohort

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In a meta-analysis by Estrada *et al.* (2012), 56 loci were found associated with BMD, 14 of which were also associated with osteoporotic fracture. Several of these genes belong to the Wnt signaling pathway, including two inhibitors: *DKK1 and SOST*.
To better understand the role of these genes in BMD determination and fracture susceptibility, we aimed to explore their allelic architecture by resequencing all coding exons and flanking regions in two extreme BMD groups from the BARCOS cohort: 55 women with the highest BMD (*HBM*) and 53 with the lowest BMD (*LBM*). Once these variants were determined, the most promising ones were genotyped in the complete BARCOS cohort and, where appropriate, tested for association.
Resequencing of *DKK1* and *SOST* identified 11 and 3 SNVs, respectively. Half of them had frequencies above 1%, and the rest were observed in only one or two samples, each. Only the rare variant c.*752C>T, in *DKK1*, was novel. One low-frequency variant in *DKK1* showed significant differences between the genotype frequencies of the two extreme groups (rs74711339, $P=0.0224$).
This SNP and two SNPs (*DKK1*: rs1569198, SOST: rs17882143) and one rare variant (*SOST*: rs570754792) with a potential biological function were genotyped in $n=1625$ women from the BARCOS cohort. We tested the association of the three SNPs with LS-BMD and nominal significant results were only obtained for rs17882143. This SNP is a missense variant (p.V10I) and in our cohort it is in strong linkage disequilibrium with rs4792909 (the 'GWAS hit'). Regarding the rare variant rs570754792, it was found in heterozygosity in only three women, whose values were below the mean BMD of the BARCOS cohort. It lies in a putative transcriptional regulation site.
In conclusion, our results suggest that the *SOST* p.V10I missense variant may play role in BMD determination. Functional studies to test this hypothesis are underway.

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Analysis of the polyalanine repeat polymorphism in the *RUNX2* gene in relation to bone mineral density and fracture risk in Maltese postmenopausal women

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Introduction
Runt-related transcription factor 2 (RUNX2) is a major transcription factor essential for the regulation of osteoblast and chondrocyte differentiation, hence affecting skeletogenesis, bone and cartilage formation. The RUNX2 protein has unique consecutive polyglutamine and polyalanine repeats (Q/A) which are important for its transactivation function. Several variants within the *RUNX2* gene have been implicated in osteoporosis and fracture susceptibility.
Aim
To evaluate the association of an 18 bp deletion within the polyalanine tract (17A>11A; rs11498192) with bone mineral density (BMD) at lumbar spine (LS) and hip, and with different types of low-trauma fractures.
Methods
A case-control collection of 1043 Maltese postmenopausal women was used. Women who suffered a fracture were classified as cases whereas those without a fracture history were included as controls. Genotyping was performed by polymerase chain reaction and odds ratios (OR) were computed using logistic regression analysis adjusted for confounders.
Results
RUNX2 alleles were observed at a frequency of 0.90 and 0.10 for the 17A and 11A alleles, respectively, and which were found to be in Hardy-Weinberg equilibrium. Carriers of the 11A allele were found to have a twofold increased risk of osteoporosis at the total hip (adjusted OR: 2.1 [1.1–3.9], $P=0.02$) and to a lower extent at the femoral neck (adjusted OR: 1.7 [1.1–2.5], $P=0.02$). No association was observed for the LS BMD. Heterozygosity for the 11A allele was also associated with an increased hip fracture risk which was not attenuated after adjusting for BMD (adjusted OR: 2.2 [1.1–4.8], $P=0.03$).
Conclusion
Results from this independent replication study indicated that the *RUNX2* 11A variant predisposes to reduced BMD and increased fracture risk in a site-selective manner in Maltese postmenopausal women. The deletion is thought to alter the secondary structure of RUNX2 thereby affecting its transcriptional ability.

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Identification of a novel locus on 2q13 of large effect size which predisposes to clinical vertebral fractures independently of BMD: the GEFOS consortium

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Vertebral fractures are the most common complication of osteoporosis, but little is known about the genetic determinants of susceptibility. Here we present the results of a genome wide association study in 1553 postmenopausal women with clinical vertebral fractures and 4340 controls, with replication in 667 cases and 2105 controls. A locus tagged by a less frequent variant (rs10190845, A-allele MAF=0.05) was identified on chromosome 2q13 as a strong predictor of clinical vertebral fracture ($P=1.27\times10^{-8}$) with a large effect size (odds ratio 1.75, 95% CI 1.4–2.1). Three other loci were identified on chromosomes 1p31, 11q12 and 15q11, associated at suggestive level ($P<5\times10^{-6}$). All were novel loci that had not previously been associated with bone mineral density (BMD) or clinical fractures. Analysis of 71 variants that had been associated with spine BMD or fractures at a genome wide significant level in other studies identified eight that were significantly associated with vertebral fractures in the present study after Bonferroni correction ($P<7\times10^{-4}$). Bioinformatic analysis of the 2q13 locus identified several potentially functional SNPs which were associated with expression of the positional candidate genes *TTL* and *SLC20A1* in whole bone tissue, none of which is known to play a role in bone biology. Our study illustrates that some predisposing variants for clinical vertebral fractures overlap with known genetic determinants of susceptibility to osteoporosis whereas others are unique. The study has cast new light on the genetic architecture of clinical