ratio (OR) for vert fx were in the expected direction. Giving an expected false positive number of 19 SNPs ($15{,}097 \times 0.5 \times 0.05 \times 0.05$).

Results: In women we found 41 significant SNPs originating from 8 genes. In men we found 75 significant SNPs in 8 genes, of which two were found in both men and women, Estrogen Receptor alpha (ESR1) and steroid-5-alpha-reductase, alpha polypeptide 2 (SRD5A2). However, the associations are likely to originate from different loci within the genes. In men the most significant SNP is rs7579411 in the LHCGR-gene (lsBMD: beta = −0.02, SE = 0.006, p-value = 5×10^{-4}; vert fx: OR = 1.6, 95%CI = 1.2–2.1, p-value = 4×10^{-4}). In women the most significant SNP is rs3757597 in the POR-gene (lsBMD: beta = 0.048, SE = 0.015, p-value = 9×10^{-4}; vert fx: OR = 0.288, 95%CI = 0.11–0.75, p-value = 0.004).

Discussion: This study indicates gender-specific associations for genetic variations in the estrogen pathway with lumbar spine BMD and vertebral fracture risk. After menopause locally produced estrogens are important for bone, whereas pre-menopausal and in men systemic estrogens are important. There could be a difference in genes responsible for local or systemic produced estrogen, explaining the gender-specific differences in this study.

Conflict of interest: None declared.

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P247

Lack of association between OPG and RANKL polymorphisms and BMD — Results from the Odense Androgen Study

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Introduction: The objective of this study was to investigate the effect of polymorphisms in osteoprotegerin (OPG) and nuclear factor-κB ligand (RANKL) on bone mineral density (BMD) in men.

Methods: Odense Androgen Study is a population-based, cross-sectional study on endocrine functions in men. The study population consists of random samples of 783 young (aged 20–30 yrs) and 600 elderly (aged: 60–75 yrs) men. BMD of the whole body (WBBMD), femoral neck (FNBMD), total hip (THBMD) and lumbar spine (LSBMD) were measured using a Hologic 4500 densitometer.

Polymorphisms in OPG (dbSNP: rs6993813, rs6469804) and RANKL (dbSNP: rs9594738, rs9594759) were analysed using Taqman real-time PCR.

Young and elderly men were analysed separately. Linear regression analysis and one-way ANOVA were used to test for associations between measures of bone mass and genotypes allowing for additive, dominant and recessive models. Adjustment for age, BMI, use of tobacco and alcohol consumption was performed.

Results: The polymorphisms were associated with WBBMD, FNBMD, THBMD or LSBMD neither before nor after adjustment of potential confounders, although tendencies towards associations between FNBMD and both OPG polymorphisms (rs6993813: $r = -.06$; $p = .10$. rs6469804: $r = -.06$; $p = .09$) were found in the young population.

Conclusion: Our study was not supportive of an association between polymorphisms in OPG and RANKL on BMD in males including PBM. The results may, however, be hampered by low power due to a rather small number of participants and the cross-sectional design. Furthermore, the results may only apply to males of Danish origin. Finally, the study did not incorporate information on fractures.

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P248

An interaction between PPAR gamma and polyunsaturated fatty acids influences changes of bone microstructure in ageing mice

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Background: Western diets characterized by low ratios of omega-3/-6 fatty acids (FA) have been associated with age-related chronic disorders. Mechanisms of PPAR gamma activation, increased adiposity and decreased osteoblastogenesis. In B6.C3H-6T (6T) congenic mice, which express higher levels of PPAR gamma compared to genuine C57BL/6J (B6), bone loss induced by a fat-rich diet is magnified (Ackert-Bicknell et al, JBMR 2008). We hypothesized that the level of PPAR gamma would specifically influence the effects of omega-3/-6 on bone mass and structure.

Methods: 6T and B6 female mice aged 3 months were given either a fish oil diet (omega-3/-6 ratio: 7.9) or an isocaloric safflower oil diet (omega-3/-6 ratio: 0) for 8 months ($n = 10$). Changes in body composition and bone mass were analysed by Piximus, and microstructure in caudal spine and tibia by in vivo microCT.

Results: After 8 months, body weight and % fat were lower with fish oil compared to safflower in both 6T (−24.9% and −35.4%, respectively, $p < 0.05$) and B6 (−30.0%, −27.6%, respectively, $p < 0.05$). Spine BMD was significantly improved in response to fish oil in B6 (71 ± 3 mg/cm^2 vs 54 ± 3 mg/cm^2 with safflower, $p < 0.01$), whereas femoral BMD was unaffected. In contrast, in 6T, vertebral BMD did not significantly differ between the two diets whereas femoral BMD was significantly lower in the fish oil compared to safflower group (76 ± 2 mg/cm^2 vs 83 ± 1 mg/cm^2, $p < 0.05$). In caudal spine of B6 mice, fish oil inhibited loss of BV/TV and trabecular number (TbN) (−6.0% and +2.2% versus −32% and −11.4% in the safflower group, respectively, all $p < 0.05$), whereas in 6T, fish oil did not have significant effects. In the proximal tibia, the loss of BV/TV and TbN with aging was not prevented by fish oil in B6. However, in 6T, fish oil further increased the loss of BV/TV and TbN (−93.7% and −94.4%, versus −84.8% and −87.3% in safflower group, respectively, all $p < 0.05$). At the femur midshaft, cortical bone volume gain was significantly reduced by fish oil in B6 (−1.7% vs +8.0% in safflower group, $p < 0.05$) and 6T (−1.7% and +5.6% in safflower group, $p < 0.05$). These differences did not remain significant after adjustment for body weight.

Conclusion: Compared to a diet rich in omega-6s, fish oil prevents spine BMD and trabecular bone loss in ageing mice. These beneficial effects are lost, and eventually reversed, in mice expressing *PPAR gamma* gene at higher levels, suggesting that the effects of FA on bone microstructure may depend on interactions with PPAR gamma genotypes. Moreover, the effects of FA on trabecular microarchitecture appear to be site dependent, which could be related to differences in adiposity.

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P249

Genetic variation in the *TNFRSF11A* (RANK) gene contributes to the risk to develop sporadic Paget's disease of bone

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RANK (receptor activator of nuclear factor-\kappaB), encoded by the *TNFRSF11A* gene, is one of the most important proteins in osteoclastogenesis and bone remodeling. Mutations in *TNFRSF11A* have been reported to cause Paget's disease of bone (PDB)-like diseases (i.e. familial expansile osteolysis, expansile skeletal hyperphosphatasia and early onset PDB) and an osteoclast-poor form of osteopetrosis. Yet, the role of the *TNFRSF11A* gene in classical PDB has not been investigated in detail.

We have conducted an association study in 196 Belgian sporadic PDB patients (83 females and 112 males) and 212 controls (86 females and 126 males). Based on HapMap, 27 tagSNPs and 5 multimarker tests (MMTs) were selected in and around *TNFRSF11A*. In addition, we have included 1 non-synonymous SNP (H141Y) which is not present in HapMap. Genotyping was carried out by KASPar technique (KBioscience, UK), TaqMan assay and direct sequencing. Statistical analysis indicates that 13 SNPs and 2 MMTs are significantly associated (*P*-values between 0.037 and 3.17×10^{-4}), the majority of them due to an association in the female subcohort. Six SNPs and 1 MMT withstand the Bonferroni correction ($P<0.002$).

In order to confirm our findings, replication studies were performed with the 2 non-synonymous SNPs (H141Y and A192V) in 1) a Dutch cohort with 78 cases (35 females and 43 males) and 95 controls (46 females and 49 males) and 2) a British cohort with 282 cases (144 females and 138 males) and 325 controls (166 females and 159 males). Statistics of the 2 populations shows significant *P*-values for both SNPs: H141Y with $P=0.012$ and $P=0.028$ (respectively); and A192V with $P=8.8\times10^{-5}$ and $P=0.005$ (respectively). Although the association of H141Y seems to be driven only by females (the Dutch: $P=0.004$ and the British: $P=0.047$), significance of A192V is observed in males as well as in females (the Dutch: $P=0.012$ and $P=0.002$, respectively; and the British: $P=0.047$ and $P=0.045$, respectively).

Finally, meta-analysis of all 3 European populations results in $P=4.73\times10^{-8}$ for A192V (common OR of *C* allele = 1.575, 95%CI: 1.339–1.852) and $P=0.004$ for H141Y (common OR of *C* allele = 1.374, 95%CI: 1.111–1.700).

In conclusion, these results provide a very strong indication that genetic variation within *TNFRSF11A* influences the risk to develop sporadic PDB. Functional studies are ongoing to check whether any of the non-synonymous SNPs is the real causative SNP.

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P250
Associations of the CALCR and VDR genes with bone density, bone-related biochemical markers and fracture incidence with regard to calcium intake level in Slovak postmenopausal women

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The *calcitonin receptor* (*CALCR*) and the *vitamin D receptor* (*VDR*) genes code proteins that mediate an action of hormones involved in calcium homeostasis in target tissues. Therefore, genetic variability in the genes could affect the variability of bone mineral density (BMD), bone remodeling, and could lead to osteoporosis and higher fracture risk.

In this study we analyzed associations of AluI polymorphism in the *CALCR* gene and FokI polymorphism in the *VDR* gene with variability of femoral and spinal BMD, circulating alkaline phosphatase (ALP) and osteocalcin (OC; formation markers), beta-CrossLaps (CTx; resorption marker), and fracture incidence with regard to different calcium intake.

Our study involved 152 Slovak postmenopausal women (64.4 ± 7.2 years) selected according to strict inclusion criteria. Genetic polymorphisms were detected by PCR-RFLP method. The differences between the genotypes were analyzed by GLM procedure and covariance analysis after correction of the measurements for age and BMI. Gene–gene interactions were also evaluated within the statistical analysis. Frequencies of fractures were tested using the chi-square test. The fracture frequencies were also compared between groups with different daily calcium intake of <350 mg (group A), 350–650 mg (B), and >650 mg (C).

We found a significant effect of VDR–CALCR interaction on femoral BMD ($P=0.033$) and spine BMD ($P<0.001$). No polymorphism alone affected any of the analyzed traits significantly; however, effects of *VDR* gene on spine BMD ($P=0.070$) and ALP ($P=0.086$) were not far from the significance level. We did not find significant associations between the genes and OC, CTx. Comparison of fracture incidence between the genotype groups showed significant differences ($P<0.05$) for the *CALCR* gene regardless of calcium intake level. The effect of the *VDR* gene was significant only in C diet group ($P<0.01$). The ff-genotype carriers had higher frequency of fracture than the others.

The analysis of associations between candidate genes (interactions), diet, and BMD or bone turnover markers can extend our knowledge about molecular background of bone remodeling and loss. The results could be also applicable in osteoporosis susceptibility prediction.

All procedures were approved by the Ethical Committee of the Specialized Hospital of St. Svorad in Nitra (Slovakia). This study was supported by the grant KEGA 2009-2011 (Omelka).

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