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MK-7 enhances expression of genes related to bone, enamel and dentin, and reduces the expression of genes related to apoptosis in developing murine molars

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Introduction
The fat-soluble and vitamin K2 homologe Menaquinone-7 (MK-7) is needed for post-translational modification of proteins essential in blood coagulation, and in metabolic pathways in various tissues like bone. Recent studies found an association between long-term anticoagulant treatment (OAC) and reduced bone quality due to reduction of active osteocalcin. OAC is often linked to an undesired soft-tissue calcification in both children and adults and may lead to increased incidence of fractures, reduced bone mineral density/bone mineral content, osteopenia and increased serum levels of undercarboxylated of vitamin K-dependent proteins, known as Gla-proteins. Little is known about the effects of vitamin K2 during tooth development.
Methods
New born Balb C mice were exposed to MK-7 (0.2, 2 or 10 mg/kg body weight) (Kappa Bioscience, Oslo, Norway) using a local s.c. injection on the right side mandibula. The control group was mice injected with vehicle. At 24 h post-injection the pups were sacrificed and first right-hand side molar dissected. Total RNA was isolated from the dissected molar using the RNeasy Mini Kit and used for analysis of gene expression using deoxyoligonucleotide microarrays and RT-PCR. Biological triplicates were used. Microarray results were validated by RT-PCR. Bioinformatic analysis was performed using Ingenuity Pathways Analysis. The results are based on measurements from biological triplicates.
Results
After Treatment with 10 mg/kg body-weight MK-7, 629 genes showed altered gene expression ($P<0.05$) compared to controll with the molecular and cellular functions: carbohydrate metabolism, Cell-to cell-signaling/interaction and cellular growth and proliferation. Treatment with 0.2 and 2 mg/kg body weight MK-7 influenced the expression of genes associated with ' cell death', with a highly significant association to decreased apoptosis.
Conclusions
A clear effect on gene expression in the developing tooth germ was apparent after 24 h at all dosages. The results indicate increased transcription of genes involved in development of bone (increased biosynthesis of important carbohydrates) and of enamel/dentin, and reduced expression of apoptosis related proteins.

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Analysis of genetic polymorphisms in relation to bone mineral density and fracture risk in maltese postmenopausal women

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Background
Osteoporosis is a hereditary multifactorial disease characterised by low bone mass leading to an increased susceptibility to fracture. Bone mineral density (BMD) is the most widely used predictor of fracture risk. Gene variants have been found associated with a low BMD and increased fracture risk; nonetheless studies have identified the relationship between susceptibility genes and fractures independent of BMD.
Objective
Eight single nucleotide polymorphisms (SNPs) within four candidate genes were investigated for their effect on BMD at different anatomical sites and with different low-trauma fractures.
Methods
One thousand and forty-five maltese postmenopausal women were recruited and BMD measurements were performed by dual-energy X-ray absorptiometry. Women who suffered low-trauma fractures were classified as cases whereas subjects without a fracture history were included as controls. Informed consent was obtained from all participants. Genotyping was performed by PCR followed by restriction fragment length polymorphism, and RT PCR high resolution melt.
Results
Using logistic regression analysis adjusted for age, three SNPs in three genes (LRP5 (rs3736228), RANK (rs3018362) and OPG (rs2073618)) were found associated with a low BMD and increased risk of all-type of low trauma fractures ($P<0.05$). SNPs rs3736228 and rs3018362 were associated with reduced BMD at the spine and femoral neck, whereas rs2073618 was only linked to low spine BMD. Three SNPs in the OPG gene (rs3134069, rs3102735 and rs2062377) were associated with an increased fracture risk that conversely did not affect BMD. The haplotype carrying the risk alleles for rs3736228, rs3018362, rs3134069, rs3102735 and rs2062377 was associated with increased fracture risk (permutated P-value = 0.01) as opposed to the haplotype reference which was strongly linked to a high BMD and low fracture risk (permutated P-value = 0.0001).
Conclusion
Results from this independent replication study indicate that a number of gene variants are associated with reduced BMD and/or increased fracture susceptibility in maltese postmenopausal women.

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Interactions between the effects of polymorphisms in the RANK and RANKL genes affects bone mass

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Osteoporosis is a common disorder with a partly genetic pathogenesis. Interaction between RANKL and its receptor RANK is essential in bone remodeling.
We therefore investigated the effect of polymorphisms in the *RANK* and *RANKL* genes and interaction between the effects on bone mineral density (BMD) and vertebral fractures.
The study was a case-control study with 462 osteoporotic patients and 336 controls. Ten polymorphisms in *RANK* and seven in *RANKL* were selected for genotyping. We genotyped using Taqman or sequencing and examined BMD by DXA. We examined interaction of polymorphisms on BMD or vertebral fractures using the software FAMHAP and performed other statistical analyses using SPSS.
None of the polymorphisms affected BMD or fracture risk. Interaction analyses revealed interaction between the effects of *RANK* polymorphism rs9653064 and *RANKL* polymorphisms rs2277439, rs2875459, rs922996, and rs1054016 on lumbar spine BMD (global $P<0.1$ for all). Interaction was also found between the effects of *RANK* polymorphism rs56231704 and *RANKL* polymorphisms rs2277439 on lumbar spine-, femoral neck, and total hip BMD, rs922996 and rs1054016 on lumbar spine BMD, and rs56231704 on total hip BMD (global $P<0.1$ for all). Subsequent analyses of the effect of *RANKL* polymorphisms on BMD were stratified for *RANK* genotypes and revealed several interactions between polymorphisms in the two genes, for example that BMD was higher at all sites in individuals homozygous for the normal allele at *RANK* rs9653064 and carrying the variant allele at *RANKL* rs2277439 compared with individuals homozygous for the normal allele at both polymorphisms, whereas BMD was lower at all sites in individuals carrying the variant allele at both polymorphisms compared with individuals carrying the variant allele at *RANK* rs9653064 and homozygous for the normal allele at *RANKL* rs2277439 ($P<0.05$).
This study shows that RANK and RANKL interact at the DNA level as at the protein level.

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Association of methylenetetrahydrofolate reductase (MTHFR) polymorphism (C677T) with clinical indicators of osteoporosis in postmenopausal Slovak women

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Objective
The enzyme methylenetetrahydrofolate reductase (MTHFR) is known to play an important role in the removal of circulating homocysteine *via* the methionine cycle. C677T polymorphism is associated with higher plasma homocysteine levels, which could affect collagen maturation. The aim of the present study was to examine possible associations of C677T polymorphism in the MTHFR gene with a variability of femoral (F-BMD), spinal BMD (S-BMD) together with circulating alkaline phosphatase (ALP), osteocalcin (OC; formation markers), beta-CrossLaps (CTx; resorption marker) and fracture incidence in 334 Slovak postmenopausal women.