

### THE SIZE OF IN VITRO CULTURED OSTEOCLASTS WAS INCREASED AND THE OSTEOCLASTIC ACTIVITY DECREASED AFTER TREATMENT WITH MMP-INHIBITOR BB-94

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Matrix metalloproteinases (MMPs) are a group of enzymes capable of degrading extracellular matrix components. The exact roles of MMPs in bone biology are not understood, but they have been suggested to be required for the solubilization of bone matrix and possibly for osteoclast migration and anchorage. At least two members of the group, MMP-9 and MT1-MMP, have been demonstrated to be present in osteoclasts. In addition, MMPs of osteoblast lineage cells probably also affect osteoclasts.

We have previously shown that bone resorption is disturbed in the presence of MMP-inhibitor batimastat (BB-94). In the present study we further analysed the effects of BB-94 on osteoclasts. A mixed rat bone cell population was cultured on bovine bone slices for three days. After the culture period cells were stained for tartrate-resistant acid phosphatase (TRAP) and with DNA-binding fluorochrome Hoechst 33258 to visualize nuclei. F-actin was stained with TRITC-conjugated phalloidin to detect actin rings.

MMP-inhibitor BB-94 at 6microM concentration, did not significantly affect the number of osteoclasts. Instead, the average number of nuclei per osteoclast was significantly increased ( $7.41 \pm 0.36$  versus  $9.53 \pm 0.55$ ). In addition, the number of mono- and binuclear TRAP-positive cells was lower on BB-94 treated slices. Phalloidin staining revealed that the formation of actin rings was dramatically decreased by BB-94. These result were reproduced with another MMP-inhibitor BB-2516. Another major group of proteinases expressed in osteoclasts is cysteine proteinases. To confirm that the effect on osteoclast size was specific for MMP-inhibition, we studied the effect of cysteine proteinase inhibitor E-64 on osteoclasts. It had no effect on the size of osteoclasts even at 50microM concentration.

To conclude, we demonstrated that the osteoclast size was increased and the osteoclastic activity decreased by MMP-inhibitor BB-94. We suggest that the observed phenomenon may be due to disturbed migration of osteoclasts.

## Genetics

### P-24

#### COMPLICATIONS IN LEG LENGTHENING PROCEDURE BY WAGNER APPARATUS IN CHILDREN: DIFFERING CONGENITAL AND ACQUIRED AETIOLOGY OF SHORTENING

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Leg lengthening procedure is often accompanied by many complications. Connection between aetiology of abbreviation and complications was studied in 36 patients (average age 11 years and 2 months). All the patients were younger than 18 yrs without previous leg lengthening operative procedure made of any kind. They underwent 41 lower limb leg lengthening operations (33 femoral and 8 tibial) by Wagner apparatus. To the one of the children femur and tibia on the one leg were elongated and to the other one together femur and tibia on both legs were lengthened. Their medical data were collected retrospectively and analysed. Average preoperative leg length discrepancy was 5.4 cm: average lengthening achieved was 5 cm. Wagner apparatus was placed in situ in average during 5 months and 21 days. Children were divided by the aetiology of leg shortening in two groups; in 25 of them aetiology was congenital (congenital idiopathic hypoplasia, congenital hip dislocation consequences, coxa vara, mb. Ollier, mb. Klippel-Trenaunay, achondroplasia) and 11 of them had acquired aetiology of abbreviation (tumours resection effect, paresis et paraparesis consequences caused by polio, meningitis, myelomeningocele, tumours resection effect, posttraumatic status). 22 elongations were followed by one or more complications (14 patients had joint contractures, 8 pin-track infections, 4 fractures, 2 too early consolidations, 1 pseudarthrosis). Complications were divided on heavier (an additional operative procedure was required) and easier (solved with physical therapy and antibiotics). It was presumed that the frequency of complications and their severity are influenced by aetiology of shortening. Statistical data evaluated by Hi square test show no significant difference. It seems that the aetiology does not play the role in prognosis of complication rate and severity during leg lengthening procedure by Wagner apparatus. Reoperations could be avoided by continuous physical therapy considering setting the operated leg under graduate pressure, rigorous pin-track toilette with microbiological sample taking and AO fixation with spongioplasty.

### P-25

#### VITAMIN D RECEPTOR GENE POLYMORPHISMS AND BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN IN MALTA

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Osteoporosis is a disease with a strong genetic component. One of the genes that has been implicated in osteoporosis is the Vitamin D receptor (VDR) gene, although there have been conflicting results as to whether VDR gene polymorphisms influence bone mineral density (BMD). The aim of this study was to determine whether correlation exists between VDR gene polymorphisms and bone mineral density in postmenopausal women in Malta.

104 postmenopausal women (aged 48-62 years) were recruited for this study. Amplification of the desired target sequence of the VDR gene was carried out by PCR, and the subjects were genotyped on the presence (b) or absence (B) of the BsmI restriction enzyme cleavage site in the VDR gene. BMD at the lumbar spine and femur was measured using a Norland DEXA 486. Serum concentrations of procollagen Type 1 and urinary deoxypyridinoline crosslinks were used as biochemical markers of bone turnover.

The genotype frequencies observed (BB=16.4%, Bb=51.9% and bb=31.7%) were similar to those observed in other Caucasian populations. BMD at both anatomical sites was observed to be highest in BB homozygotes, although the difference between the genotypes was not statistically significant (ANOVA p=0.768; 0.967 for lumbar spine and femur, respectively). The biochemical marker of bone formation was observed to be highest in the BB genotype, whereas the biochemical marker of bone resorption was found to be lowest in the BB group, although both these results were not statistically significant (ANOVA p=0.591; p=0.806 respectively). A non-statistically significant difference (Chi-square test p=0.624) was observed in the frequencies of VDR genotypes between normal individuals and a group of osteoporotic women at the lumbar spine (t-score < -2.5.)

In conclusion, there does not seem to be an association between polymorphisms in the VDR gene and BMD in postmenopausal women in Malta.

### P-26

#### POLYMORPHISM IN THE VITAMIN D RECEPTOR GENE AND THEIR RELATIONSHIP WITH BONE LOSS IN POSTMENOPAUSAL WOMEN

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Bone mineral density (BMD), the major determinant of fracture risk, is under strong genetic control. Although polymorphisms of the vitamin D receptor (VDR) gene have been suggested to account for some genetic variation in bone mass, the influence of VDR genotypes on osteoporosis remains controversial. We examined the possible effect of these genotypes on postmenopausal bone loss.

Patients and methods: We performed a multicenter trial in 211 postmenopausal women (mean age 54.3). VDR polymorphisms were determined by RFLP using Bsm I (intron 8), Apa I (intron 8) and Taq I (T1055-C) after PCR. For the hypothesis contrast and for every polymorphism, we have analysed the genotypes separately and after we have analysed grouped in unfavorable genotypes and their relationship with bone loss rate. We have also analysed the lumbar spine and femoral neck BMD. Bone mass was measured by DEXA. The percent change in BMD at 2 yr was used to classify subjects as slow bone loss (<OR=1.4%), intermediate (between 1.4 and 2.7%) and fast bone loss patients (estimated annual bone loss >2.7%).

Results: The prevalence of genotypes were BB: 22.3%, Bb 40.2% and bb: 37.5%; AA 31.3%, Aa 41.1% and aa 27.7% and TT 48.2%, Tt 51.8% and tt 18.8%. Distribution of different genotypes, grouped and ungrouped, was similar between the three groups of annual bone loss rate, neither did we find any significant association between the different genotypes and bone mass loss.

Conclusions: In this study, VDR genotype is not associated with the annual bone loss in postmenopausal women

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