Investigation of bone metabolism in different molecular biological levels

Doctoral thesis

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INTRODUCTION

Osteoporosis is a modern endemic metabolic multifactorial skeletal disease in the aging populations of industrial countries. A large number of environmental factors are involved in the pathogenetic process, including lack of physical activity, cigarette smoking, alcohol consumption, dietary intakes and diet composition. Nevertheless, the influence of genetic factors on osteogenesis and bone loss is significant. The development of the osteoporotic condition and the quantity and quality of bone are considerably influenced by genetic factors. The variability of phenotypes such as bone mineral density (BMD), femoral neck geometry (size and shape), quantitative ultrasound properties, microarchitecture (trabecular, cortical thickness) and biochemical markers of bone turnover is known to be under strong genetic control. It has been well established that estrogenic hormones playing role in menopausal changes can strongly support physiological skeletal homeostasis via numerous pathways. Nevertheless, several different cytokines as well as local and systemic factors have been suggested to be involved in these estrogen-dependent biological processes.

Osteoporosis is a polygenic disease, involving a large variety of gene products. The genetic background of osteoporosis has been examined in numerous studies mainly in *genomic* level. Linkage analysis, association studies and searching for polymorphic markers focused on candidate genes. These genes encode calciotropic hormones and their receptors (such as vitamin D receptor, estrogen receptor alpha, calcitonin receptor, calcium sensing receptor), bone matrix components (such as collagen type I alpha 1, osteocalcin, osteonectin, osteopontin), and local regulators of bone metabolism (such as transforming growth factor beta, insulin-like growth factor, interleukin 1 beta, bone morphogenetic protein 4). Several associations between serum concentration of bone markers and BMD have been observed in *proteomic* level. Principally factors promote bone formation (ALP, IGF) or bone resorption (IL-6) show correlation with bone mass. The results of the *metabolomic* level of systems biology are collagen cross linkers, which have strong diagnostic power. *Transcriptional* changes in the activity of several bone cell-specific genes are attributed to estrogenic hormones. These genes include receptor activator of NF-κB (RANK), receptor activator of NF-κB ligand (RANKL), osteoprotegerin (OPG), vitamin D receptor (VDR), alkaline phosphatase (ALP), variable growth factors and extracellular matrix proteins. Estrogen has a direct suppressive influence on the synthesis of bone-resorbing pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and lysosomal enzymes (lyzozyme, cathepsin B, D) which promote osteoclastogenesis and osteoclast activity.

So far the impact of single genes on bone metabolism has been examined, and only limited data are available on the expression profile of human bone tissues.

Numerous connections including cellular and molecular mechanisms have been discovered between bone and the immune system (*i.e.* using same progenitors as well as parallel overlapping signaling networks and cytokine panels in the common bone marrow niche). Activated T cells produce receptor activator of nuclear factor kappa B ligand (RANKL) which is capable of inducing osteoclastogenesis and promoting bone resorption via receptor activator of nuclear factor kappa B (RANK) presented on the surface of osteoclasts. Besides the T cell-osteoclast interaction, osteoblast also has immune-competent properties. Osteoblasts have been found to express MHC type II molecules and ligands for T cell co-stimulation. There are a few studies reporting the immunological aspects of osteoporosis and bone loss. The $CD4^+$ / $CD8^+$ T cell ratio is changed in osteoporotic patients. Increased number of another lymphocyte subpopulation, the natural killer T cells is associated with decreased bone mineral density in osteoporotic women. Hormonal deficiency after menopause affects alike the bone and immune homeostasis and modifies their complex relationship. Sex hormone deficiency triggers the function of dendritic cells and macrophages, as well as intensifies the antigen presentation process and MHC type II gene transcription. Thymectomy or immune-suppressant drug administrations that inhibit T cell function resulted in rapid bone loss and demineralized bone production. There is increasing evidence that the immune system modifies bone resorption and formation, nevertheless only limited data are available on the influence of osteoblastic and osteoclastic cells on immune functions in osteoporotic bone tissue.

AIMS

I. Examination of postmenopausal bone loss in transcriptomic level

I/a. Transcriptional profiling of bone tissue of postmenopausal osteoporotic and non-osteoporotic women

The aim of our examination was to identify the multiple gene expression profiles of osteoporotic and non-osteoporotic bone samples from postmenopausal women using quantitative real-time RT-PCR. For comparing gene expression patterns in the bone tissue of postmenopausal osteoporotic and non-osteoporotic women, we selected 120 candidate genes that might be involved in the development and pathogenesis of osteoporosis based on recent literature, as well as data concerning genetic pathway analysis.

I/b. Complex gene expression analysis of postmenopausal and premenopausal human bone tissue

The aim of our investigation was to determine genes characterized by significantly changed mRNA expression rates in postmenopausal *vs.* premenopausal non-osteoporotic bone tissue applying Taqman probe based quantitative real time PCR, as well as to describe the interrelationships among these genes using multivariate data analysis.

II. Single nucleotide polymorphisms of new candidate genes in postmenopausal women

We aimed to investigate the effect of multiple SNPs in ALPL, FABP3, FGFR1, MMP2, TIMP2, OPG and RANKL genes on BMD in 353 Hungarian postmenopausal women using a high-throughput genotyping method.

III. Examine the expression changes of immune system-related genes in human bone tissue

Our objectives were to identify differences in the multiple transcription profiles of additional 27 immune-related genes in human bone samples, and to search for gene clusters which separate the menopausal states of human bone tissue in an immunological approach.

MATERIALS AND METHODS

1. Study groups of postmenopausal osteoporotic and non-osteoporotic women

Gene expression profile in bone samples was determined in 7 postmenopausal, unrelated, consecutive, Hungarian, Caucasian osteoporotic women (OP group). The control group included 10 bone tissue samples from postmenopausal non-osteoporotic women (NOP group). OP was defined according to the WHO criteria, i.e., T-score less than -2.5 SD at any measured site. There was no significant difference between the osteoporotic and control groups in age, smoking habits, calcium intake, alcohol, caffeine consumption, physical activity, and they have not received any steroid or biological therapies. Strongly pronounced differences were observed principally in T-score and BMD at the total femur ($p = 0.0004$) as well as in T-score and BMD at the lumbar spine ($p = 0.002$) between osteoporotic and non-osteoporotic subjects.

2. Study groups of postmenopausal and premenopausal non-osteoporotic women

Gene expression profile in bone samples was determined in 10 postmenopausal, unrelated, consecutive, Hungarian, Caucasian non-osteoporotic women (POST group) who did not received hormonal replacement therapy. Menopause was defined according to the recent WHO criteria. The control group included 7 bone tissue samples from premenopausal non-osteoporotic women (PRE group). There were no significant differences between the pre- and postmenopausal groups in age, bone mineral density, smoking habits, calcium intake, alcohol, caffeine consumption and physical activity. Remarkable differences were observed in the level of serum estradiol ($p = 0.0006$) and in the markers of bone turnover, *i.e.,* osteocalcin ($p = 0.002$) and beta-crosslaps ($p = 0.01$) plasma levels between the two study groups.

3. Patient group of single nucleotid polymorphism (SNP) study

Three hundred and fifty three Hungarian unrelated postmenopausal women were recruited to the study through our clinic. Exclusion criteria were history of bone, metabolic, or endocrine disease; any chronic illness; hormone replacement, steroid therapy, or any medication known to influence bone metabolism. Subjects with biochemical abnormalities such as increased levels of serum ALPL, thyrotrophin, parathyroid hormone, or reduced level of 25-OH vitamin D (<30 ng/ml) were not included in the study.

4. Densitometry

BMD values at the lumbar spine (L2–L4) and total hip were measured using a Lunar Prodigy DXA (GE Medical Systems). BMD at the distal radius was determined by a Norland pDEXA (Cooper- Surgical Inc.) densitometer.

5. Human bone tissue samples

All subjects participating in the study had undergone surgery due to primary osteoarthritis. Osteoarthritic patients were classified as grade III using the Kellgren-Lawrence Grading System for Osteoarthritis. The disease did not affect the spongious bone substance of femoral head and there was no difference in the degree of arthrosis among subjects by X-ray examination. Bone tissue samples were collected from the spongious substance of femoral head during total hip arthroplasty before resection. Surgically removed bone samples were extensively washed in PBS and placed immediately into liquid nitrogen. The study was approved by the Regional Committee of Science and Research Ethics, Semmelweis University (6392-1/2004-1018EKU), and all patients gave written informed consent.

6. Direct polyA-RNA isolation

Human bone samples (approximately 500 mg) were cryo-grinded under liquid nitrogen using a freezer-mill 6750 (SPEX Certiprep Inc.). Direct mRNA isolation using Dynabeads Oligo $(dt)_{25}$ kit (Dynal Biotech ASA) according to manufacturer's protocol. After DNase treatment mRNA was cleaned with NucleoSpin RNA Clean-up kit (Macherey-Nagel). The quantity and quality of purified mRNA were checked by NanoDrop spectrofotometer (Nanodrop Technologies) at 260/280 nm. Using 200 U SuperScriptIII RNase H - Reverse Transcriptase (Invitrogen Life Technologies), 100 ng of each human mRNA sample was reverse transcribed.

7. Genomic DNA isolation

Genomic DNA was isolated from EDTA blood using a commercially available kit (Roche High Pure PCR Template Purification kit, Roche Diagnostics). The quantity and quality of purified mRNA were checked by NanoDrop spectrofotometer at 260/280 nm.

8. Selection of the examined genes and quantitative real-time PCR

For quantitative real-time RT-PCR comparison of gene expression patterns of candidate genes in the bone tissue of postmenopausal and premenopausal non-osteoporotic women, we selected 147 genes based on recent literature, OMIM database, as well as our data concerning genetic pathway analysis. Predesigned and validated gene-specific TaqMan Gene Expression Assays from Applied Biosystem (Foster City, CA, USA) were used in triplicate for quantitative real-time PCR according to the manufacturer's protocol.

9. Gene and SNP selection

Based on our previous study and on online data mining (http://www.hapmap.org, http://www.ncbi.nlm.nih.gov/OMIM/,), we chose seven genes expressed in human bones that are likely to play an essential role in bone metabolism; ALPL, MMP2, TIMP2, FGFR1, RANKL, OPG and FABP3. We chose 26 SNPs from the sites of the genes with optimal (40–

65%) GC content. Large-scale genotyping process was performed at the SNP Core Facility of Semmelweis University using a GenomeLab SNPstream Genotyping System (Beckman Coulter, Fullerton, CA, USA). The automated ultrahigh- throughput genotyping system utilizes multiplexed PCR in conjunction with tagged-array, multiplexed, single-base primer extension technology

10. Multiparametric statistical analysis

We used standardized PCA because there were still excessive differences in the variance of variables. In the biplot graphic drawn for two components at a time, the observations (in this case, the subjects) appear as points, whereas the variables (in this case, the genes) are emphasized by lines pointing to their positions. This simultaneous representation allows for the evaluation of the grouping of subjects and of the relative importance and correlations of genes in influencing this configuration. Groups may be further illuminated by superimposing convex hulls on the ordination plane. Whereas PCA explores the total variance in the data, canonical variates analysis (CVA, alias discriminant function analysis) maximizes separation of *a priori* defined groups of observations. The results of CVA are canonical scores obtained from the canonical functions derived through eigenanalysis, which serve as coordinates of observations in the canonical space. Since the number of canonical axes is one less than the number of groups, in our case CVA produced only one variate.

RESULTS

1. Comparison of gene expression in osteoporotic vs. non-osteoporotic women by Mann-Whitney U test

Nine genes, namely alkaline phosphatase (ALPL), type I collage alpha 1 chain (COL1A1), matrix metalloproteinase 2 (MMP2), matrix metalloproteinase 13 (MMP13), matrix metalloproteinase 9 (MMP9), platelet-derived growth factor alpha polypeptide (PDGFA), nuclear factor of kappa light polypeptide (NFKB1), thrombospondin receptor (CD36) and twist homolog 2 (TWIST2), the Mann-Whitney U test indicated significant differences in gene expression between osteoporotic and non-osteoporotic individuals ($p \leq 0.05$). Seven of these 9 genes were significantly down-regulated in osteoporotic women as compared to non-osteoporotic ones. The fold change was 0.41-fold in the case of ALPL expression, 0.39-fold for COL1A1, 0.40-fold for MMP2, 0.21-fold for MMP13 and 0.34-fold for MMP9, 0.47-fold for PDGFA, and 0.44-fold for NFKB1. In contrast to the above 7 down-regulated genes, two genes were significantly up-regulated in osteoporotic patients. The

observed increase in expression in osteoporotic bone was 4.00-fold for the TWIST2 and 3.22-fold in case of CD36.

1.1 Multivariate analyses

The first two components derived by standardized PCA account for 30.4% (eigenvalue = 23.7) and 18.4% (eigenvalue = 14.4) of the total variation, respectively. The subsequent components (starting at 9.8%) were ignored in this evaluation, because the scree test suggests clearly that these are responsible only for stochastic variation (see inset in Fig. 1). The osteoporotic patients (OP) form a relatively compact group near the origin, and only two non-osteoporotic patients (NOP02 and NOP15) are intermingled with them. On component 1, NOP12 is separated from the others, whereas on component 2 NOP07, takes a relatively distant position. NOP11 and NOP13 are in the negative domain for both components and thus fall outside the group of osteoporotic women (Fig. 1).

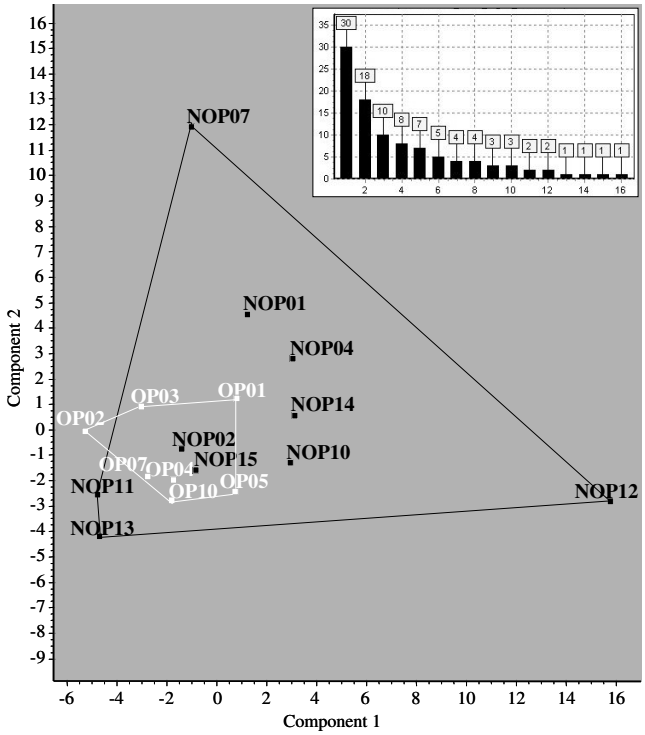


Fig. 1. Principal components ordination representing the positions of 17 patients along the first two dimensions. Convex hulls superimposed enhance discrimination between groups.

On the basis of the expression pattern the osteoporotic and non-osteoporotic phenotypes sharply separated from each other. The osteoporotic people (white OP) formed a small and compact cluster according to their same gene expression rates which characterize their phenotype. The non-osteoporotic patients (black NOP) were more loosely positioned, most of them distant from the postmenopausal osteoporotic ones.

1.2 Canonical variates analysis

Eight gene subsets have been evaluated by CVA (Fig. 2). The subsets were selected based on the results of the Mann-Whitney U test (9 genes) and principal components 1 (PC-1, 16 genes) and 2 (PC-2, 13 genes). Some of the gene subsets were created on their similar signal transduction control on literature basis: TGFB/activin/nodal pathway (12 genes), BMP cascade (7 genes) and 8 genes regulated by estrogen signaling. The remaining two gene sets contain human osteoporosis diagnostic markers (8 genes) whose serum level, expression changes or gene polymorphisms have been associated to enhanced bone turnover as well as BMD and genes showing 3-fold or more down-regulation in gene expression levels of osteoporotic patients (9 genes) in the present quantitative real-time PCR study. The gene set derived from component 1, including growth factors and receptors exhibited the best discriminatory power which separates the osteoporotic and non-osteoporotic subjects unambiguously. Genes belonging to the TGFB/activin/nodal pathway and genes showing 3-fold or more down-regulation in osteoporotic patients also showed strong correlation with the single canonical variate, hence achieving clear separation of the two groups of postmenopausal women. The subsets of diagnostic markers and the nine significant genes detected by the Mann-Whitney U test had lower discriminatory power (Fig. 2).

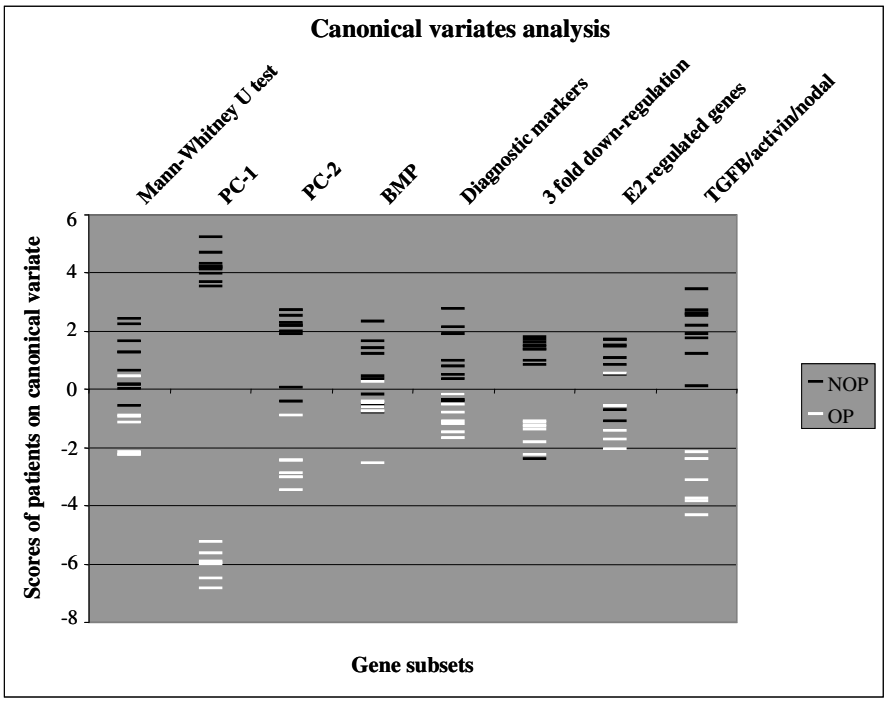


Fig. 2. Canonical variates analysis of gene expression patterns of bone tissue in seven postmenopausal osteoporotic (OP, white bars) and ten postmenopausal non-osteoporotic (NOP, black bars) women. The axis on the left is the single canonical variate, while gene subsets are arranged along the horizontal axis.

2. Comparison of gene expression in 10 postmenopausal vs. 7 premenopausal non-osteoporotic women by Mann-Whitney U test

In postmenopausal non-osteoporotic women, seven collagen molecules (COL2A1, COL3A1, COL5A1, COL5A2, COL9A1, COL12A1, COL15A1), three non-collagen type extracellular matrix (ECM) molecules (MGP, BGLAP, FN1) and two matrix degrading enzymes (MMP13, BMP1) showed significantly up-regulated expression patterns. Five genes belonging to the common TGFB/BMP signal transduction network (BMPR1A, TGFB2, TGFB3, TGFBR2, SMAD4) have been characterized by enhanced gene transcription rates in estrogen deficient women. The two indispensable transcription factors of osteoblasts (RUNX2, SP7) and the remaining transcription factors involved in the Wingless pathway (TCF7L2) and a chondrocyte maturation gene (SOX9) have shown increased expression in postmenopausal women compared to premenopausal ones. Two growth factors involved in MAPK signaling (PDGFA, FGFR1) also exerted overexpression in the absence of estrogen. Two osteoclast stimulating factors (TNFSF11/RANKL, IL6) and a main marker of differentiated osteoblasts (ALPL) had elevated expression activity in postmenopausal non-osteoporotic subjects. IGSF4 and TRIB2 have been detected in non-osteoporotic human bone tissue for the first time. These genes were found to be over expressed in menopause. In case of ENO1/MBP1, diminished expression levels were observed.

	Gene symbol[b]	Gene name[b]	Fold change RQ POST/ RQ PRE[c]	*p* value[d]
ECM components and digesting enzymes	**COL2A1**	Collagen type II alpha 1	**8,07**	0,02
	COL3A1	Collagen type III alpha 1	**5,62**	0,02
	COL5A1	Collagen type V alpha 1	**9,60**	0,03
	COL5A2	Collagen type V alpha 2	**4,82**	0,02
	COL9A1	Collagen type IX alpha 1	**5,63**	0,04
	COL12A1	Collagen type XII alpha 1	**5,30**	0,02
	COL15A1	Collagen type XV alpha 1	**6,59**	0,01
	MGP	Matrix gamma-carboxyglutamate (gla) protein	**3,28**	0,02
	BGLAP	Bone gamma-carboxyglutamate (gla) protein, osteocalcin	**5,52**	0,01
	FN1	Fibronectin 1	**4,02**	0,01
	MMP13	Matrix metalloproteinase 13 (collagenase 3)	**3,70**	0,02
	BMP1	Bone morphogenetic protein 1	**3,12**	0,04
TGFB/BMP pathway	**BMPR1A**	Bone morphogenetic protein receptor type IA	**6,16**	0,01
	TGFB2	Transforming growth factor beta 2	**3,42**	0,04
	TGFB3	Transforming growth factor beta 3	**3,70**	0,002
	TGFBR2	Transforming growth factor beta receptor II	**3,26**	0,03
	SMAD4	SMAD, mothers against DPP homolog 4 (Drosophila)	**2,59**	0,002
Transcription factors	**ENO1 / MBP1**	Enolase 1 / C-myc promoter-binding protein	**0,53**	0,005
	RUNX2	Runt-related transcription factor 2	**2,76**	0,01
	SOX9	SRY (sex determining region Y)-box 9	**9,35**	0,04
	SP7	Sp7 transcription factor (osterix)	**5,65**	0,04
	TCF7L2	Transcription factor 7-like 2 (T-cell specific)	**3,02**	0,01
Growth factor, receptor	**PDGFA**	Platelet-derived growth factor alpha polypeptide	**2,81**	0,04
	FGFR1	Fibroblast growth factor receptor 1	**3,85**	0,005
Others	**TNFSF11**	Tumor necrosis factor (ligand) superfamily member 11 (RANKL)	**21,57**	0,01
	ALPL	Alkaline phosphatase, liver/bone/kidney	**2,48**	0,02
	IL6	Interleukin 6 (interferon beta 2)	**7,28**	0,02
	IGSF4	Immunoglobulin superfamily member 4	**3,94**	0,02
	TRIB2	Tribbles homolog 2 (Drosophila)	**2,10**	0,02

Table 1. Summary of the quantitative real-time RT-PCR data of the significantly changed 29 genes in 10 postmenopausal and in 7 premenopausal non-osteoporotic women.

3. Association of novel gene polymorphism with postmenopausal bone loss

The power of each significant association was also computed and only statistically significant associations with a power of study higher than 80% (0.80). The rs6996321 in FGFR1 was associated with BMD at lumbar spine (unadjusted mean a mean BMD±S.E.M. was 0.858±0.013; 0.912±0.015 and 0.916±0.029 in subjects with G/G, A/G and A/A genotypes respectively). Adjusted BMD significantly differed between genotypes in the dominant genetic model (mean of adjusted spine BMD±S.E.M. was -0.031±0.011 and 0.031±0.011 in groups of G/G and A/G+A/A respectively, P=0.002; Fig. 3). The homozygous recessive genotype of rs10914367 in FABP3 was related to higher BMD at the total hip (mean BMD± S.E.M. was 0.783±0.009, 0.776±0.028, and 0.945±0.029 in subjects with G/G, A/G, and A/A

genotypes respectively; mean of covariates adjusted BMD±S.E.M. in the recessive model was -0.004±0.008 and 0.143±0.037 in G/G+A/G and A/A groups respectively, P=0.028; Fig. 3).

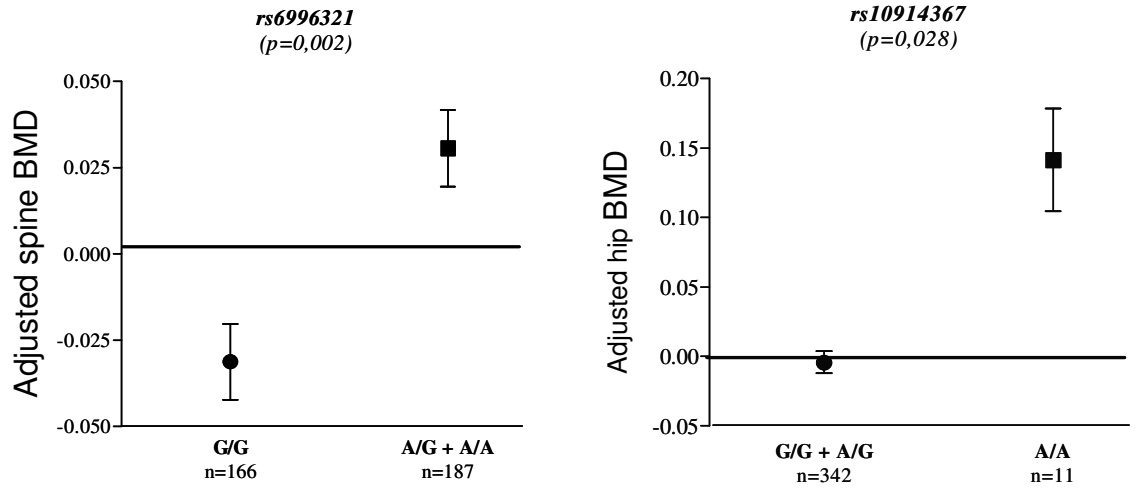
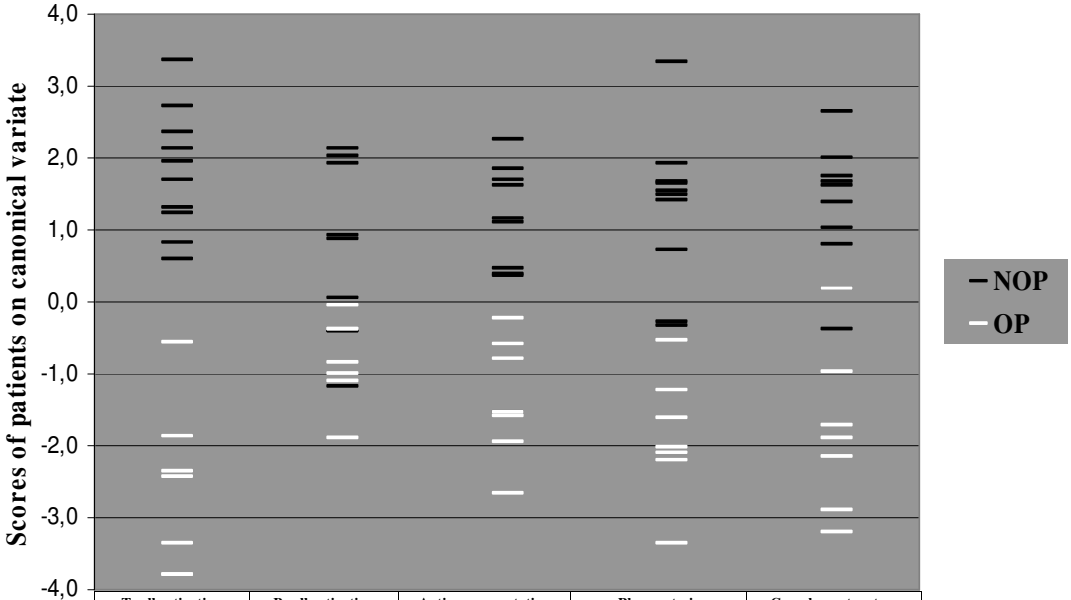


Fig. 3. Association of rs6996321 in FGFR1 with spine BMD. Association of rs10914367 in FABP3 with hip BMD. Genotypespecific BMD means with standard errors of means (mean±S.E.M.) are represented. The data were adjusted for significant covariates.

4. Transcriptional profiling of immune system-related genes in postmenopausal osteoporotic versus non-osteoporotic human bone tissue

The selection of gene subsets was based on searching factors which are presented by bone cells and are involved in both innate and adaptive immune processes; including T and B lymphocyte activation (14 and 5 genes), antigen presentation and co-stimulation (11 genes), phagocytosis (9 genes) and complement functions (10 genes). The gene subset of T cell activating molecules exhibited the best discriminatory power, with unambiguous separation of the OP and NOP subjects (Fig. 4). Genes involved in antigen presentation also showed strong correlation with the single canonical variate, hence achieving clear separation of the two groups of examined subjects. Segregation was especially sharp based on marker genes of phagocytosis (Fig. 4).

Canonical correlation measures the strength between the canonical variate and the group membership variable. The stronger the separation of groups along the CVA axis, the higher the correlation. Canonical correlation values were 0.918 in case of the T cell-specific gene cluster, 0.857 for the subset of phagocytosis marker genes, 0.851 for genes of the complement cascade, 0.766 for the gene subset of antigen presentation and 0.580 for B cell gene group, respectively.



T cell activation		B cell activation		Antigen presentation		Phagocytosis		Complement system	
Gene name	Corr. with CV	Gene name	Corr. with CV	Gene name	Corr. with CV	Gene name	Corr. with CV	Gene name	Corr. with CV
IL-10	-0.418	IL-7	-0.434	CD80	-0.149	TLR4	-0.113	ITGAX	-0.431
IL-1B	-0.366	IFNG	0.333	HLA-DRB1	-0.138	ITGB2	0.209	IL-1B	-0.395
CD86	-0.358	IL-6	0.464	CD40	0.024	VCAM1	0.231	C1QA	-0.334
TGFB1	-0.342	TGFB1	0.542	IL-12A	0.049	ITGAM	0.232	IL-6	-0.319
IL-6	-0.293	IL-10	0.662	HLA-A	0.183	CD14	0.266	C3	-0.239
IL-7	-0.274			IFNG	0.252	ITGAX	0.428	ITGAM	-0.233
IFNG	-0.210			VCAM1	0.259	ICAM1	0.446	IFNG	-0.224
HLA-A	-0.153			IL-6	0.351	FCGR2A	0.512	ITGB2	-0.210
TNFSF11	-0.086			CD86	0.429	SCARA3	0.559	TNF	-0.196
IL-12A	-0.041			IL-1B	0.439			C5R1	0.024
CD40	-0.020			ICAM1	0.499				
HLA-DRB1	0.115								
CD80	0.124								
TNFRSF11	0.135								

Fig. 4. Canonical variates analysis of immune-related gene expression patterns of bone tissue in 7 postmenopausal OP (white bars) and 10 NOP (black bars) women. Symbols and names for human genes belonging to the five subsets are used according to the standard "Gene Cards" (www.genecards.org). Gene symbols and correlations of genes with the single canonical variates (CV) are summarized in the table pertaining to each subset of genes.

CONCLUSIONS

The genetic background of postmenopausal bone loss and the pathogenesis of osteoporosis are not completely understood. The effect of single genes on bone mass proved meager in several previous reports. This fact is suggests the role of multiple genes and gene-gene interaction in the changed bone metabolic processes after menopause.

- We have found significant differences in the gene expression profiles of the bone tissue of postmenopausal osteoporotic and non-osteoporotic women including genes that have not yet been associated with menopausal changes. The separation of the two groups by the multiparametric statistical methods applied suggests the involvement of new candidate gene subsets as well as genetic pathways (canonical TGFB cascade, MAPK signaling and collagen type extracellular matrix components) that might be useful for the development of future diagnostic tools. Our findings may provide further insight into the process of postmenopausal changes of bone metabolism as well as it can contribute to the development of new statistical methods for the evaluation of batched genetic data.
- We observed significant differences in the expression pattern of immune-competent genes in postmenopausal versus premenopausal as well as in osteoporotic and non-osteoporotic bone tissue. The separation of the two groups by CVA suggests the involvement of novel gene subsets that might be useful for a deeper understanding of the immunological aspects of menopausal changes of bone metabolism. Our findings provide further insight into the process of altered bone and immune homeostasis after menopause and osteoporotic microenvironment.
- We investigated the association of postmenopausal bone loss and multiple allelic variants of seven potential candidate genes. Polymorphisms of four of the selected genes were not previously reported in this field. Three individual SNPs associated with osteoporotic phenotypes have been identified in this study.

LIST OF PUBLICATIONS

Cumulative impact factor (IF): **42,500**

In connection with theme

Balla B, Kósa JP, Kiss J, Borsy A, Podani J, Takács I, Lazáry Á, Nagy Zs, Bácsi K, Speer G, Orosz L, Lakatos P: Different gene expression patterns in bone tissue of aging postmenopausal osteoporotic and non-osteoporotic women. *Calcif Tissue Int* 2008; **82**(1):12-26. **IF:2,435**

Balla B, Kósa JP, Takács I, Kiss J, Podani J, Borsy A, Lazáry Á, Bácsi K, Nagy Zs, Speer G, Orosz L, Lakatos P: Menopauza hatása a csontszöveti génkifejeződésre posztmenopauzás és premenopauzás korú nem oszteoprotikus nőkben. *Magy Belorv Arch* 2008; **61**(3):208-219.

Lazáry Á, Kósa JP, Tóbiás B, Lazáry J, **Balla B,** Bácsi K, Takács I, Nagy Zs, Mező T, Speer G, Lakatos P: Single nucleotide polymorphisms in new candidate genes are associated with bone mineral density and fracture risk. *Eur J Endocrinol* 2008; **159**(2):187-196. **IF:3,239**

Balla B, Kósa JP, Kiss J, Podani J, Takács I, Lazáry Á, Nagy Zs, Bácsi K, Speer G, Lakatos P: Transcriptional profiling of immune system-related genes in postmenopausal osteoporotic versus non-osteoporotic human bone tissue. *Clin Immunol* 2009; **131**(2):354-9. **IF:3,551**

Kósa JP[1], **Balla B[1],** Speer G, Kiss J, Borsy A, Podani J, Takács I, Lazáry Á, Nagy Zs, Bácsi K, Orosz L, Lakatos P: Effect of menopause on gene expression pattern in bone tissue of nonosteoporotic women. *Menopause* 2009; **16**(2):367-77. **IF:3,672**
[1], These authors equally contributed to this work.

Borsy A, Podani J, Stéger V, **Balla B,** Horváth A, Kósa J, Gyurján I Jr., Molnár A, Szabolcsi Z, Szabó L, Jako E, Zomborszky Z, Nagy J, Semsey Sz, Vellai T, Lakatos P, Orosz L: Identifying novel genes involved in both deer physiological and human pathological osteoporosis. *Mol Genet Genomics* 2009; **281**(3):301-13. **IF:2,978**

Kósa JP[1], **Balla B[1]**, Kiss J, Podani J, Takács I, Lazáry Á, Nagy Zs, Bácsi K, Karsai A, Speer G, Lakatos P: Postmenopausal expression changes of immune system-related genes in human bone tissue. *J Clin Immunol* 2009; **29**(6):761-8. **IF:3,248**
[1], These authors equally contributed to this work.

Balla B, Kósa JP, Kiss J, Podani J, Takács I, Lazáry Á, Bácsi K, Nagy Zs, Speer G, Lakatos P: Az immunrendszerben szerepet játszó gének expressziós változásainak vizsgálata osteoporoticus humán csontszövetben. *Immunológiai szemle* 2009; **1**(1-2):13-21.

Takács I, Lazáry Á, Kósa JP, Kiss J, **Balla B**, Nagy Zs, Bácsi K, Speer G, Lakatos P: Allelic variations of RANKL/OPG signaling system is related to bone mineral density and in vivo gene expression. *Eur J Endocrinol* 2010;**162**(2):423-31. **IF:3.791**

Balla B, Kósa J, Takács I, Kiss J, Podani J, Lazáry Á, Bácsi K, Nagy Zs, Speer G, Lakatos P: Rendszerbiológiai szemlélet a primer csontvesztés molekuláris biológiai kutatásában és az osteoporosis immunológiai vonatkozásai. *Orvosképzés* 2009; **84**(S5).

Balla B, Kósa JP, Kiss J, Podani J, Takács I, Lazáry Á, Bácsi K, Nagy Zs, Speer G, Lakatos P: Menopausa hatása az immunrendszer működését szabályozó gének transzkriptomikai változásaira humán csontszövetben. *LAM – accepted for publication*

Takács I, Lazáry Á, Kósa J, Kiss J, **Balla B**, Nagy Zs, Bácsi K, Speer G, Lakatos P: A RANKL/OPG jelátviteli rendszer allélikus variációinak összefüggése a csonttömeggel és az *in vivo* génexpresszióval. *Magy Belorv Arch – submitted for publication*

Other publications

Kiss J[1], **Balla B[1],** Kósa JP, Borsy A, Podani J, Takács I, Lazáry Á, Nagy Zs, Bácsi K, Szlávy E, Szendrői M, Speer G, Orosz L, Lakatos P: Gene expression patterns in the bone tissue of women with fibrous dysplasia. *Am J Med Genet A – accepted for publication*
[1], These authors equally contributed to this work.

Bácsi K, Kósa J, **Balla B**, Lazáry Á, Takács I, Nagy Zs, Speer G, Lakatos P: A kalciummetabolizmus jelentősége a csontritkulás és a colorectalis daganat patogenezisében. *Orvosképzés* 2009; **84**(S2):101-10.

Bácsi K, Hitre E, Kósa JP, Horváth H, Lazáry Á, Lakatos LP, **Balla B,** Budai B, Nagy Zs, Lakatos P, Speer G: Effects of the lactase 13910 C/T and calcium-sensor receptor A986S G/T polymorphisms on the incidence and recurrence of colorectal cancer in Hungarian population. *BMC Cancer* 2008; **8**(1):317. **IF:2,709**

Bácsi K, Kósa JP, Lazáry Á, **Balla B,** Horváth H, Kis A, Nagy Zs, Takács I, Lakatos P, Speer G: LCT 13910 C/T polymorphism, serum calcium, and bone mineral density in postmenopausal women. *Osteoporos Int* 2009; **20**(4):639-45. **IF:3,893**

Lazáry Á, Speer G, Varga PP, **Balla B,** Bácsi K, Kósa JP, Nagy Zs, Takács I, Lakatos P: Effect of vertebroplasty filler materials on viability and gene expression of human nucleus pulposus cells. *J Orthop Res* 2008; **26**(5):601-607. **IF:2,437**

Lazáry Á, **Balla B,** Kósa JP, Bácsi K, Nagy Zs, Takács I, Varga PP, Speer G, Lakatos P: A szintetikus csontpótló graftok alkalmazásának összefoglalása. A gipsz szerepe a csontpótlásban: molekuláris biológiai megközelítéssel, saját eredményeink alapján. *Orv Hetil* 2007; **148**(51):2427-2433.

Kósa JP, Kis A, Bácsi K, Nagy Zs, **Balla B,** Lazáry Á, Takács I, Speer G, Lakatos P: Új, a glükokortikoidok csontsejtekre gyakorolt hatásának közvetítésében szerepet játszó gének azonosítása izolált és immortalizált egér osteoblastsejteken. *Magy Belorv Arch* 2007; **60**:435-441.

Bácsi K, Kósa J, Lazáry Á, **Balla B,** Horváth H, Takács I, Nagy Zs, Speer G, Lakatos P: A CYP3A7*1C polimorfizmus hatása a csont ásványanyag-tartalmára posztmenopauzás nőkben. *Orv Hetil* 2007; **148**(27):1273-1280.

Bácsi K, Kósa J, Lazáry Á, Horváth H, **Balla B,** Lakatos P, Speer G: A dehidroepiandroszteron és dehidroepiandroszteron szulfát jelentősége a különböző kórállapotokban. *Orv Hetil* 2007; **148**(14):651-657.

Bácsi K, Kósa JP, Borgulya G, **Balla B,** Lazáry Á, Nagy Zs, Horváth Cs, Speer G, Lakatos P: CYP3A7*1C polymorphism, serum dehydroepiandrosterone sulfate level and bone mineral density in postmenopausal women. *Calcif Tissue Int* 2007; **80**(3):154-159. **IF:2,435**

Lazáry Á, **Balla B,** Kósa JP, Bácsi K, Nagy Zs, Takács I, Varga PP, Speer G, Lakatos P: Effect of gypsum on proliferation and differentiation of MC3T3-E1 mouse osteoblastic cells. *Biomaterials* 2007; **28**(3):393-399. **IF:6,262**