The role of osteoprotegerin (OPG) and estrogen receptor (ER-α) gene polymorphisms in rheumatoid arthritis

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Summary

Objective. **Osteoclast activation at the cartilage pannus junction is an essential step in the destruction of bone matrix in patients affected by rheumatoid arthritis (RA). Receptor activator of NFkappaB ligand (RANK-L) is responsible for osteoclast differentiation and activation. Osteoprotegerin (*OPG*) is an alternative, high-affinity soluble receptor for RANK-L which significantly inhibits osteoclastogenesis. Estrogens and the specific receptors α and β (*ER-α* and *ER-α*) are known to play an important role in the pathophysiology of osteoarthritis (OA). Scope of the present study is to investigate the role of *ER-α* and *OPG* gene polymorphisms in a group of women affected by RA.**
Materials and Methods. **139 consecutive RA patients (115 females and 34 males; median age 65.8 years) were selected. Bone mineral density (BMD) was measured by dual energy x-ray absorptinometry at the lumbar spine (LS-BMD) and femoral neck (FN-BMD) and the presence of bone erosions was evaluated by conventional X-ray. *ER-α* gene polymorphisms were determined by PvuII and XbaI restriction endonuclease digestion of polymerase chain reaction (PCR) products. By convention, the presence of the endonuclease restriction site was indicated with lowercase (p and x) letters while the absence of the restriction site was indicated with uppercase letters (P and X). *OPG* gene polymorphism was determined by RsaI restriction endonuclease digestion of PCR products and the presence and absence of restriction fragment was identified as TT and CC respectively.**
Results. **Pearson's χ^2 analysis for the *ER-α* gene polymorphism showed a prevalence of Pp genotype (58%) (p=0.04) and Xx (54%) (p=0.04) in the total population, without differences between males and females. We did not observed any significant differences between *ER-α* genotypes and LS-BMD. However subjects with xx or pp genotype had a lower LS-BMD in comparison with the opposite genotype.**
For *OPG* gene polymorphism, non significant differences in the distribution of the genotypes were observed between males and females. In addition, we did not observed significant differences on LS-BMD between the genotypes.
Finally, we observed that patients with *ER-α* pp genotype was significant more represented in patients with hand erosions (p = 0.05). No significant correlation was observed for *ER-α* XbaI genotype, however a trend characterized by a correlation between xx and hand erosions was observed (p = 0.13). For *OPG* gene polymorphism, we found a statistical correlation between C allele of *OPG* and hands bone erosions (p = 0.02).
Conclusion. **We found a significant association between *ER-α* and *OPG* gene polymorphisms and the presence of bone erosions in RA patients. These preliminary data suggest a role of *ER-α* and *OPG* gene polymorphisms in bone turnover and disease progression.**

KEY WORDS: estrogen receptor-alpha gene, osteoprotegerin gene, genetics, rheumatoid arthritis.

Introduction

RA is characterized by inflammation of the synovial membrane, leading to invasion of synovial tissue into the adjacent cartilage matrix with degradation of cartilage and bone as a consequence. This, results in erosion of bone, which is often radiographically observed as marginal joint erosions and is predictive of a poorer prognosis (1). The pathophysiological mechanisms of cartilage and bone destruction in RA are yet to be completely understood, however it is known that matrix metalloproteinases, cathepsins and mast cell proteinases can contribute to cartilage and bone destruction (2-4). It is now clear that osteoclast formation and activation at the cartilage–pannus junction is an essential step in the destruction of bone matrix patients with RA (5-8). A number of inflammatory cytokines found in the RA synovial tissue (IL-1α, IL-1β, tumour necrosis factor α (TNF-α) and macrophage colony-stimulating factor) have the potential to promote osteoclast formation and bone resorption (9-11). Recent evidences indicate that the interaction between RANK-L and *OPG* has an essential role in osteoclastogenesis (5, 6, 12, 13).
OPG is an alternative, high-affinity soluble decoy receptor for RANK-L which blocks the interaction between RANK-L and RANK and significantly inhibits osteoclastogenesis (14-15). Similar to RANK and RANK-L, *OPG* production is stimulated by proinflammatory cytokines, such as IL-1β and TNF-α (16). RANK, RANK-L, and OPG are expressed in tissue of the RA joint (5, 6, 8).
Haynes et al. found that RANK-L is an essential factor for osteoclast formation by cells in the rheumatic joints and that OPG may prevent the bone erosion seen in RA joints (8).
In view of its ability to block RANK-L-RANK interaction and inhibit osteoclast formation, *OPG* may have a role in normal homeostasis within the joint and may be a potential therapeutic factor alone or in combination with anti-inflammatory therapies in RA. An important role in the regulation of bone mass, bone turnover and immune response is also play by sex hormones.

It is well documented that androgens and estrogens modulate susceptibility and progression to autoimmune rheumatic diseases. At any concentration, androgens seem to be primarily suppressive on cellular and humoral immunity, whereas at physiologic concentrations estrogens seem to enhance humoral immunity (17-19). In particular, low gonadal and adrenal androgens [testosterone (T)/dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA) and its sulfate (DHEAS)] levels, as well as reduced androgens/estrogens ratio, have been detected in body fluids (i.e. blood, synovial fluid, smears, salivary) of males and females with RA, supporting the possible pathogenic role for the decreased levels of the immuno-suppressive androgens (20). In addition, Jochems et al. observed that loss of endogenous estrogens and the inflammation contribute equally to bone loss in an animal model and, markers of bone and cartilage turnover, as well as bone marrow lymphocyte phenotypes, indicate different mechanisms for bone loss induced by estrogen deficiency and inflammation, respectively (21). In addition, genetic factors might further interfere with the roles of androgens and estrogens in selected individuals (18).
It has been reported that 15.4% of the monozygotic twins were disease concordant for RA (22). Recently, the existence of an *ER* gene polymorphism has been made clear, and its association to some variant *ER* genotypes with breast cancer (23, 24), hypertension (25), generalised osteoarthritis (26) and osteoporosis (27), has been reported.
Genetic factors play an important role in the determination of bone mass and osteoporosis. A number of candidate genes have been implicated in osteoporosis, including genes encoding type 1 collagen, vitamin D receptor, *ER-α*, and others (28). A number of association studies have been performed with single nucleotide polymorphisms in the *ER-α* gene to assess their relation with bone mineral density in pre- and postmenopausal women, as well as the rate of bone loss after menopause and skeletal response to estrogen administration (29).
Assessing genetic factors may be helpful in targeting preventive measures to individuals with higher risk of developing osteoporosis and provide the preventive effort more cost-effective.
The aim of our study is to investigate the association between *ER-α* and *OPG* genes polymorphisms in RA.

Materials and methods

Patients

Our study population included 139 consecutive Italian patients (115 females and 34 males; median age 65.8 years, range 24-89 years, mean disease duration 8.5 ± 3.2 SD years) affected by RA referred to the Section of Rheumatology of the Department of Internal Medicine of the University of Florence, Italy. They all fulfilled the American College of Rheumatology criteria (30). Sixty seven had rheumatoid factor positive and seven two had rheumatoid factor negative. As regard the therapy all patients with active disease (polyarthritis or extended oligoarthritis) were receiving treatment with methotrexate or hydroxychloroquine and low dose of steroid or non-steroidal anti-inflammatory drugs (NSAIDs); patients in remission were treated with low dose of steroid or NSAID therapy.
ESR (erytrocite sedimentation rate), CRP (C-reactive protein), disease duration and bone erosion were considered as disease indexes.

Bone erosions

Bone erosions were detected by conventional X-rays.

Bone mineral density

Bone status was evaluated at the time of the study using dual-X ray absorptiometry (DXA) devices (Hologic QDR 1000/W, Waltham, MA, USA) at the lumbar spine (L2-L4, posterior anterior) and femoral neck.
We set a cut-off point for osteoporosis at a T score of less than -2.5.

Determination of genotype

After informed consent was obtained, blood samples were drawn during routine follow-up hematological tests. Genomic DNA was isolated from EDTA blood samples by a standard phenol-chloroform extraction procedure.
For *OPG* polymorphism, genomic DNA was amplified by polymerase chain reaction (PCR) as indicated by Wuyts et al. (31). PCR was carried out and products were digested by *Pst I* enzyme (New England, Biolabs, Beverly, MA, USA) under conditions recommended by the manufacturer and electrophoresed in 3% NuSive and 1% Agarose. Pst I restriction site was introduced in the presence of T allele. The presence or absence of the restriction site was indicated as TT and CC respectively. The heterozygous were indicated as CT.
Genotyping for the *ER-α* gene polymorphisms was performed using PCR-RFLP, single base extension sequencing, and 5' nuclease Taqman assays for the *Xba*I and *Pvu*II polymorphisms (27). For *Xba*I and *Pvu*II, *X* and *P* denote the absence of the respective restriction sites (G-allele and C-allele, respectively).

Statistical analysis

Analysis of covariance (ANCOVA), followed by LSD protected (least significant difference), was performed in order to evaluate the correlation between OPG polymorphisms and LS-BMD values. Results were expressed as means ± SEM. The following covariates were considered for the ANCOVA analysis: sex, age, body mass index, and disease duration. For the assessment of *OPG* and *ER-α* polymorphism distribution in the total population the Pearson's chi-square (χ^2) analysis was applied (Odd ratios with 95% confidence intervals). This test was also performed to evaluate the frequency of bone erosions between ER-a and *OPG* genotypes in the population. All tests were perfomed using Statistica 5.1 program (Statsoft Inc., Tulsa, OK, USA).

Results

ER-α gene polymorphism

Pearson's χ^2 analysis showed a prevalence of Pp genotype (58%) ($p = 0.04$) and Xx (54%) ($p = 0.04$) in the total population, without differences between male and female. ANCOVA analysis and LSD test did not shown any statistically significant differences between the ER-α genotypes and LS-BMD. However, a trend characterized by a lower LS-BMD was observed in subjects with xx or *pp* genotypes (Fig. 1).
In addition, we observed that patients with pp genotype was significant more represented in patients with hand erosions ($p = 0.05$) (Fig. 2). No significant correlation was observed for XbaI polymorphism. However, a trend of correlation between xx and hand erosions was observed ($p = 0.13$). Finally, no statistically significant association between the disease markers and ER-α genotypes was observed (data not shown) but a trend of correlation between pp genotype and the presence of rheumatoid factor was found.

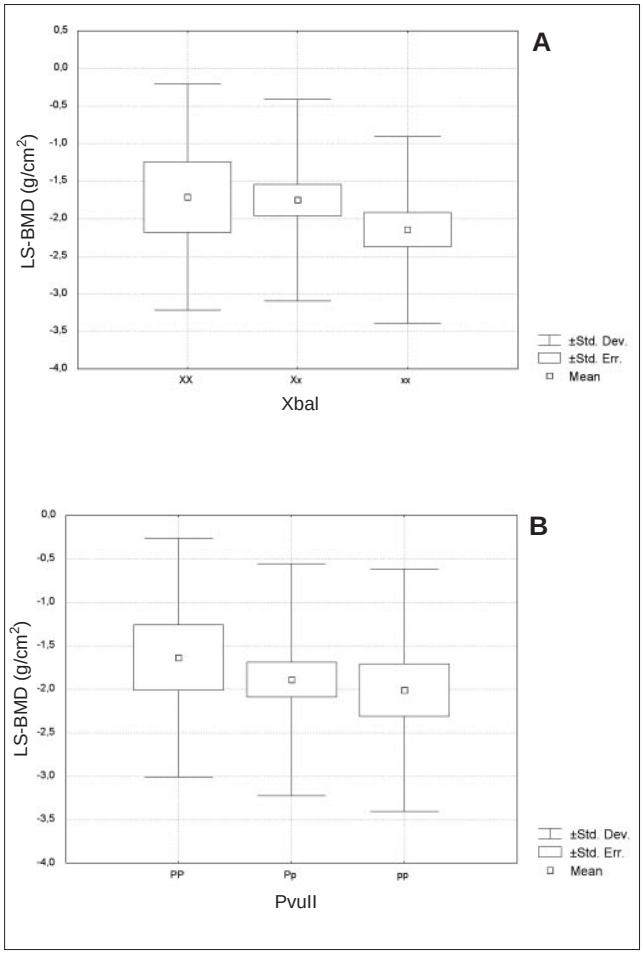

Figure 1 - Differences in the LS-BMD between the ER-α gene polymorphisms (XbaI and PvuII). A: no significant differences were observed between XbaII genotypes; B: no significant differences were observed between PvuII genotypes, however a trend characterized by a lower LS-BMD was observed in patients with xx and pp genotypes (ANCOVA analysis).

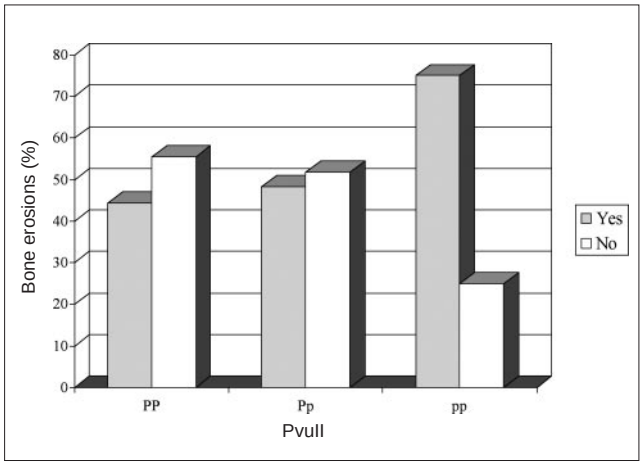

Figure 2 - Percent of bone erosions between PvuII genotypes. Bone erosions were more frequent in patients with pp genotypes (Pearson's χ^2 test: p = 0.05). The grey bar represent the percent of subjects with bone erosion and white bar the percent of subjects without bone erosions.

OPG gene polymorphism

Pearson's χ^2 analysis showed a prevalence of CC (78%) in the total population. No significant differences in the distribution of the genotypes were observed between male and female. ANCOVA analysis and LSD test did not shown any statistically significant differences between the OPG genotypes and LS-BMD (Fig. 3).

A statistically significant correlation between C allele of *OPG* and hands bone erosions (p = 0.02) (Fig. 4) was observed. Finally, not significant association between the presence of rheumatoid factor and *OPG* gene polymorphism was found.

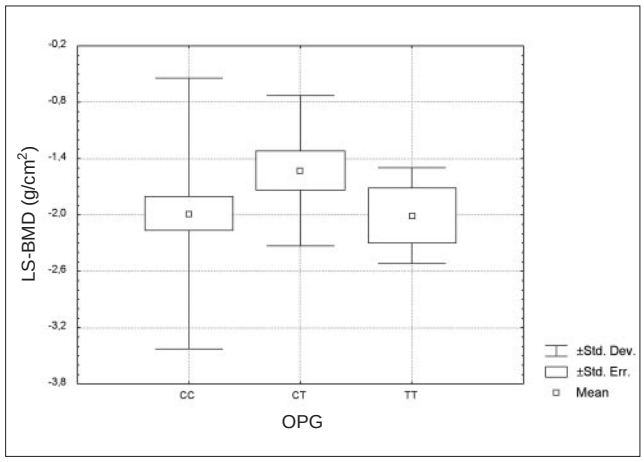

Figure 3 - Differences in the LS-BMD between the OPG gene polymorphism. No significant differences were observed between genotypes (ANCOVA analysis).

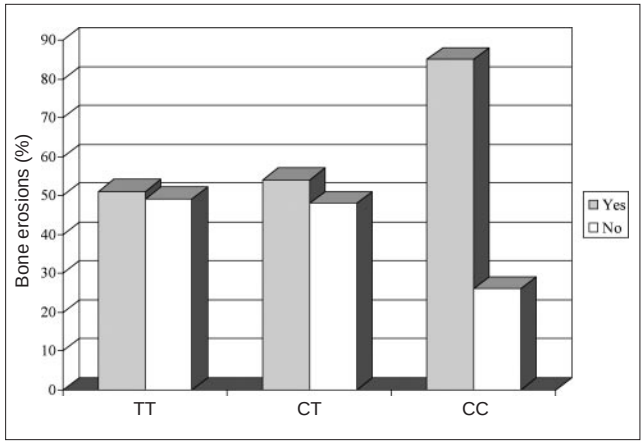

Figure 4 - Percent of bone erosions between *OPG* genotypes. Bone erosions were more frequent in patients with CC genotype (Pearson's χ^2 test: p = 0.02). The grey bar represent the percent of subjects with bone erosion and white bar the percent of subjects without bone erosions.

Discussion

Genetic factors play an important role in the regulation of BMD and risk of osteoporotic fractures in both men and women (32-34).

In the present study, we evaluated the role of *OPG* and *ER-α* gene polymorphisms in patients with RA. It is well known that *OPG* knockout mice exhibit a decrease in total BMD and a high

incidence of fracture due to enhanced osteoclastogenesis (35) suggesting that *OPG* gene would be a candidate gene for investigation into susceptibility to osteoporosis in humans. We did not found a significant association between *OPG* genotypes and LS-BMD in RA patients, however, a positive association between *OPG* polymorphism and hands bone erosions was observed. These data are in accord with the data published by Langdahl et al. (36). The presence of C allele identified in intron 2 of the *OPG* gene exhibits a significant association with bone erosions.

Bone and articular cartilage destruction can be prevent by *OPG* treatment in arthritic rats (37). The polymorphism of the *OPG* gene could be important as a disease index. In particular C allele could be associated with a imbalance between OPG/RANK-RANK-L and could causes an insufficient production of *OPG*. This *OPG* gene sequence variation is an intronic polymorphism close to the splice junction of the exon 3 and could in theory affect splicing efficiency. Several *OPG* gene polymorphisms have been described in the literature (38). In this study we evaluated the polymorphism localized in the intron 2, described for the first time by Wuyts et al. (31). These authors found a higher frequency of C allele in subject affected by Paget's disease. In addition, we previously observed a significant association between this polymorphism and LS-BMD in children affected by Juvenile Idiopathic Arthritis (39). The discrepancy with the present results could be due to differences between adult and pediatric population where several environmental factors could modify the results. However, this data is in accord with a recent study which found a negative correlation between *OPG* polymorphism and BMD at the lumbar spine but not at the femoral neck site in postmenopausal women (38).

Bone loss is a major unsolved problem in RA, a disease characterized by chronic synovial inflammation and hyperplasia culminating in joint destruction (40, 41). Until recently, the processes underlying these types of bone loss were thought to be separate. Thus, focal bone erosions in RA were considered to be due to direct invasion by the inflamed synovial membrane (SM, also referred to as "pannus"). The juxtaarticular osteoporosis was considered to be secondary to increased local production of osteoclast-activating cytokines, whereas generalized osteoporosis was regarded as multifactorial, especially as a consequence of loss of mobility or long-term use of corticosteroids. Now, the recognition of osteoclasts as one of the pivotal effectors cells in the pathogenesis of bone and joint damage in RA, together with elucidation of receptor activator of RANK-L, *OPG*, and RANK as central regulators of osteoclast recruitment and activation, point to a unifying paradigm for the entire spectrum of skeletal pathology in RA and provide a chance for powerful new therapeutic interventions.

In addition, it has been shown that estrogens loss up-regulates osteoclastogenesis through an increase in the production of interleukin 6 (IL-6) in the microenvironment of the bone tissue, resulting in osteoporosis (42). On the other hand, the concentration of IL-6 is increased in the synovial fluids of RA patients, and IL-6 is believed to play important part in immune response and activation of inflammation at articular joints (43). It is already known that articular chondrocytes and synovial cells express ER (44) and that estrogens induces the up-regulation of IL-6 production by chondrocytes, showing a conspicuous difference from bone tissue (45). It is therefore probable that women who show a specific reaction to the hormonal stimuli may tend to develop either osteoporosis in bone tissue at the postmenopausal period or rheumatoid inflammation in joint tissues at the premenopausal period through different effects of estrogens toward IL-6 production.

Because estrogens have important effects on bone mass and bone remodelling (4, 5), many investigators have evaluated the role of *ER-α* gene polymorphisms in the genetic regulation of BMD. In the present study we found that subjects with *xx* or *pp* genotype for the *ER-α* gene polymorphisms had a lower LS-BMD in comparison with the opposite genotype. We have not found any association between *ER-α* gene polymorphisms and FN-BMD. These data are in accord with the meta-analysis reported for *Pvu*II, *Xba*I, and promoter TA repeats with BMD and fractures in 18,917 individuals from eight European research centers (46). None of the three polymorphisms or haplotypes had statistically significant association with BMD. However, there was a highly significant protection conferred by the *Xba*I XX genotype against fracture risk. In women with the XX genotype, the OR was 0.81 ($p = 0.002$) for any fractures and 0.65 ($p = 0.003$) for vertebral fractures. The observed effects on fractures were independent of BMD (46). In the present study, no data on fractures were available. However, the association between xx genotype and hand bone erosion make possible to speculate that *ER-α* gene polymorphism could play a role in the control of cartilage and bone quality influencing some parameters which are not involved in the regulation of bone mass.

The molecular mechanism by which *Pvu*II and *Xba*I polymorphisms influence bone mass and osteoporotic risk are still unclear, mainly because these polymorphism lie in an intronic and apparently nonfunctional area of the gene. A possible explanation is that the two polymorphisms in intron I should be in linkage disequilibrium with causal polymorphism that lie elsewhere in the *ER-α* gene, or in adjacent genes (47). In conclusion, these preliminary data suggests a possible role of *OPG* and *ER-α* gene polymorphisms in the regulation of bone mineral density, bone erosion and disease progression. Therefore, further study and a larger number of populations are required to elucidate a possible relationship between *OPG* and *ER-α* gene polymorphisms.

References

1. Van Zeben D, Hazes JM, Zwinderman AH, Vandenbroucke JP, Breedveld FC. Factors predicting outcome of rheumatoid arthritis: results of a follow-up study. J Rheumatol. 1993;20:1288-96.
2. Woolley DE, Crossley MJ, Evanson JM. Collagenase at sites of cartilage erosion in the rheumatoid joint. Arthritis Rheum. 1977;20: 1231-9.
3. Trabant A, Gay RE, Fassbender HG, Gay S. Cathepsin B in synovial cells at the site of joint destruction in rheumatoid arthritis. Arthritis Rheum. 1991;34:1444-51.
4. Tetlow LC, Woolley DE. Mast cells, cytokines, and metalloproteinases at the rheumatoid lesion: dual immunolocalisation studies. Ann Rheum Dis. 1995;54:896-903.
5. Gravallese EM, Manning C, Tsay A et al. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum. 2000;43:250-8.
6. Takayanagi H, Iizuka H, Juji T et al. Involvement of receptor activator of nuclear factor B ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum. 2000;43:259-69.
7. Romas E, Bakharevski O, Hards DK et al. Expression of osteoclast differentiation factor at sites of bone erosion in collagen-induced arthritis. Arthritis Rheum. 2000;43:821-6.
8. Haynes DR, Crotti TN, Capone M, Bain GI, Atkins GJ, Findlay DM. Osteoprotegerin and receptor activator of nuclear factor kappa B ligand (RANK-L) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. Rheumatology. 2001;40:623-30.
9. Chu CQ, Field M, Allard S, Abney E, Feldmann M, Maini RN. Detection of cytokines at the cartilage/pannus junction in patients with rheumatoid arthritis: implications for the role of cytokines in cartilage destruction and repair. Br J Rheumatol. 1992;31:653-61.
10. Deleuran BW, Chu CQ, Field M et al. Localization of interleukin-1 alpha, type 1 interleukin-1 receptor and interleukin-1 receptor an-

tagonist in the synovial membrane and cartilage/pannus junction in rheumatoid arthritis. Br J Rheumatol. 1992;31:801-9.
11. Chu CQ, Field M, Feldman M, Maini RN. Localization of tumor necrosis factor in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum. 1991;34:1125-32.
12. Yasuda H, Shima N, Nakagawa N et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANK-L. Proc Natl Acad Sci USA. 1998;95:3597-602.
13. Gravallese EM, Galson D, Goldring SR, Auron PE. The role of TNF-receptor family members and other TRAF-dependent receptors in bone resorption. Arthritis Res. 2001;3:6-12.
14. Lacey DL, Timms E, Tan HL et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell. 1998;9:165-76.
15. Simonet WS, Lacey DL, Dunstan CR et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell. 1997;89:309-19.
16. Fujikawa Y, Sabokbar A, Neale SD, Athanasou NA. Human osteoclast formation and bone resorption by monocytes and synovial macrophages in rheumatoid arthritis. Ann Rheum Dis. 1996;55: 816-22.
17. Cutolo M, Accardo S. Sex hormones, HLA and Rheumatoid arthritis. Clin Exp Rheumatol. 1991;9:641-8.
18. Cutolo M, Seriolo B, Pizzorni C, Sulli A. The role of androgens in rheumatic disease. Israel Med Ass J. 2001;3:743-8.
19. Cutolo M, Straub R. Recent aspect of gonadal hormones and neurotrasmitter interactions with synovial cells in rheumatoid arthritis. N Rheum Dis. 2000;59:657-61.
20. Cutolo M, Seriolo B, Villaggio B, Pizzorno C, Craviotto C, Sulli A. Androgens and estrogens the immune and inflammatory responses in rheumatoid arthritis. N NY Acad Sci. 2002;966:131-42.
21. Jochems C, Islander U, Erlandsson M, Verdrengh M, Ohlsson C, Carlsten H. Osteoporosis in experimental postmenopausal polyarthritis: the relative contributions of estrogen deficiency and inflammation. Arthritis Res Ther. 2005;7(4):R837-R843.
22. Silman AJ, MacGregor AJ, Thomson W, Holligan S, Carthy D, Farhan A, et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. Br J Rheumatol. 1993;32:903-907.
23. Hill SM, Fuqua SAW, Chamness GC, Greene GL, McGuire WL. Estrogen receptor expression in human breast cancer associated with an estrogen receptor gene restriction fragment length polymorphism. Cancer Res. 1989;49:145-148.
24. Yaich L, Dupont WD, Cavener DR, Parl FF. Analysis of the Pvu II restriction fragment-length polymorphism and exon structure of the estrogen receptor gene in breast cancer and peripheral blood. Cancer Res. 1992;52:77-83.
25. Lehrer S, Rabin J, Kalir T, Schachter BS. Estrogen receptor variant and hypertension in women. Hypertension. 1993;21:439-441.
26. Ushiyama T, Ueyama H, Inoue K, Nishioka J, Ohkubo I, Hukuda S. Estrogen receptor gene polymorphism and generalized osteoarthritis. J Rheumatol. 1998;25:134-137.
27. Kobayashi S, Inoue S, Hosoi T, Ouchi Y, Shiraki M, Orimo H. Association of bone mineral density with polymorphism of the estrogen receptor gene. J Bone Miner Res. 1996;11:306-31.
28. Liu Y.J Shen H, Xiao P, Xiong DH, Li LH, Recker RR, Deng HW. Molecular Genetic Studies of Gene Identification for Osteoporosis: A 2004 Update J Bone Min Res. 2006;21:1511-1535.
29. Gennari L, Merlotti D, De Paola V, Calabro A, Becherini L, Martini G, Nuti R. Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. Am J Epidemiol. 2005 Feb 15;161(4):307-20. Review.
30. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum. 1988;31:315-324.
31. Wuyts W, Van Wesenbeeck L, Morales-Piga A, Ralston S, Hocking L, Vanhoenacker F, Westhovens R, Verbruggen L, Anderson D, Hughes A, Van Hul W. Evaluation of the role of RANK and OPG genes in Paget's disease of bone. Bone. 2001;28:104-7.
32. Stewart TL, Ralston SH 2000 Role of genetic factors in the pathogenesis of osteoporosis. J Endocrinol. 166:235-245.
33. Nguyen TV, Eisman JA. Genetics of fracture: Challenges and opportunities. J Bone Miner Res. 2000;15:1253-1256.
34. Nguyen TV, Blangero J, Eisman JA: Genetic epidemiological approaches to the search for osteoporosis genes. J Bone Miner Res. 2000;15:392-401.
35. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev. 1998 May 1;12(9):1260-8.
36. Langdahl BL, Carstens M, Stenkjaer L, Eriksen EF. Polymorphisms in the osteoprotegerin gene are associated with osteoporotic fractures. J Bone Miner Res. 2002 Jul;17(7):1245-55.
37. Koch A, Campagnuolo G, Bolon B, Feige U. Kinetics of bone protection by recombinant osteoprotogerin (OPG) therapy in Lewis rats with mycobacteria-induced adjuvant arthritis. Arthritis Rheum. 2001;44:S267.
38. Arko B, Prezel J, Komel R, Koijancic A, Hudler P, Marc J. Sequence variations in the OPG gene promoter in patients with postmenopausal osteoporosis. J Clin Endocr Metab. 2002;87:4080-4084.
39. Masi L, Simonini G, Piscitelli E, Del Monte F, Giani T, Cimaz R, Vierucci S, Brandi ML, Falcini F. Osteoprotegerin (OPG)/RANK-L system in juvenile idiopathic arthritis: Is here a potential modulating role for OPG/RANK-L in bone injury? J Rheumatol. 2004;31: 986-991.
40. Feldmann M,Brennan FM, Maini RN. Rheumatoid arthritis. Cell. 1996;85:307-310.
41. Harris ED, Jr. Rheumatoid arthritis.Pathophysiology and implications for therapy. N Engl J Med. 1990;322:1277-1289.
42. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, et al. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science. 1992;257:88-91.
43. Ito A, Itoh Y, Sasaguri Y, Morimatsu M, Mori Y. Effects of interleukin-6 on the metabolism of connective tissue components in rheumatoid synovial fibroblasts. Arthritis Rheum. 1992;35:1197-1201.
44. Ushiyama T, Inoue K, Nishioka J. Expression of estrogen receptor related protein (p29) and estradiol binding in human arthritic synovium. J Rheumatol. 1995;22:421-426.
45. Guerne PA, Carson DA, Lotz M. IL-6 production by human articular chondrocytes. J Immunol. 1990;144:499-505.
46. Ioannidis JP, Ralston SH, Bennet ST, Brandi ML, Grindberg D, Karassa FB, Lang van Merus JB, Mosekilde L, Scollen L, Albagia OM, Carey AH, Enjuanes DAM, can Leeuwen JP, Mavilia C, Masi L, McGigan FE, Nogues X, Reid PDM, Schuit SC, Sherlock RE, Uitterlinden AG; GENOMOS Study: Differential genetic effects of ESR1 gene polymorphisms on osteoporotic outcomes. JAMA. 2004;297:2105-2114.
47. Gennari L, Becherini L, Falchetti L, Masi L, Massart F, Brandi ML. Genetic of osteoporosis: role of steroid hormone receptor gene polymorphisms. J Steroid Biochem Mol Biol. 2002;81:1-24.