ROLE OF THE GENETIC PREDISPOSITION AS ARISK FACTOR FOR OSTEOPOROSIS

ILENIA PEPE - DOMENICO MERENDINO - VASILIKI SARAKATSIANOU - CLAUDIA MAGGIO - DARIO BRUNO - STEFANIA SFERRAZZA - GRAZIELLA TAORMINA - ANDREA ABRUZZESE - GRAZIELLA CARDELLA - GIUSEPPE AMBROSIANO - ANTONINO REINA - PASQUALE MANSUETO - DINO GULLOTTI - PAOLO RIGANO - NICOLA NAPOLI
University of Palermo - AOUP"Paolo Giaccone" - Department of Clinical Medicine and Emerging Pathologies - Division of Internal Medicine and Metabolic Diseases - (*Head Prof. G. B. Rini*)

[Ruolo della predisposizione genetica quale fattore di rischio per l'osteoporosi]

SUMMARY

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture. This pathology is a complex disorder with a strong genetic component. It is characterized by low bone mineral density, bone tissue microarchitectural disorders and predisposition to an increased risk of fracture.

Twins and families studies have shown that genetic factors play a main role in the bone mass variability up to 50-85%.

Many studies have also aimed at identifying polymorphisms of genes involved in such a pathology.

Linkage and association studies on various populations, have analyzed the presence of polymorphisms in candidated genes in relation to bone mineral density and bone properties.

Candidated genes has been chosen on the base of their biological effects on bone metabolism, bone cells and extracellular matrix.

Linkage Studies has defined the loci of the genes which regulate bone mass; nevertheless, disagreements on associations between genotypes and BMD still needs further examinations.

We will review polymorphisms of genes mainly involved in bone fragility and risk of fractures.

Key words: Osteoporosis, genetics, risk factors

RIASSUNTO

L'osteoporosi è un disordine scheletrico caratterizzato da compromissione della resistenza ossea e predisposizione ad un aumentato rischio di frattura. Si tratta di un disordine complesso con un forte contributo della componente genetica. E' caratterizzato da una bassa densità minerale ossea, da alterazioni microarchitetturali del tessuto osseo e predisposizione ad un aumentato rischio di frattura.

Studi condotti sia su famiglie che su gemelli, evidenziano come i fattori genetici influenzino per il 50-85% la variabilità della massa ossea.

Molti studi sono stati condotti al fine di identificare i polimorfismi dei geni coinvolti in tale patologia.

Studi di linkage e di associazione condotti su diverse popolazioni, analizzano la presenza dei polimorfismi dei geni candidati in relazione alla BMD e alle proprietà dell'osso.

I geni candidati sono scelti sulla base dei loro effetti biologici sul metabolismo osseo, sulle cellule dell'osso o sulla matrice extracellulare.

Studi di linkage definiscono i loci dei geni che regolano la massa ossea, ma le divergenze in relazione all'associazione tra i genotipi e la BMD, richiedono ulteriori approfondimenti.

Tratteremo qui di seguito i polimorfismi dei geni maggiormente coinvolti nella fragilità ossea e nel rischio di frattura.

***Parole chiave**: Osteoporosi, genetica, fattori di rischio*

Introduction

Osteoporosis (OP) is a skeletal disorder with multifactorial ethiology, due to the interaction among genetic, endocrine-metabolic, immunological and environmental factors (Fig. 1). Out of them, the genetic component plays the main role. Several studies, both on families and twins, have underlined that up to 50-85% of bone mineral density (BMD) is genetically determined.

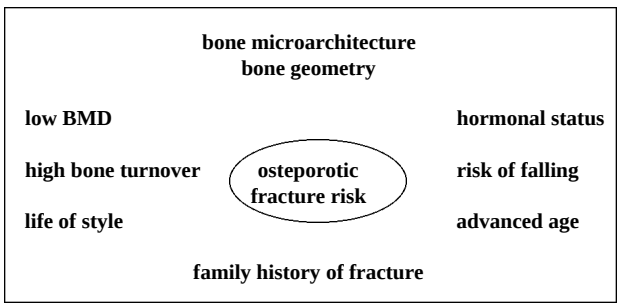

Fig. 1: Interaction of genetic and non-genetic factors in the risk of osteoporotic fractures

The bone mass peak is a determinant of the risk of fracture, especially in advanced age. Genetic factors influence the bone mass and, therefore, the risk of developing osteoporosis[27].

The bone strength depends on the morphology, from the architecture and from the geometry of the bones, from the bone remodelling and from the quality of the extracellular matrix; to these factors must be added the strong contribution of the genetic factors[32][65][23].

Since a low BMD determines an increased risk of fractures especially in women in post-menopause[47], many studies have been conducted with the purpose to identify which genetic mechanisms are involved.

The influence of the genetics is more evident from studies on twins[16].

The BMD is a complex trait, which doesn't show a classical Mendel's heritability both it dominant and recessive, attributable to a single genetic locus[38].

Several diseases that show a low BMD whose expression is linked to the mutation of a single genetic locus: the osteogenesis imperfecta, osteoporosis-pseudoglioma syndrome and other pathologic phenotypes associated to mutations in the gene of the aromatase or of the estrogen receptors (ER).

The contribution of the genetics results different in comparison to environmental factors or the lifestyle that result modifiable, while genetic influence persists during all time of life.

In the structure of the DNA it is possible to individualize some characteristic sequences that quantitatively or qualitatively influence the gene expression. The polymorphisms can involve a single nucleotide (SNP) or repeated sequences, known as tandems (microsatellites), located in specific loci.

Studies of linkage disequilibrium (LD) have been performed with the purpose to identify specific allelic combinations of two or more linked loci that may be inherited together as result of their near position on the same chromosome[56].

The studies of LD are a tested approach to identify the genes responsible of monogenic illnesses and have been applied for identifying chromosomal regions that regulate quantitative parameters like bone mass. These regions are called QTL (quantitative trait loci).

The studies of linkage examine several polymorphisms and the heritability of the various allelic forms that can influence the BMD[56].

Through statistic analysis it will be possible to calculate the predictive value of each genotype on the determination of the BMD and therefore on the risk of fractures.

In the last ten years, since studies conducted by Morrison and coll[46], have been individualized numerous polymorphisms involved in the ethiopathogenesis of osteoporosis and among these some probably show great influence on BMD in comparison to others: VDR, COLIA1, ER., CYP19, CYP17, APOE, PTHR1, LRP5 and the codifying genes for some cytokines and growth factors (TGF β, Activina A, IL-6 and IGF1)[28][8] (Tab.1).

	Candidate gene	Polymorphism Characteristics	Reference
Calciotropic hormones	VDR	5'FokI	Gross et al. 1996
		3'BsmI, TaqI, ApaI	Morrison et al 1994
		Cdx-2 (promotor)	Yamamoto et al. 1999
	ER-α	5'PvuII, XbaI	Kobayashi et al. 1996
		5'TArepeat	Sano et al. 1995
	ER-β	CArepeat	Ogawa et al. 2000
	PTHR1	(AAAG) n repeat	Minagawa et al. 2002
	CYP19	(TTTA) n repeat	Gennati et al. 2000
Cytokines and growth factors	TGF-β	C/T(exon 5)	Langdahl et al. 1997
		T/C (exon 1)	Yamada et al.1998
	Activina A		
	IL-6	3'ATrepeat	Murray et al. 1997
		G/C (promotor)	Ferrari et al. 2001
	IGF-1	CArepeat (promotor)	Miyao et al. 1998
Bone matrix protein	COLIA1	Sp1	Grant et al. 1996
	COLIA2	RsaI, PvuII	Willing et al. 1998
Miscellaneous	ApoE	E2, E3, E4	Shiraki et al. 1997
	LRP5		Gong Y. et al 2001

Table 1: Mayor candidate genes showing association to bone mass variance

Vitamin D Receptor (VDR)

The vitamin D plays an important role in calcium homeostasis, in the regulation of the growth and the differentiation of the bone cells, in the intestinal absorption of calcium and phosphorus and in the secretion of the PTH (Parathyroid hormone); therefore one of the first genes to be studied have been the VDR gene (Vitamin D Receptor)[47].

It is involved in the pathogenesis of the osteoporotic fractures, but also in the osteoarthritis, in the diabetes, in the breast and prostate cancer, and naturally in the BMD reduction[17].

The polymorphisms are located in a long restriction fragment RFLP (restriction fragment length polymorphisms). Some results suggest that the interaction among the various polymorphisms can influence the VDR function.

Morrison and coll. using restriction enzymes, have individualized three polymorphisms near the region 3' of the VDR codifying gene, known as BsmI, ApaI and TaqI. These SNP showed significant association with the levels of circulating osteocalcin (OC) and the lumbar and femoral BMD by DXA (dual X-ray absorptiometry). In 1997 another polymorphism, called FokI, has been identified and associated to low BMD in some populations. It codifies for two isoforms of the VDR, that differ for a length of three amino acids; the statistical analysis showed that these two isoformes influence the VDR function[2].

Finally, in 1999 has been isolated a further polymorphism in the promoter region of the VDR gene, codifying for the protein Cdx-2[3], a bowel-specific transcription factor that could regulate the VDR expression in this tissue and consequently could influence the calcium homeostasis.

The importance of the VDR on the reduction of the BMD and therefore on the risk of fracture is controversial in fact some studies show a correlation with low lumbar BMD but not to femoral level, others don't bring any significant influence for both the segments[33].

A study conducted on a sample of postmenopausal women has analyzed the role of the VDR as incidental factor on the risk of fracture, independently from BMD. In precedence many studies had more times analyzed the association among VDR gene and reduction of the bone mass, but from a metanalysis has emerged that this could be limited to the lumbar site[64].

PTH-Receptor 1 (PTHR1)

The PTH is a polipeptidic hormone, having the function to maintain calcium concentration in the extracellular fluids, directly acting on the bone tissue, on the kidney and indirectly, on the bowel through the activation of the vitamin D that determines calcium absorption.

PTH-rP (PTH-related peptides) represents a series of peptides of different length originated from alternative splicing of the PTH molecule that maintains elevated homology in the N-terminal[66-20] part and that leads to differentiation, proliferation, and apoptosis inhibition of the bone and cartilage cells and favouring the bone endochondral formation[44].

Both the molecules are able to link the same receptor, PTHR1, that belong to the superfamily of G proteins coupled receptors, and that is expressed in the bone and in the kidney. The genetic analysis of this receptor has allowed identifying three promoter regions: P1-P2 expressed both in the human genoma and in the mouse and P3 expressed only in the human genoma. P3 develops a remarkable role in bone tissue, acting on the osteoblasts and on the cells of the kidney[9].

At P3 region has been identified a microsatellite sequence (AAAG)n, present both in the Caucasian and Japanese population; have been identified at least 6 genotypes by the number of repetitions of such sequence (AAAG3, AAAG4, AAAG5, etc.). Has been shown a significant association between the number of these repetitions and the urinary values of deoxipyridinoline (D-pyr) and pyridinoline (Pyr), markers of bone resorption[43-1-39].

The PTHR1 linking PTH, activates the osteoblastic cells modulating, through the production of cytokines, the osteoclastogenesis and therefore the osteoclastic activity[63].

Type 1 Collagene (COLIA1)

The genes that codify for the type 1 or 2 collagene (COLIA1 and COLIA2), are responsible of osteogenesis imperfecta[12].

The Sp1, polymorphisms of the COLIA1, has been discovered in 1996 by Uitterlindeen and coll. in post-menopausal women affected by osteoporosis. An association between this polymorphism and the bone mass, has been individualized in homozygote subjects in which the risk of fracture results up to 2.8 times higher at lumbar site compared to controls[66]. The presence of Sp1 implies changes in the molecule of type 1 collagene with increased formation of the α-1 chains and consequently reduced biomechanical function of the molecule.

Other studies have confirmed a role as predictive factor of the fracture risk, showing that the COLIA1 gene polymorphisms influence more the quality of the bone than the quantity[67].

Estrogen Receptors (ERs)

The estrogens receptors (ERs), belong to the family of the receptors of steroid hormones[26].

Two subtypes of ERs have been identified, ERα and ERβ. The ERα has been studied for the first time in vitro on uterine cells[25], while the ERβ has initially been isolated on prostatic cells of rat[37]. This last receptor shows a structure similar to the ERα, especially at the DNAlinking site.

The study in ER knock-out mice (ERKO) has shown an association between mutations of ERα and the reduction of longitudinal growth of the bone, especially in females and a modest reduction of bone mineral density, more evident in males[53][18]. These data are controversial in comparison to those obtained by studies on man with ER mutations[61] or with deficit of aromatase, in which the longitudinal growth of the bone is increased[45].

In ERβ knock-out mice (BERKO) has been noticed an increase of BMD and of the periostal diameter in the females, while in the males a normal skeletal phenotype has been detected[69].

The presence of ERα in human osteoblasts and in rat osteoblasts was discovered for the first time in 1988[19] and consequently found in osteoclasts[54] and osteocytes[11]. The ERβ mRNA has also been isolated from human osteoblastic cells (SV-HFO)[4], from osteocytes at nuclear level and from osteoclasts cytoplasm[68].

The codifying gene for the ERα results a probable candidate in the pathogenesis of OP in consequence of a clear association between its inactivation and the bone mass reduction. The estrogens through the interaction with specific receptors, modulate the function of osteoblasts, osteoclasts, bone marrow cells, RANK, RANKL, OPG system[19].

The PvuII polymorphism shows two allelic forms, individualized through restriction enzymes that act at the introne 1 and are named as P (PvuII-negative sites) and p (PvuII-positive sites); the XbaI polymorphism also shows two allelic forms obtained through the cut operated by restriction enzymes at the same intron defined as X (XbaI-negative sites) and x (XbaI-positive sites)[7].

A linkage disequilibrium has been shown among the polymorphisms XbaI, PvuII and the number of TA repeats. The association studies of the PvuII and XbaI polymorphisms with the BMD have shown non univocal data.

It has been shown another polymorphic site based on dinucleotidic CA repeats[52].

CYP19 and CYP17

In pre-menopausal women, the gonads are mainly involved in the production of estrogens, above all the estradiol, while in post-menopause these hormones are produced through the aromatizzation of extragonadal androgens especially in the adipose tissue. The aromatase enzyme is the transcription product of the CYP19 gene on the chromosome 15q21.2. The promoter has been identified at the exon 1.4 both in osteoblastic and osteoclastic cells[60].

Inactivating mutations of the aromatase gene result associated to the increased bone turnover and to a BMD reduction both in women and in men.

In men androgens represent predominant steroid hormones with probable implication of them in the maintenance of the bone structure since the hypogonadism determines a BMD reduction and increased bone turnover[29]. The studies show also that estrogens contribute to regulate bone growth and mineralization through aromatase activity on androgens converting them in estrogens in peripheral tissues. Increased bone turnover and severe osteoporosis has been found in men with homozygote mutation in the CYP19[45]. It has been also showed in these patients that testosterone administration was ineffective in opposite to transdermic estrogen administration[13].

The first polymorphism related to bone mass was studied by Masi et al. They showed that the longer TTTA repeats were represented in higher prevalence in non-osteoporotic women and that were associated with a higher lumbar BMD compared to those with alleles containing 8–11 TTTA repeats. These subjects had also lower risk of vertebral fractures when opposed to those with the shorter repeats.

Cytochrome P450c17α (CYP 17) encodes an enzyme with both 17α-hydroxylase and 17,20-lyase activities, implicated in androgen biosynthesys[62][5].

A polymorphic variant in the CYP 17 (T to C substitution in the promoter region of CYP17) has been found to be associated with disease in which sex steroids are implicated, particularly in osteoporosis[48].

It has been shown that the allelic combinations of the C (CYP17) and the A (CYP19) alleles influence the skeletal phenotypes.

In fact postmenopausal women have the lowest BMD in the trabecular region of the spine, femoral neck, and trochanter in comparison to other alle-

lic combinations. Therefore, the allelic combinations of the C (CYP17) and A (CYP19) is associated with lowest bone density phenotype in both the spine and proximal femur[48].

Some enzymes are involved in estrogen metabolism: CYP1A1 at chromosome 15q22-24 and CYP1B1 at chromosome 2p22-p21[41].

It has been identified a C→A transversion at position 4887 of the CYP1A1 gene. Women with the A allele have an increased bone resorption with lower femoral BMD, higher urinary estrogen metabolites and lower free estradiol index suggestive of an increased estrogen catabolism. The C4887A polymorphism of the CYP1A1 gene may represent a possible genetic risk factor for osteoporosis[49].

LDL-R-Related Protein 5 (LRP5)

The LRP5 (LDL-R-Related Protein 5) is a member of the LDL receptors family and plays an important role in the regulation of the osteoblasts proliferation and in the bone formation; it has been thought that polymorphisms of the gene of this receptor can contribute to the genetic variation of the peak of bone mass[6]. Mutations in its structure are enumerated among the causes of the osteoporosis-pseudoglyoma syndrome, a rare autosomic-recessive illness, characterized by reduction of the BMD, spontaneous fractures and blindness.

QTL for the BMD has been found in the chromosome 11q12-13, the same region where is located the codifying gene for LRP5[35].

The polymorphism of this gene result therefore to have a role in the OP and on the risk of vertebral fracture, particularly in men.

Cytochine and Growth Factors

Has been also studied the role of cytokines and growth factors in bone remodelling, above all the TGF β, the Activin A, IL-6 and IGF-1.

Monogenic mutations in the TGF β (Trasforming Growth Factor β) has been found in some diseases as the osteosclerosis[30]. Despite these evidences, the physiopathological mechanism is not known.

Activin A is a growth factor of the superfamily of the TGF β which also seems involved in the processes of bone remodeling. In vitro studies show that this molecule acts at the bone level stimulating osteoblasts proliferation, increasing the secretion of the bone matrix, and also stimulating osteoclasts differentiation. An interesting point is the high bone remodelling processes show altered levels of Activin A expression. This factor could probably play a role in the formation of bone metastasis[20]

IL6 (Interleukin-6) is a proinflammatory cytokine with important effects on the osteoclastic function and differentiation. Its expression is also modulated by the estrogens.

Some studies have shown the relationship among the various polymorphisms of the IL-6 gene and bone phenotypes, like the comparison of tandem sequences whose presence is associated to altered bone resorption through an osteoclast-dependent mechanism[20].

The IGF-1 (Insulin-like Growth Factor 1) is a growth factor that acts since the infancy on the skeletal development, but also in the adult it doesn't stop its activity, in fact it contributes to the maintenance of the cortical and trabecular structure of the bone.

This molecule enhances the osteoblasts proliferation and differentiation and also increases the collagene type 1 synthesis[59]. Besides it is a modulator of the action of different hormones acting on bone metabolism, like PTH, the growth hormone and the estrogens. Lower serum levels of IGF-1 have been associated to increased risk of fracture[22] as also the presence of some polymorphisms, but these results need further investigations.

Considerations

The great innovations in the sector of the human genetics during the last ten years have strongly contributed to increase the possibilities to individualize with success the genes involved in complex pathologies like osteoporosis.

The genetic component has a notable impact on the predisposition to osteoporosis, and is highly probable that many genes are involved with other causes that influence it.

The osteoporosis as diabetes and hypertension, represent a good example of multifactorial disease to which could apply models of genetic analysis to precociously identify the subjects susceptible of low bone mass with elevated risk of fracture.

The results obtained by various genetic studies are not settling for a whole series of existing limitations in the used analytical models, in relationship to the various races, to the presence of complex genotypes and the interference of the environmental factors.

Furthermore deeper knowledge on the molecular mechanisms of the OP will make the possible development of new diagnostic and predictive tests, over that new based therapeutic strategies based on the possibility to preventively identify the subjects with strong susceptibility to develop osteoporosis, modifying, when possible, the exposure to further risk factors, consequential from the style of life, from feeding and, in the woman, from estrogenic lack, before the clinical-instrumental manifestations.

References

1) Amizuka N, Karaplis AC, Henderson JE, et al. *Haploinsufficiency of parathyroid hormone-related peptide (PTHrP) results in abnormal postnatal bone development.* Dev. Biol. 1996; 175: *166.*
2) Arai H, Miyamoto KI, Taketani Y, et al. *A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J* Bone Miner Res. 1997; 12: *915-921.*
3) Arai H, Miyamoto KI, Yoshida M, et al. *The polymorphism in the caudal-related homeodomain protein Cdx-2 binding element in the human vitamin D receptor gene.* J Bone Miner Res. 2001; 16: *1256-1264.*
4) Arts J, Kuiper GGJM, Janssen JMMF, Gustafsson JA, Lowik CWGM, Pols HAP, and Vanleeuwen JPTM. *Differential expression of estrogen receptors* β *and* β*mRNA during differentiation of human osteoblast SV-HFO cells.* Endocrinology. 1997; 138: *5067-5070.*
5) Auchus RJ, Miller WL *Molecular modeling of human P450c17 (17-hydroxylase/17,20-lyase): insights into reaction mechanisms and effects of mutations*. Mol Endocrinol.1999; 13: *1169–1182.*
6) Audrey KoaY M, Peng Y Woon, Yun Zhang, Lisa J Miles et al. *Influence of LPR5 Polymorphisms on normal variation in BMD*. J Bone Miner Res. 2004; 10: *1619-1627.*
7) Bacherini L, Gennari L, Masi L, Mansani R, Massart F, Morelli A, Falchetti A, Gonnelli S, Fiorelli G, Tanini A and Maria Luisa Brandi. *Evidence of a linkage disequilibrium between polymorphism in the human estrogen receptor* α *gene and their relationship to bone mass variation in postmenopausal italian women.* Human Molecular Genetics. 2000; Vol. 9 No. 13: *2043-2050.*
8) Baldock PA, Eisman JA *Genetic determinants of bone mass.* Curr Opin Rheumato. 2004; l16: *450-456.*
9) Bettoun JD, Minagawa M, Kwan MY, Lee HS, Yasuda T, Hendy GN, Goltzman D, White JH *Cloning and characterization of the promoter regions of the human parathyroid hormone (PTH)/PTH-related peptide receptor gene: analysis of deoxyribonucleic acid from normal subjects and patients with pseudohypoparathyroidism type 1b.* J Clin Endocrinol Metab. 1997; 82: *1031-1040.*
10) Bord S, Vedi S, Beavan SR, Horner A, and Compston JE. *Megakaryocyte population in human bone marrow increases with oestrogen treatment: a role in bone remodeling.* J Bone Miner Res. 1999; 14: *1032.*
11) Braidman IP, Davenport LK, Carter DH, Selby PL, Mawer EB, and Freemont AJ. *Preliminary in situ identification of estrogen target cells in bone*. J Bone Miner Res. 1995; 10: *74-80.*
12) Byers PH Brittle *bones fragile molecules: disorders of collagen gene structure and expression.* Trends in Genetics. 1990; 6: *293–300.*
13) Carani C, Qin k, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson *ER Effect of testosterone and estradiol in a men with aromatasi deficiency.* N Engl J Med. 1997; 337: *91-95.*
14) Chikari Takeo, Etsuko Negishi, Aya Nakajima, Koichi Ueno, Ichiro Tatsuno, Yasushi Saito, Keiko Amano, and Aizan Hirai. *Association of cytosine-adenine repeat polymorphism of the estrogen receptor-*β *gene with menopausal symptoms gender.* Medicine. 2005; Vol. 2 No. 2.
15) Chitra Sundarrajan, Wu Xiang Liao, Ashim Chandra Roy, And Soon Chye Ng *Association between Estrogen Receptor-*β *Gene Polymorphisms and Ovulatory Dysfunctions in Patients with Menstrual Disorders* The Journal of Clinical Endocrinology & Metabolism. 2001; Vol. 86, No. 1.
16) Cohen-Solal M., De Vernejoul M.C.*Genetic of osteoporosis* Elselvier SAS. 2004; *S526-530.*
17) Cooper GS, Umbach DM *Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis*. J Bone Miner Res. 1996; 11: *1841–1849.*
18) Couse JF, and Korach KS. *Estrogen receptor null mice: what have we learned and where will they lead us?* Endocr Rev. 1999; 20: *358-417.*
19) Eriksen EF, Colvard DS, Berg NJ, Grahan ML, Mann KG, Spelsberg TC, and Riggs BL. *Evidence of estrogen receptors in normal human osteoblast-like cells*. Science. 1988; 41: *84-86.*
20) Ferrari SL, Garnero P, Emond S, Montgomery H, Humphries SE & Greenspan SL *A functional polymorphic variant in the interleukin-6 gene promoter associated with low bone resorption in postmenopausal women.* Arthritis and Rheumatism. 2001; 44: *196–201.*
21) Fukayama S, Tashjian AH Jr, Davis JN, et al. *Signaling by N- and C-terminal sequences of parathyroid hormone-related protein in hippocampal neurons.* Proc. Natl. Acad. Sci. USA. 1995; 92: *10182.*
22) Garnero P, Sornay-Rendu E, Delmas PD. *Low serum IGF-1 and occurrence of osteoporotic fractures in postmenopausal women.* Lancet. 2000; 355: *898-899.*
23) Garnero P., Arden N.K., Griffiths G., Delmas P.D., Spector T.D *Genetic influence on bone turnover in postmenopausal twins*. J Clin Endocrinol Metab. 1996; 81: *140-146.*
24) Gennari L, Masi L, Merletti D, Picariello L, Falchetti A, Tanini A, Mavilia C, Del Monte F, Gonnelli S, Lucani B, Gennari C, and Maria Luisa Brandi. *A polymorphic CYP19 TTTA repeat influences aromatasi activity and estrogen levels in elderly men: effects on bone metabolism.* The Journal of Clinical Endocrinology & Metabolism. 2004; 89 (6): *2803-2810.*

25) Green S, Walter P, Kumar V, Krust M, Bornet A, Argos P, and Chambon P. *Human oestrogen receptor cDNA: sequence, expression and homology to c-erbA.* Nature. 1986; 320: *134-139.*

26) Gronemeyer H. *Transcription activation by estrogen and progesterone receptors.* Annu Rev Genet. 1991; 25: *89-123.*

27) Gueguen R, Jouanny P,Guillemin F, et al. *Segregation analysis and variance components analysis of bone mineral density*. J Bone Miner Res. 1995; 10: *2017-2022.*

28) Hobson EE, Ralston SH. *Role of genetic factors in the pathophysiology and management of osteoporosis* Clin Endocrinol. 2001; 54: **1-9**.

29) Hofbauer LC, Khosla S. *Androgen effects on bone metabolism: recent progress and controversies.* Eur J Endocrinol. 1999; 140: *271-286.*

30) Incorvaia L., Sferrazza C., Di Fede G., Badalamenti G., Rotolo G., Fricano S., Di Lorenzo G., Leto G., Gebbia N., Rini G.B.. *Livelli circolanti di Activina A in pazienti con osteoporosi primitiva o metastasi ossee da carcino - ma mammario*. V Congresso Nazionale SIOMMMS Siena. 16-19 Novembre 2005.

31) Ioannidis JP, Stavrou I, trikalinos TA, Zots C, Brandi ML, Gennari L. et al. ER-alpha Genetics Meta-Analysis. *Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density and fracture risk in women: a meta-analysis*. Bone Miner Res. 2002; 17: *2048-60.*

32) Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN, Eisman JA *Genetic factors in bone tur - nover*. J Clin Endocrinol Metab. 1991; 72: *808-13.*

33) Kelly PJ, Nguyen T, Hopper J, Pocock N, Sambrook P, Eisman J *Changes in axial bone density with age: a twin study.* J Bone Miner Res. 1993; 8: *11-17.*

34) Kenichi Negoro et al. *Polymorphisms in the estrogen receptor β gene but not estrogen receptor β gene affect the risk of developing endometriosis in a Japanese population.* Fertility and sterility. 2004; Vol. 81, No. 6.

35) Kinoshita A, saito T, tomita H, Makita Y, Yoshida K, Ghadami M, et al. *Domain-specific mutations in TGFβ1 result in Camurati-Engelmann disease*. Nat Genet. 2000; 26: *19-20.*

36) 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: *306–311.*

37) Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, and Gustafsson J-A. *Cloning of a novel estrogen recep - tor expressed in rat prostate and ovary.* Proc Natl Acad Sci USA. 1996; 93: *5925-5930.*

38) Lander ES, Schork NJ: *Genetics dissection of complex traits.* Science. 1994; 265: *2037-2048.*

39) Lanske B, Amling M, Neff L, et al: *Ablation of the PTHrP gene or the PTH/PTHrP receptor gene leads to distinct abnormalities in bone development*. J.Clin.Invest. 1999; 104: *399.*

40) Lau H. H. L., Ho A. Y. Y., Luk K. D. K., And Kung A. W. C. Estrogen Receptor β *Gene Polymorphisms Are Associated With Higher Bone Mineral Density in Premenopausal, but not Postmenopausal Southern Chinese Women Bone.* 2002; Vol. 31, No. 2: *276-281.*

41) Martucci CP, Fishman J. *P450 enzymes of estrogen metabolism*. Pharmacol Ther. 1993; 57: *237-257.*

42) Masi L, Becherini L, Gennari L, Amedei A, Colli E, Falchetti A, Farci M, Silvestri S, Gonnelli S, and Maria Luisa Brandi. *Polymorphism of the Aromatase Gene in postmenopausal Italian women: distribution and corre - lation with bone mass and fracture risk.* The Journal of Clinical Endocrinology & Metabolism. 2001; 86: *2263-2269.*

43) Minagawa M, Watanabe T, Kohno Y, Mochizuki H, Hendy GN, Goltzman D, White JH, Yasuda T *Analysis of the P3 promoter of the human parathyroid hormone (PTH)/PTH-related peptide receptor gene in pseudohy - poparathyroidism type 1b*. J Clin Endocrinol Metab. 2001; 86: *1394-1397.*

44) Minagawa M, Yasuda T, Watanabe T, Minamitani K, Takahashi Y, Goltzman D, White JH, Geoffrey N, Hendy, and Kohno Y. *Association between AAAG repeat polymorphism in the P3 promoter of the human parathyroid hormone (PTH)/PTH-related peptide receptor gene and adult height, urinary pyridinoline excretion, and promoter activity.* The Journal of Clinical Endocrinology & Metabolism. 2002; 87: *1791-1796.*

45) Morishima A, Grumbach MM, Simpson ER, Fisher C, and Qin K. *Aromatase deficiency in male and female siblings caused by a novel mutation and the physiologi - cal role of estrogens.* J Clin Endocrinol Metab. 1995; 80: *3689-3698.*

46) Morrison NA, Qi JC, Tokita A et al *Prediction of bone density from vitamin D receptor alleles.* Nature. 1994; 367: *284-287.*

47) Morrison NA, Yeoman R, Kelly PJ, Eisman JA Contribution of trans-acting factor alleles to normal *physiological variability: vitamin D receptor gene polymorphism and circulating osteocalcin.* Proc Natl Acad Sci USA. 1992; 89: *6665–6659.*

48) Napoli N., Del Fiacco R., Mumm S., Fakhri A., Rini G.B., Villareal R.C. *Allelic combination of the CYP17 and CYP19 genes: skeletal and hormonal phenotypes.* Journal of Bone and Mineral Research. Vol. 20 (Suppl 1) September 2005; s1-s512.

49) Napoli N, Villareal DT, Mumm S, Halstead L, Sheikh S, Cagaana M, Rini GB, and Armamento-Villareal R. *Effect of CYP1A1 gene polymorphisms on estrogen metabolism and bone density*. Journal of Bone and Mineral Research. Vol.20, No.2, 2005: *232-239.*

50) Nawata H, Tanaka S, et al. *Aromatase in bone cell: association with osteoporosis in postmenopausal women.* J Steroid Biochem Mol. 1995; 1-6: *165-174.*

51) Nilsson M, Naessen S, Dahlman I, et al. *Association of estrogen receptor beta gene polymorphisms with buli - mic disease in women.* Mol Psychiatry. 2004; 9: *28-34.*

52) Ogawa, S., Hosoi, T., Shiraki, M., Orimo, H., Emi, M., Muramatsu, M., Ouchi, Y., and Inoue, S. *Association of estrogen receptor β gene polymorphism with bone mineral density*. Biochem Biophys Res Commun. 2000; 269: *537–41.*

53) Pan LC, Ke HZ, Simmons HA, Crawford DT, Chidsey-Frink KL, McCurdy SP, Schafer JR, Kimbro KS, Taki M, Korach KS, and Thompson DD. Estrogen receptor-alpha knockout (ERKO) mice lose trabecular and cortical bone following ovariectomy. J Bone Miner Res. 1997; 12 (Suppl 1): S134.

54) Pettersson K, Grandien K, Kuiper GGJM, and Gustafsson J-A. *Mouse estrogen receptor beta forms estrogen response element-binding hetero-dimers with estrogen receptor alpha*. Mol Endocrinol. 1997; 11: *1486-1496.*

55) Polymeropoulos H, Xiao H, Rath DS, Merril CR. *Tetranucleotide repeat polymorphism at the human P-450 gene (CYP19).* Nucleic Acid Res. 1997; 19: 195.

56) Reich DE, Cargill M, Bolk S, et al. Linkage disequilibrium in the human genome. Nature. 2001; 411: *199-204.*

57) Rosenkranz K, Hinney A, Ziegler A, Hermann H, Fichter M, Mayer H, et al. *Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: identification of several genetic variants.* J Clin Endocrinol Metab. 1998; 83: *4524-7.*

58) Saupe J, Shearer MJ, Kohlmeier M. *Phylloquinone transport and ist influence on gamma-carboxyglutamate residues of osteocalcin in patients on maintainance hemodyalisis.* Am J Clin Nutr. 1993; 58: *204-208.*

59) Schmid C, Ernst M: *Insulin-like growth factor. In cytokines and Bone Metabolism,* Ed M Gowen, Boca Raton, Ann Arbor, Ionmdon: CRC Press. 1992 pp*229-265*.

60) Shozu M, Simposon ER. *Aromatase expression of human osteoblast-like cells*. Mol Cell Endocrinol. 1998; 139: *117-129.*

61) Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, and Korach KS. *Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man.* N Engl J Med. 1994; 331: *1056-1061*.

62) Somner J, McLellan S, Cheung J, Mak YT, Frost ML, Knapp KM, Wierzbicki AS, Wheeler M, Fogelman I, Ralston SH, and Hampson GN. *Polymorphisms in the P450 c17 (17-hydroxylase/17,20-lyase) and P450 c19 (Aromatase) genes: association with serum sex steroid concentrations and bone mineral density in postmenopausal women*. The Journal of Clinical Endocrinology & Metabolism. 2004; 89(1): *344-351*.

63) Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ *Modulation of osteoclast differentiation and function by the new members of tumor necrosis factors and ligand families*. Endocr Rev. 1999; 20: *345–357*.

64) Thakkinstian A, D'Este C, Eisman J, Nguyen T, Attia J *Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study*. J Bone Miner Res. 2004; 19: *419-28*.

65) Tokita A, Kelly PJ, Nguyen TV, Qi JC, Morrison NA, Risteli L, Risteli J, Sambrook PN, Eisman JA *Genetic influences on type I collagen synthesis and degradation: further evidence for genetic regulation of bone turnover.* J Clin Endocrinol Metab. 1994; 78: *1461-1466*.

66) Uitterlinden AG, Burger H, Huang Q et al.: *Relation of alleles of the collagen type Ia gene to bone density and a risk of osteoporotic fractures in postmenopausal women.* N Engl J Med. 1998; 338: *1016-1022*.

67) Uitterlinden AG, Weel AE, Burger H, et al: *Interaction between the vitamin D receptor gene and collagen type Ia gene in susceptibility for fracture.* J Bone Miner Res. 2001; 16: *379-385*.

68) Vidal O, Kindblom L-G, and Ohlsson C. *Expression and localization of estrogen receptor-beta in murine and human bone*. J Bone Miner Res. 1999; 14: *923-929.*

69) Vidal O, Windahl S, Andersson G, Gustavsson JA, and Phlsson C. *Increased cortical bone mineral content but unchanged trabecular bone mineral density in adult female mice lacking estrogen receptor-*β. J Bone Miner Res. 1999; 14 (Suppl): *S141.*

70) Vogt MT, Cauley JA, Kuller S, Nevitt MC. *Bone mineral density and blood flow to the lower extremities: the study of osteoporotica fractures.* J Bone Miner Res. 1997; 12: *283-9.*

71) Wysolmerski JJ, Mccaugherncarucci JF, Daifotis AG, et al. *Overexpression of parathyroid hormone-related protein or parathyroid hormone in transgenic mice impairs branching morphogenesis during mammary gland development*. Development. 1995; 121: *3539.*

Request reprints from:
Dott. ILENIA PEPE
Dipartimento di Medicina Clinica e delle Patol. Emergenti
Via Del Vespro, 143
90127 Palermo
(Italy)