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REVIEW

Risk Factor for Postmenopausal Osteoporosis: Genetic Polymorphism?

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Osteoporosis is a major community health problem affecting the elderly in most countries. Increasing life expectancy and changes in life-style indicate that problems of osteoporosis will markedly affect a large number of women populations. The results of large prospective studies have shown that incidence of almost every type of fracture has increased in patients with low bone density. The chance for adults who have a sustained fracture to suffer another one of a different type is 50 -100%.[1] Incidence rates of hip fracture are known to increase exponentially with age in women in most regions of the world. In Asia, the number of individuals, aged 65 and older, is expected to increase from 145 million to 894 million. It is estimated that the number of hip fractures worldwide will increase from 1.7 million in 1990 to 6.3 million in 2050, and most of the world's hip fractures will be in Asia.[2] The measurement of bone mineral density (BMD) of older women is very important for assessment of skeletal abnormality, especially in the area of osteoporotic fracture risk.[3] A low peak bone mass achieved in adulthood and high rate of bone loss in peri- and postmenopausal periods are major determinants for reduced bone density.[4] Both factors are partly genetically determined.[5-9] Several risk factors for osteoporosis have been identified (table 1).[10,11] Hypogonadism is an important risk factor for osteoporosis. Anorexia nervosa, exercise-induced amenorrhea, chronic illness and premature menopause are associated with the increase of osteoporosis. A history of osteoporotic fracture is also indicative of further fracture. Other risk factors for osteoporosis are smoking, excessive alcohol consumption, glucocorticoid therapy, low body weight, long-term immobilization and poor calcium nutrition. Among the risk factors for postmenopausal osteoporosis, genetic factors may play an important role in determining postmenopausal osteoporosis.

Table 1. Risk factors for osteoporotic fractures[11]

Risk factors for osteoporotic fractures
Female sex
Premature menopause
Age
Primary or secondary amenorrhea
Asian or white ethnic origin
Previous fragility fracture
Low bone mineral density
Glucocorticoid therapy
High bone turnover
Family history of hip fracture
Poor visual acuity
Low bodyweight
Neuromuscular disorders
Cigarette smoking
Excessive alcohol consumption
Long-term immobilization
Low dietary calcium intake
Vitamin D deficiency

Genetic polymorphism is the occurrence in a population of two or more alleles at a locus in frequencies greater than can be maintained by mutation. Polymorphisms are genetic differences that provide variation within a species and reflect alterations of DNA sequence that may be demonstrated by DNA technology such as restriction fragment length polymorphisms (RFLPs). Moreover, altered proteins, enzymes, or antigens and abnormal physical features, all of these can indicate polymorphisms. A number of candidate genes have been examined for their effects of allelic variation in normal bone metabolism. Evidence from twin studies and studies of daughters of women with osteoporosis demonstrate that osteoporosis has to be considered a multifactorial polygenic disease in which genetic determinants are modulated by hormonal, environmental and nutritional factors.[9,12,13] Given the important role that steroid hormone plays in the development of bone cells and in the maintenance of normal bone architecture, polymorphisms at receptors of steroid hormone receptors superfamily, such as vitamin D receptor (VDR) and estrogen receptor alpha have been thoroughly in vestigated in the recent years. Apparently, they appear to represent important candidate genes. It has been suggested that vitamin D receptor gene may be one of the genes for osteoporosis and polymorphisms in this gene appear to predict spinal and femoral BMD in postmenopausal women.[14-18] However, several conflicting reports on the relationship between VDR genotype and BMD have been published.[14,16,17,19-29] These discordant findings are thought to be due to ethnic and environmental differences among populations. Recently, polymorphisms at estrogen receptor (ER) gene, androgen receptor gene and aromatase gene were introduced as another potential candidates for genetic regulation of BMD.

Estrogen receptor gene polymorphisms

From basic science, we know that among the factors that influence bone loss, estrogen deficiency is the most important. Estrogen receptor is found on normal human osteoblast-like cells.[30] The finding suggests that estrogen plays direct effects on bone cells. A report of decreased BMD in mice lacking functional ERα but not in those lacking ERβ further supports the hypothesis that ERα is a major candidate gene for osteoporosis.[31-33] It is possible that heterogeneity in bone mass and bone metabolism after menopause may reflect different responses in lower circulating levels of estrogen in postmenopausal period because of genetic variations in the ERα gene.[34] Then variants of ER gene might have an effect on the development of osteoporosis. Until now, there have been many studies on ERα gene polymorphisms and BMD. Kobayashi et al.35 first reported that the combined PPxx genotype was associated with lower spine BMD in Japanese women. However the observation could only be reproduced in northern Chinese but not in southern Chinese women.[36] Willing et al.[37] showed that premenopausal and perimenopausal women with PP genotype had higher lumbar spine BMD and Ongphiphadhanakul B et al.[38] reported that PP genotype was associated with higher BMD at the lumbar spine and the femoral neck in premenopausal women. In contrast, in a study in Korea, found no association between ERα genotypes and BMD.[39] The *Pvu*II restriction fragment length polymorphism (RFLP) of ERα gene was detected by DNA extraction and amplification by polymerase chain reaction. Then, by using a specific restriction enzyme (*Pvu*II restriction endonuclease), we can further differentiate the cut and uncut fragments of this estrogen receptor gene.

The PvuII restriction fragment length polymorphism (RFLP) of ERα gene: methods of detection

DNA was extracted from leucocytes from peripheral blood samples. Approximately 50 ng of DNA from each individual was used in polymerase chain reactions (PCRs). *Pvu*II RFLP of ERα gene was detected by amplifying a 1.3 kb fragment of intron 1 using primers e was detected by amplification of a 1.3 kb fragment of intron 1 using primers ERPvuF (5'-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3')

and ERPvuR (5'-TCTTTCTCTGCCACCCTGG CGTCGATTATCTGA-3'). DNA was added to a mixture containing 0.5 µM of each primer, 1x PCR buffer (Perkin-Elmer Applied Biosystems), 250 µM dNTP (Perkin-Elmer Applied Biosystems), 1.5 mM $MgCl_2$ (Perkin-Elmer Applied Biosystems), and 1 Unit Amplitag Gold DNA polymerase (Perkin-Elmer Applied Biosystems), to give a total reaction volume of 20 µL. The required thermal conditions for the reaction were 95°C for 10 minutes (for activation of the Amplitag Gold enzyme), followed by 35 cycles of 95°C for 1 minute, 62°C for 1 minute and 72°C for 2 minutes, followed by a final extension time of 72°C for 10 minutes. Products were then digested by adding 5 units of *Pvu*II restriction endonuclease (Boehringer Mannheim) in the presence of 1x buffer 'M' (Boehringer Mannheim) at 37°C for one hour. Digested products were then electrophoresed and visualised through 2% agarose gel (Type II-A, Sigma) containing ethidium bromide. DNA was visualized by placing the gel on UV transilluminator. The restriction fragment length polymorphisms (RFLPs) were represented as Pp, with upper case and lower case letters signifying the absence or presence of restriction sites, respectively as shown in figure 1. Then *Pvu*II restriction fragment length polymorphisms (RFLP) of ERα gene were, namely: homozygous PP, homozygous pp and heterozygous Pp.

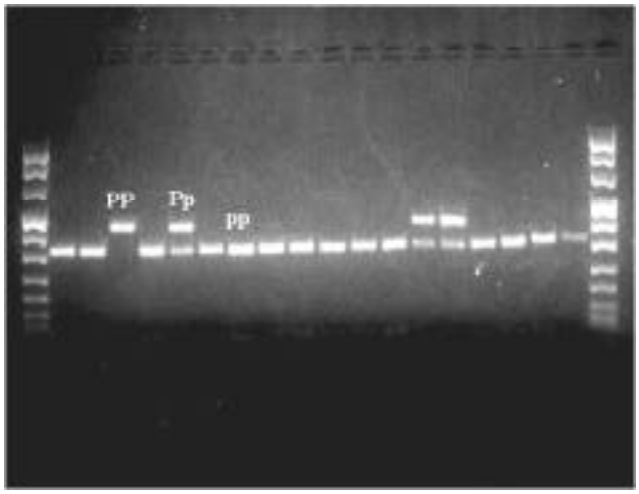

Fig. 1. *Pvu*II restriction fragment length polymorphism (RFLP) of ERα gene.

Among the studies of the association between estrogen receptor polymorphism and bone mineral density, the results were still controversial. The possible reason for discrepancies among the reported studies could be due to different ethnicity as well as environmental and hormonal confounders in each study. Otherwise, it might be explained by the fact that PvuII polymorphism of ER gene is not true causative alleles and might be linked to some variations in ER gene or other genes which affects BMD.

Currently, there are a few reports on the effect of ERα gene polymorphism on bone loss in women. Bagger et al.[40] revealed that no relation between ER genotypes and the rate of bone loss, either in the hip and spine over 6 years in postmenopausal Danish women. Han et al.[41] reported that there was no association of ER genotype with BMD and bone turnover in pre-, peri-, and postmenopausal Korean women. Mizunuma et al.[4] investigated the association between ER gene polymorphism and the rate of bone loss in pre- and postmenopausal women and failed to demonstrate any relationship between RFLP by *Pvu*II and the rate of bone loss. In conclusion, the effects of *Pvu*II polymorphism of ERα gene on BMD postmenopausal women still need further studies. How this polymorphism interacts with other genetic and environment factors awaits clarification.

Androgen receptor gene polymorphisms

Androgen plays an important role in the maintenance of bone mass and can stimulate bone formation. Estrogen was thought to regulate bone mass in women, whereas androgen was thought to regulate bone mass in men. Now it appears that both sex steroids together influence bone and mineral metabolism in women.[42] Androgen acts either by direct binding to androgen receptors, or by aromatization to estrogen and subsequently interacts with estrogen receptors.[43] The detection and functional characterization of androgen receptors in bone cells has implicated bone tissue as a potential target tissue for androgens. Gonadal and adrenal

androgens directly regulate various aspects of osteoblastic lineage cells, including proliferation, differentiation, mineralization, and gene expression.[44] Polymorphisms of androgen receptor are associated with reduced its function due to defective intermolecular protein-protein interactions with coactivator molecules.[45] Sowers et al,[46] evaluated the genetic markers for products that contribute to skeletal mineralization and found that the polymorphic (ACG)n site in androgen receptors showed a strong association with BMD of the femoral neck and lumbar spine. However, there is a shortage of studies on the association between androgen receptors and bone mineral density. We still need a lot of trials before jumping to conclusion of the effect of this receptor on bone mass.

Aromatase gene polymorphisms and bone mineral density

The aromatase enzyme catalyses the conversion of androgens to estrogens in the estrogen biosynthesis pathway.[47] Moreover, genetic studies suggest that several genes contribute to the regulation of bone mass via interaction with the modeling and remodeling processes. Among these genes, aromatase gene is a potential candidate to be evaluated for segregation with bone metabolism and bone mass. The gene tat encodes the aromatase enzyme called CYP 19. CYP 19 gene is located on chromosome 15 at position q21.1. Currently, there are no described RFLPs detectable by polymerase chain reaction. However, a tetranucleotide simple tandem repeat polymorphism in intron 4 at the human aromatase cytochrome P-450 gene has been recently described. The study showed that the allele containing the longer TTTA repeats was statistically more represented in non-osteoporotic women and women with a high number of TTTA repeats had a significantly higher lumbar bone mineral density than those with alleles containing 8-11 TTTA repeats.[48] Then the aromatase gene can be one of the several genes potentially involved in the maintenance of bone mass and the regulation of bone loss.

In conclusion, individual contribution of these genetic polymorphisms to the pathogenesis of osteoporosis remains to be confirmed, Further, the important aim in future works will be to define how these polymorphisms interact with each other and with the environment that causes the osteoporotic phenotype. The basic knowledge about the genetic involvement on osteoporosis is important. We may use genetic markers for assessment of fracture risk in the future and for identifying molecules that use for the design of new pharmacotherapy for prevention and treatment of osteoporosis.

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