Bone mass pharmacogenetics and ethnic health implications

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Summary

Osteoporosis is a common skeletal disease with a strong genetic component characterized by reduced bone mass and increased risk of fragility fractures. Bone mineral density (BMD) is considered the best established risk factor for osteoporotic fractures.
Over the last years a large number of studies have pointed to the variability in many target genes and their relation with BMD and other determinants of fracture risk such as ultrasound bone properties, skeletal geometry and bone turnover markers. The importance of genetic factors in the bone quality is substantial, but no consensus exists yet on the genes that are involved.
Although osteoporosis is world healthy problem, there are many differences in human ethnics regarding both disease morbidity and drug treatment efficacy. Heterogeneity in drug response may reflect varying responsiveness to osteoporosis treatments due to allele variation in signaling pathway genes such as vitamin D receptor (VDR) or estrogen receptor α (ERα). Polymorphisms of VDR and ERα loci appear genetic determinants of their corresponding hormonal treatment response such as vitamin D and estrogens. Because of their specific ethnic distribution, polymorphisms of VDR and ERα genes may be involved in reported human differences of osteoporosis treatment responses.
Knowledge of the molecular and functional consequences of the gene polymorphisms is crucial to fully appreciate their significance and understand their potential clinical implications. Future studies and preventive strategies to management osteoporosis need to take in account these genetic factors.

KEY WORDS: genetics, estrogen receptor, osteoporosis, pharmacogenomics, polymorphism, vitamin D receptor.

Introduction

Osteoporosis affects an estimated 75 million people among Europe, the United States (US) and Japan (1) and represents a major health problem, especially in countries where life expectancy has dramatically increased during the past decades. Hip and vertebral fractures, which are frequent complications of osteoporosis, represent one of the most important causes of morbidity and mortality among elderly people around the world (2, 3). Bone mineral density (BMD) is the major determinant of fragility fracture (4). Although many environmental factors, such as dietary intakes, physical activities, education, etc., play an important role in BMD, it is strongly inherited. From studies of monozygotic and dizygotic twins, inheritance was estimated to account for 60-80% of BMD in both men (5) and women (6, 7). In this regards, a large number of polymorphisms in multiple candidate genes have been investigated (8). Of them, vitamin D receptor (VDR) and estrogen receptor α (ERα) have been among of the most intensively studied genes in genetic regulation of BMD.
To date, interest of most scientists and clinicians working in genetics, is to arrange genetic markers useful in the patient treatment. In this view, genetics not only offer possibility to anticipate pathological phenotype even before real disease onset but also to foresee the specific patient response to drugs. An early specific and efficacious medicine means greater healthy chances for patient and less hospital economic loss.
The present analysis reviews available molecular data of two major osteoporotic treatments based on vitamin D and estrogens regarding predictor markers for their clinical drug response. Many clinical clues suggest human genetic backgrounds play major role determining treatment effectiveness. Treatment responses to vitamin D or estrogens are affected by specific genotypes of target genes such as VDR and ERα. In our opinion, this review could offer argument of pharmacological data reanalysis and/or new future health strategies.

Vitamin D receptor gene

Since 1990s, the gene encoding for VDR was proposed as major genetic locus of bone mass (9). The VDR gene is located on the long arm of chromosome 12 (12q12-14) and is composed by 10 exons, the first of which is not transcribed, and 8 introns (10). The nine coding exons are transcribed into the VDR messenger RNA (mRNA), which in turn is translated into the functional VDR protein. Several restriction fragment length polymorphisms (RFLPs) in the human VDR gene locus have been used in population-based studies (Figure 1). The respective restriction endonuclease enzymes have been conventionally indicated with lowercase letter (*t*, *a*, *b* or *f*, respectively for *Taq*I, *Apa*I, *Bsm*I and *Fok*I restriction endonucleases), while uppercase letter (*T*, *A*, *B* or *F*) indicates the absence of the restriction site. The *Bsm*I and *Apa*I polymorphisms lie in an VDR untranslated region (intron 8) and probably do not confer any functional diversity per se. Similarly, the silent nucleotide substitution in exon 9 that creates the *Taq*I polymorphism does not affect the amino acid composition of VDR protein (9). Because of their next sites, these VDR 3'end polymorphisms (i.e. *Bsm*I, *Taq*I and *Apa*I RFLPs) were in linkage disequilibrium such that *A* and *B* alleles were strongly associated with *t* allele, while *a* and *b* alleles with the absence of *Taq*I restriction site (*T* allele).
Morrison et al. (11) used for the first time a candidate gene ap-

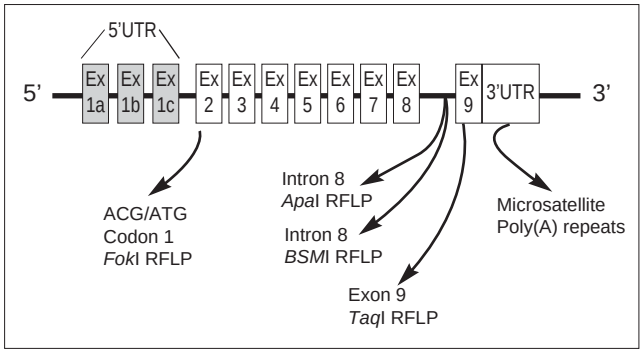

Figure 1 - Gene polymorphisms in the human VDR locus potentially involved in vitamin D treatment response.

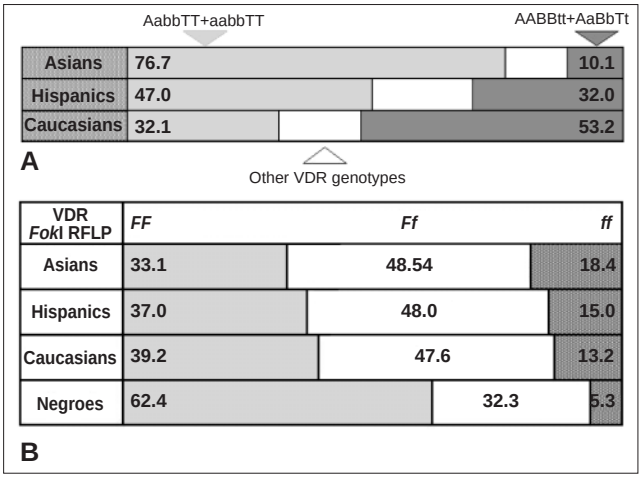

Figure 2 - Ethnic frequencies of VDR genotypes detected by *Apa*I-*Bsm*I-*Taq*I (A) and FokI (B) endonucleases, respectively. Data modified from ref. 40.

proach to related common *Bsm*I allelic variants at VDR 3'end region with bone turnover. Subsequently, the same authors suggested a major contribution by VDR 3'end RFLPs to the BMD genetic determination: VDR gene was originally claimed to contribute to almost 75% of the genetic variation on the BMD (9). In both twin pairs and unrelated post-menopausal women, they showed significantly decreased BMD values at lumbar spine and proximal femur in relation to *Bsm*I *B* allele (9). After this original report, conflicting data have been published on the association of the diallelic *Bsm*I RFLP in the VDR locus and BMD both in pre- (12-15) and post-menopausal (16-26) women. Several population studies have essentially confirmed this association, with differences in BMD ranging from 4 to 13% between the opposite *BB* and *bb* genotypes (14, 16, 17, 22, 23). Other studies found no significant association between VDR alleles and BMD (12, 15, 19, 26), whereas others reported an inverse association to that originally proposed, with subjects with the *BB* genotype showing higher and not lower BMD values than the *bb* genotype (13, 20, 21). Similarly, studies examining the relationship of this VDR polymorphism with bone turnover markers (12, 19, 24, 27), rates of bone loss (19, 28-30) and osteoporotic fractures (21, 30-35) yielded conflicting results.

Also using combined VDR RFLP analysis, the found BMD associations were not confirmed in all ethnics around the world. In a large and ethnically homogeneous Caucasian population study (16), a significant segregation of VDR genotypes with lumbar BMD was observed, similar to what previously reported in Australian Caucasians (9, 36). The *AABBtt* genotype showed a spinal BMD 13% less than *aabbTT* genotype (16). However, the BMD data were not confirmed by other showing different or no VDR genotype associations with BMD both in Caucasian and Asian populations (19, 20, 37-39). From these population based studies, it is also evident that the VDR polymorphism distribution presents specific ethnic patterns (40). Considering the 4 most frequent genotypes of VDR 3'end, there are 3 specific ethnic patterns (Figure 2A). In Asian populations, *AabbTT* and *aabbTT* genotypes (more than 75%) are predominant than *AABBtt* and *AaBbTt* genotypes which are the most frequent in Caucasians (about 53%) (40).

Do VDR genotypes influence clinical response to osteoporosis drug treatments? Few studies actually analyzed vitamin D treatment response regarding VDR genotype producing confuse data. At first, Matsuyama et al. (41) assayed VDR genotype response to $1\alpha OHD_3$ treatment (1 mg per day) in an 1-yr retrospective trial based on 120 Japanese osteoporotic women. Although they found only 2 *Bsm*I *BB* genotypes, the more common genotypes (*aabbTT* and *AabbTT*, 75% of subjects) were associated with higher $1\alpha OHD_3$ response than *AABbTt* and *AaBbTt* genotypes ($p<0.001$) (41). In a UK twin pairs study, there was a modest trend toward a positive effect of 800 IU D_3/day treatment in total hip BMD for the VDR *TT* genotype (42). By contrast, the poor response in the genotype *Tt*, the most common in Caucasians, could account for the generally good responses to vitamin D recorded in Japanese compared to Caucasian subjects (40, 41). Collectively these data could suggest VDR *TT* genotype (or linked VDR *bb* genotypes) is associated to higher vitamin D response.

In an Australian women study, Howard et al. (27, 43) reported a greater PTH response in VDR *bb* genotype vs. *BB* genotype with short-term calcitriol administration (2 µg of $1,25(OH)_2D_3$/week). However, their original findings of differences in osteocalcin and $1,25(OH)_2D_3$ levels between genotypes were not confirmed in the reanalysis (43). Because of VDR *B* allele is in linkage disequilibrium with *t* allele in Caucasian populations (38), these VDR data could agree with above *Taq*I genotype data. By contrast, Graafmans et al. (44) reported that BMD increases in the vitamin D group (400 IU D_3/day) relative to the placebo group, was significantly higher in *BB* (ΔBMD 4.4%) and *Bb* (ΔBMD 4.2%) genotype compared with *bb* genotypes (ΔBMD -0.3%). Finally, an probably explanation for the inconsistency regarding which VDR RFLP allele is associated with low BMD response, is that *Bsm*I-*Taq*I RFLPs do not represent functional loci but are in linkage disequilibrium with a bone-related gene elsewhere.

Because vitamin D and estrogen systems present many cross-talk levels, allelic variants of their signaling pathways could modify mutual hormone response (45-48). In this view, some authors analyzed VDR genotype as response marker to hormone replacement therapy (HRT). A US study based on 108 European Caucasian women, reported *Bsm*I *BB* genotype was associated with larger spinal BMD increase using low HRT dose whereas VDR *bb* genotype was associated with larger decrease in the placebo group (49). As previously reported (50) and irrespective for VDR *Apa*I or *Fok*I sites, Japanese women with VDR *TT* genotype showed significantly higher ΔBMD with HRT than those with *Tt* genotype (2.6% ± 0.5% vs. –0.8 ± 1.4%; $p = 0.016$) at 1 year and slightly higher ΔBMD (3.8% ± 0.6% vs. 0.8 ± 1.6%; $p = 0.069$) at 2 years, but no significant differences between *TT* and *Tt* genotypes were seen at 3 years or later (51). Considering that *Bsm*I B allele is *Taq*I *t*-allele-linked, these studies produced conflicting data. Giguere et al. (52) found, in a cross-sectional study, that results of quantitative ultrasound examination of the heel in postmenopausal women receiving HRT for more than 5 years were affected by

variations in VDR and ERα loci. On the contrary, in a recent Danish study (429 Caucasian women), no VDR genotype effect on changes in bone mass during the subsequent 5 years could be detected, irrespective of HRT (1-2 mg estradiol/day) or not (53). VDR *Bsm*I genotype may be also involved in individual response to cyclic etidronate, raloxifene, alendronate treatments (54, 55). Although the picture is still complicated, there seems to be a trend for the VDR *ABt* haplotype (linked to short *poly(A) microsatellite* in the 3'UTR, *see below*) to display somewhoat better responses than the *abT* haplotype (linked to long poly(A) repeat alleles) (56).

The above data regarding *Apa*I, *Bsm*I and *Taq*I RFLPs, suggest that these VDR neutral polymorphisms should therefore be considered as possible markers, in linkage disequilibrium with functional genetic variants affecting structure or expression of VDR gene. 3'untranslated terminal region (3'UTR) of eukaryota genes contain sequence elements regulating mRNA stability expression (57, 58). The 3'UTRs associated with *Bsm*I-*Apa*I-*Taq*I haplotypes, result in substantial differences in VDR gene expression using a reporter gene assay (9); however, the responsible sequence variants have not yet been identified. Therefore, a major confounding factor in VDR studies, could be various linkage patterns of VDR 3'UTR RFLPs present in different human ethnics which confer different associations between BMD and VDR 3'UTR haplotypes (40).

In 1997, Ingles et al. (59) described a polymorphic *microsatellite* located approximately 1-kb upstream from the VDR 3'UTR. The *microsatellite* consists of a string of adenosine residues [*poly(A) repeats*] with polymorphic length varying from 13 to 24 adenosine repeats. Although at least 12 alleles were identified (A_{13} to A_{24}) of VDR *poly(A)* repeats, allele size follows a bimodal distribution with distinct short (A_{13}-A_{17}) and long (A_{18}-A_{24}) allele populations. As shown in figure 3, various distributions of VDR *poly(A)* repeats have been reported in human ethnic groups (40).

Assuming that VDR *Bsm*I *B* and *b* alleles are in disequilibrium with *short* and *long poly(A)* alleles respectively, agreement was high in human ethnics though only in Afro-Americans: more than 90% in Asians, 93% in Caucasians and 81% in Hispanics (60). About only 2 VDR 3'UTR haplotypes exist in no-African populations (i.e. Caucasian, Asian and Hispanic subjects): *Bsm*I *B* allele with *short poly(A)* repeats (*B-short poly(A)* haplotype) in contrast to *b* allele with long *poly(A)* repeats (*b-long poly(A)* haplotype) (60). Therefore, *Bsm*I site (and highly linked *Taq*I site) is not a good marker of VDR 3'UTR itself, as judged by the *poly(A)* site (60). Misclassification of 3'UTR *poly(A)* alleles by *Bsm*I (and also *Taq*I) site is most severe in African Americans (37%), lesser in Caucasians (7%) and Asians (8,5%) (60).

Grundberg et al. (61) recently investigated *poly(A) microsatellite* and linked *Bsm*I site of VDR gene in a population-based cohort of 343 Swedish women aged 20-39. They showed that women with *short poly(A) repeats* and/or absence of linked *Bsm*I restriction site on both alleles (*BB* genotype) have significantly higher BMD (61). Importantly, the regression analysis showed that VDR *Bsm*I genotype was significantly associated with lumbar spine BMD also when taking fat mass, lean mass, height and age into account (p=0.03) (61). The same trend was seen concerning the effect of VDR *poly(A)* genotype on lumbar spine BMD after adjustments (p=0.06) (61).

If *Apa*I-*Bsm*I-*Taq*I polymorphisms are not probably functional but disequilibrium marker liked to other VDR polymorphisms, which is the molecular effect of *poly(A)* repeats on the VDR function? *Long poly(A)* allele (A_{18}-A_{24} repeats), displays higher vitamin D-induced transcriptional activity than *short poly(A)* allele (A_{13}-A_{17} repeats), although it does not achieve statistical significance (62). Since *poly(A)* polymorphism occurs in exon 9, but is expressed only in the 3'UTR of VDR mRNA, *long poly(A)* allele may produce VDR mRNA that is more stable and/or is translated more efficiently into protein than short allele. Other data using RFLP approach, agree with this hypothesis (9, 62-66). Interestingly, high presence of *long poly(A)* allele in Asians than others (60), may be involved in the suggested higher "vitamin D-sensibility" of Asians than VDR RFLPs (i.e. *Apa*I-*Bsm*I-*Taq*I or *Fok*I RFLPs) (40-41). However, as clearly shown by Haussler's group, simultaneous analysis of *poly(A)* repeats and *Fok*I RFLPs is needed to determine the specific effects of VDR variants on the overall VDR function (62).

Regarding VDR *Fok*I site, *F* allele produces a more active VDR protein than *f* allele (62, 67-69) and *F* and *long poly(A)* alleles have synergic effect increasing VDR protein activity (62). Data indicating a more active VDR *F* allele is consistent with many clinical studies which suggest *F* allele (vs. opposite *f* allele) is associated with increased BMD (67, 70-74), higher rates of bone turnover (50), lower risk for primary hyperparathyroidism (75, 76), lower risk for intervertebral disc degeneration (77) and lower incidence of vertebral fracture (78). Figure 2B showed VDR *Fok*I genotype distribution regarding ethnic groups around the world. Because *F* allele is more represented in Caucasians than Asians, its genetic effects may contrast those of *poly(A)* polymorphism (40).

The above data suggest that the assaying of genotypic effects on the VDR function, needs a simultaneous analysis of all functional variants (i.e. *Fok*I RFLP, *poly(A) repeats* and Cdx-2 polymorphisms (79)) and not only of the disequilibrium markers (i.e. *Apa*I-*Bsm*I-*Taq*I RFLPs) which are frequently ethnic-dependent.

VDR 3'UTR	*Long poly(A) repeats*	*Short repeats*
Caucasians	59	41
Hispanics	69	31
Asians	91	9
Negroes	71	29

Figure 3 - Ethnic distribution of VDR $poly(A)_n$ repeat polymorphism. Allele cutoffs: short allele (A_{13}-A_{17} repeats) and long allele (A_{18}-A_{24} repeats). Data from ref. 60.

Estrogen receptor alpha gene

The genes encoding estrogen receptors, ERα and ERβ, have been considered as important candidate markers of osteoporotic risk. The importance of ERα gene in the bone tissue has been indicated by the osteoporotic phenotype in a man with a nonsense mutation in the ERα gene (80) as well as the reduced BMD values in mice lacking a functional ERα gene (81, 82), but not in those lacking ERβ (82, 83), strongly proposing ERα gene as major mediator of estrogen response at least in bone system.

The human ERα gene is located on the chromosome 6p25.1, comprises eight exons, and spans more than 140 kb (84). Actually, some ERα polymorphisms are proposed as involved in the bone system (Figure 4). Two single nucleotide polymorphisms have been identified in intron 1 of ERα gene: a T-397C polymorphism that is recognized by the restriction endonuclease *PvuII* [*T* and *C* alleles correspond to the presence (*p* allele) and absence (*P* allele) of the *Pvu*I restriction site, respectively] and an A-351G polymorphism that is recognized by *Xba*I [A and G alleles correspond to the presence (*x* allele) and absence (*X* allele) of the *Xba*I restriction site, respectively] (40). These ERα intron 1 RFLPs, alone or in combination, have been associated with bone mass in post-menopausal women

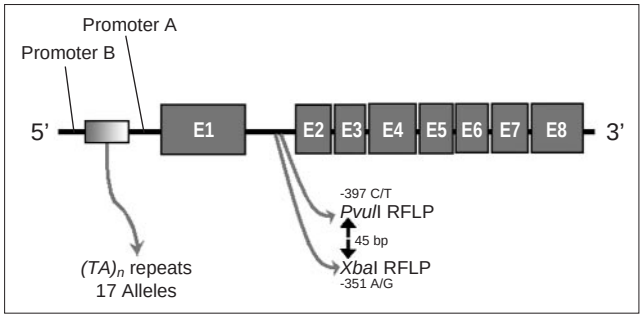

Figure 4 - Gene polymorphisms in the human ERα locus potentially involved in estrogen treatment response.

(85-89) or pre-menopausal women (90, 91). However, other studies have not confirmed these observations (16, 92-96). In addition, a *microsatellite* $(TA)_n$ repeats polymorphism, located in ERα promoter area and strongly linked to *Pvu*II-*Xba*I sites (93), was associated with BMD and with the prevalence of fractures (86, 93-94).

Actually, few studies analyzed ERα polymorphisms related to HRT responsiveness. In Korean post-menopausal women, Han et al. (96) found no significant effects of ERα intron 1 genotypes on the BMD and on the HRT responsiveness. Similarly, in a Japanese study, although ERα *PP* genotypes had significantly higher spine BMD than *Pp* and *pp* genotypes, no significant BMD change between ERα genotypes were present after 1-yr-HRT treatment [0.625 mg conjugated equine estrogens (CEE) plus 2.5-5 mg medroxyprogesterone acetate [MPA]/daily) (49). Also, *Xba*I variants were not associated spinal BMD with and without HRT treatment (49). Another Japanese study recently reported that response to HRT (0.625 mg CEE or 2 mg transdermal estradiol) in postmenopausal period were greater in *pp* genotype than in women with other *Pvu*II genotypes only within first 6 HRT months (97). The significant difference in the BMD gain observed at 6 months, was not confirmed after 12 months of HRT. In addition, there were no significant difference related to *Xba*I genotypes (97).

In a study based on different Asian genetic background (Thai women), Ongphiphadhanakul et al. (98) reported that women on 0.3 mg CEE with *P* allele (*PP* and *Pp* genotypes) had significantly higher increase in lumbar spinal BMD compared to those without *P* allele (*pp* genotype) after 1-year treatment ($p<0.05$). No difference in the BMD change at femoral neck was found on 0.3 mg CEE treatment. Neither the changes in vertebral nor femoral BMD were different among subjects on 0.625 mg CEE with different *Pvu*II genotypes (98). These Thai data were consistent with a Caucasian elderly women study, which suggested a relationship between BMD changes after low estrogen dose replacement (0.3 mg CEE/day) and ERα genotypes (48). For 3.5 years period of HRT treatment, BMD changes were analyzed at the spine, femoral neck, distal radius and total body BMC. Where ERα genotypic effects were significant, *PP* (or *xx*) genotype was generally associated with larger decreases (or smaller increase) of bone mass, whereas *pp* (or *XX*) genotype was associated with smaller decreases (or larger increase) of bone mass on HRT treatment (48). Recently, Rapuri et al. (99) evaluated the influence of ERα intron 1 RFLPs in 79 postmenopausal women receiving HRT (0.625 mg CEE plus 2.5 mg MPA) for 3 years. The percent change in BMD was higher in women with ERα genotype, *XX* or *PP* compared to women with ERα genotype *xx* or *pp* but was significant only for total body of *Pvu*II genotype *PP*. Collectively the above data suggest that ERα *pp* genotype is a relatively *estrogen-insensible* genotype, and that women with *P* allele (*Pp* and *PP* genotypes) benefit more from the protective effect of HRT on fracture risk than women with *pp* genotype, though long-term HRT seemed to eliminated the ERα genotype related differences in the BMD (100, 101).

Regarding other estrogen-sensible tissues, Herrington and colleagues (102, 103) studied ERα RFLP effects in women affected by coronary artery disease. As major results, they reported statistical associations between clinical parameters (i.e. response of E-selectin and of HDL-cholesterol to HRT) and women with *PP* genotype than the opposite *pp* genotype (102, 103). *PP* genotype had greater increase in the HDL-cholesterol (and also decrease in the E-selectin) level by HRT (0.625 mg CEE/day) than level changes observed in other *Pvu*II genotypes. Similar patterns of response were observed for *Xba*I polymorphism (102, 103).

The functional significance of *Pvu*II-*Xba*I polymorphisms, however, is not clear: *Pvu*II *P* allele disrupts a potential recognition site for the transcriptional factor AP4 which recognizes CAGCTG sequence. Three studies recently detected enhancer activities in the ERα intron 1. These enhancer activities differed among *Pvu*II-*Xba*I RFLPs: P allele confers higher transcriptional activity than *p* allele in gene reporter constructs (102, 104, 105).

Finally, all the above studies based on different ethnic populations (Asians and Caucasians) suggested that ERα *P* allele confers relatively estrogen-resistant than the opposite *p* allele using low estrogen dose (equivalent to 0.3 mg CEE/day). Using higher estrogen dose, these *Pvu*II genotype effect seems to disappear at least in skeleton system while in cardiovascular systems, a different genetic response regarding ERα intron 1 polymorphisms could persist even using higher estrogen doses than 0.3 mg CEE.

As shown in figure 5A, because estrogen-sensible *Pvu*II *P* allele is more represented in Caucasians than Asians, it supports the clinical feeling of an higher Caucasian responsiveness to estrogen treatments than Asians (40). On the other hand, the relatively estrogen-resistance of Asian background may be a selection force consequence: because Asians meanly present higher exposure to environmental estrogens, such as soy dietary phytoestrogens, the selection of more estrogen-resistant genotype (i.e. ERα *pp* genotype) could represent a defensive mechanism against environment (106-108). ERα *Xba*I polymorphism also presents different genotype distribution in human populations with *x* allele more represented in Asians than Caucasians (Figure 5B). This does not surprise because the strong and highly significant linkage disequilibrium between *Pvu*II and *Xba*I sites as expected for two sites separated by 45 *bp* (40, 93). Generally, *P* and *X* alleles, as well as *p* and *x* alleles were strongly associated with each other although combined *Pvu*II-*Xba*I genotype frequencies vary regard to different ethnics (Figure 5C). The haplotype *pX* was not observed in the majority of studies, were haplotype *Px* was detected even though at low frequency suggesting the disequilibrium is not complete and either recombination or multiple mutations have occurred between or at these two polymorphic restriction sites (40).

ERα *microsatellite* $(TA)_n$ repeats proposed as strong risk marker of postmenopausal osteoporosis, may be also involved in the estrogen-sensibility. Indeed, for clinical data and for their position in promoter area, $(TA)_n$ repeat polymorphisms could be functional modifying ERα expression and/or regulation (93). In addiction, Yim et al. (109) recently reported the percent change of lumbar spine BMD after 1-yr HRT (0.625 mg CEE with/without 2.5 mg MPA) significantly decreased ($r = –0.131$; $p = 0.035$) with an increase in the mean number of $(TA)_n$ repeats.

As represented in figure 6, similar $(TA)_n$ repeat frequencies were reported in Asian and Caucasian studies with compara-

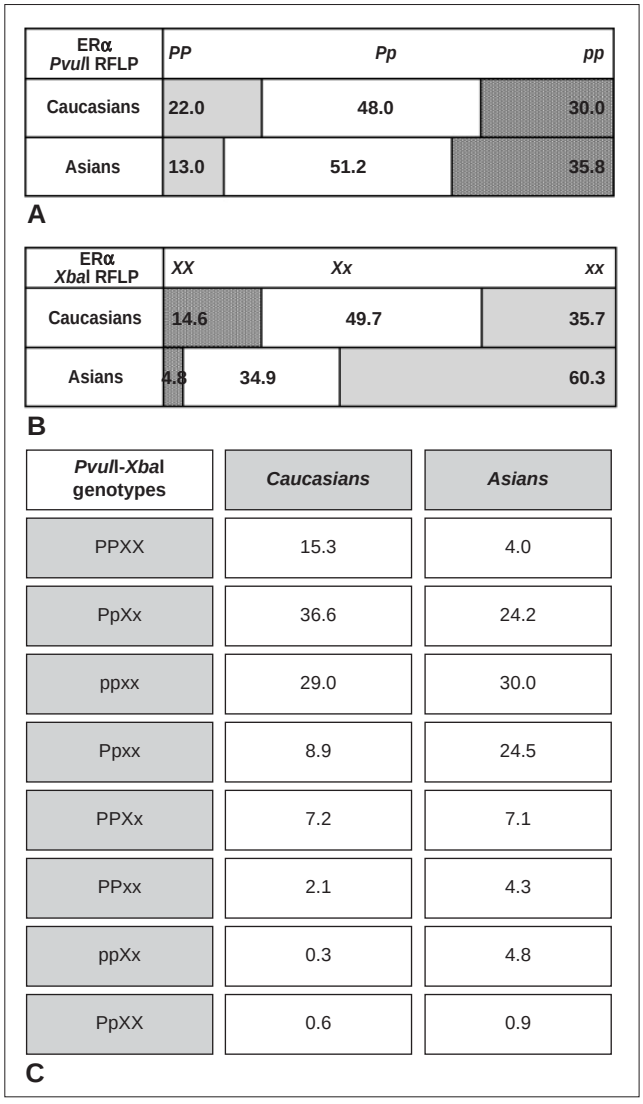

Figure 5 - Ethnic frequencies of ERα genotypes detected by *Pvu*I (A) and *Xba*I (B) endonucleases, and of the 8 most frequent *Pvu*I-*Xba*I genotypes (C), respectively. Data modified from ref. 40.

tive bimodal distributions peaked around 12-16 and 22-24 $(TA)_n$ repeats (93, 94, 98, 110-114). In contrast to ERα intron 1 RFLPs, $(TA)_n$ repeat frequencies do not apparently differ in Asians vs. Caucasians suggesting a lesser involvement in the specific ethnic pharmaco-sensitivity. However, more studies are needed to confirm or refuse this issue.

Clinically detected effects of ERα polymorphisms (i.e. *Pvu*I-*Xba*I RFLP and $(TA)_n$ repeats) are likely dependent, at least in part, to linkage disequilibrium each other and with other functional nucleotide changes in the nearby exons of ERα gene or its 5' regulatory sequence. This could be major confounding factor in the ERα polymorphism studies. In this view, a synonymous nucleotide substitution from *T* to *C* at codon 10 of ERα exon 1, was in linkage disequilibrium with intron 1 *Pvu*I RFLPs (115). The C262 allele appeared in linkage with *P* allele of the intronic polymorphism. After treating 96 post-menopausal women with 0.3-0.625 mg CEE for 2 years, vertebral BMD increased regardless of the T262C genotype. However, with regard to femoral neck BMD, only those subjects that were homozygous for T262C polymorphism had an increase in femoral BMD (115).

T262C polymorphism at the ERα locus, may represent another level of genetic modulation of estrogen responsiveness. However, it is unclear how the synonymous nucleotide change could influence the ERα function. One of the possibilities is that T262C polymorphism may affect an alternative translation initiation site. Generally the ATG codon with appropriate context nearest the 5'end of the mRNA serves as the initiation codon (116) and a polymorphism of nucleotide sequence around the initiation codon influences the surface levels of cell adhesion receptors (117). Occasional escape from this first-ATG rule occurs. The ERα T262C polymorphism is located 29 nucleotides downstream from the putative translation site in the vicinity of another ATG codon around which the context GCATC[T/C]GGGATGG may be appropriate for it to serve as another translation initiation site. So, T262C variants may influence the favorableness of its being an alternative start codon (115). More studies regarding this issue are needed.

In conclusion, none of ERα allelic variations could completely value the estrogen pharmacogenetics or heredity of complex trait estrogen dependent such as BMD. As suggest Haussler's group data on VDR variants (62), only simultaneous analysis of all alleles (i.e. *Pvu*I-*Xba*I RFLPs, T262C, $(TA)_n$ repeats and others) present in ERα locus may offer an understanding of phenotypic heredity regarding the ERα function.

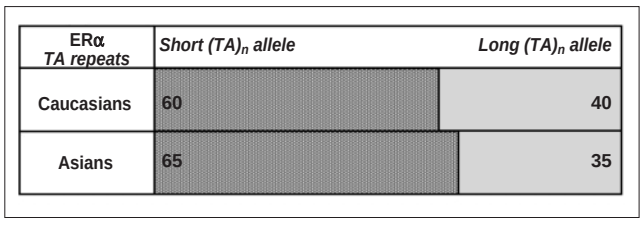

Figure 6. Ethnic distribution of ERα $(TA)_n$ repeat polymorphism located in the human ERα promoter area. Allele cutoffs: *short* $(TA)_n$ repeat allele (<TA_{18} repeats) and *long* $(TA)_n$ repeat allele (≥TA_{18} repeats). Data modified from ref. 40.

Conclusion

It is well recognized that different patient respond in different ways to the same medication. These differences are often greater among members of a population than they are within the same person at different times (or between monozygotic twins) (118). The existence of large population differences with small intrapatient variability is consistent with inheritance as determinant of drug response; it is estimated that genetics can account for 20 to 95% of variability in drug disposition and effects (119). Although many nongenetic factors influence the effects of medications, including age, organ function, concomitant therapy, drug interactions, and the nature of the disease, there are now numerous examples of cases in which interindividual differences in drug response are due to sequence variants in genes encoding drug-metabolizing enzymes, drug transporters or drug targets (120). Unlike other factors influencing drug response, inherited determinants generally remain stable throughout a person's lifetime.

Clinical observations of inherited differences in drug effects were first documented in the 1950s (121, 122), giving rise to the field of pharmacogenetics, and later pharmacogenomics. Although the two terms are synonymous for all practical purposes, pharmacogenomics uses genome-wide approaches to elucidate the inherited basis of differences between persons in the response to drugs.

The potential implication of pharmacogenomics in clinical research and clinical medicine is that disease could be treated

according to genetic and specific individual markers, selecting medications and dosages that are optimized for individual patients ("the right drug into the right patient"). The possibility of defining patient populations genetically may improve outcomes by predicting individual responses to drugs, and could improve therapy safety and efficacy. This personalizing of medicines has been the holy grail of pharmacogenomics since sequencing the human genome was conceptualized.

The application of genomic technologies, such as gene sequencing, statistical genetics and gene expression analysis to drug development, holds great promise for the future of medicine. Unfortunately, our ability to identify patients at risk for disease, stratify patients by clinical outcome and treatment response or predict adverse event occurrences is, in reality, several years away.

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