The genetics of response to estrogen treatment

Bente L. Langdahl

Department of Endocrinology and Metabolism Aarhus University Hospital, Aarhus, Denmark

Address for correspondence:
Bente L. Langdahl MD, PhD, DMSc
Department of Endocrinology and Metabolism
Aarhus University Hospital
Tage-Hansens gade 2
DK-8000 Aarhus C
Denmark
Ph. +45 8949 7678
Fax +45 8949 7659
E-mail: bentlang@rm.dk

Summary

It has been demonstrated that the response to estrogen treatment in postmenopausal women shows considerable variability. It has been speculated that this at least partly could be determined by heritable factors.
The most obvious genes to investigate in this context are the estrogen receptor genes. It has been demonstrated that women with short alleles of the TA-repeat polymorphism in the estrogen receptor α gene respond to hormone treatment with greater increases in bone mass at the lumbar spine. Also the two polymorphisms in the first intron of the same gene have been found to be associated with the response to estrogen. Several studies have found that women carrying the P- and the X-alleles respond to hormone therapy with greater increases in bone mass and sustain fewer fractures.
Polymorphisms in the collagen type Iα1 have been found to influence BMD. Conflicting results have been obtained with respect to the influence of these genetic variants on postmenopausal bone loss and response to hormone treatment.
Furthermore, two polymorphisms in the promoter of the transforming growth factor ß gene and one polymorphism in the first exon of the osteoprotegerin gene have been demonstrated to interact with the response to hormone treatment in early postmenopausal women.
The above mentioned results are obtained from relatively small studies and needs confirmation before the information can be used in the clinic.

KEY WORDS: estrogen, genetics, bone mass, osteoporosis.

Introduction

The differences in fracture risk between men and women are predominantly caused by the difference in bone size, men having bigger bones than women (1) and the abrupt withdrawal of estrogen at menopause in women. Estrogen deficiency, as seen in postmenopausal women, is associated with increased rate of bone loss and increased risk of osteoporotic fractures. Treatment with estrogen has therefore been used both as a prophylactic measure to prevent development of osteoporosis and as treatment of existing osteoporosis. Several studies have shown that estrogen treatment in postmenopausal women has profound effects both on bone mineral density (BMD) and fracture risk (2, 3). These studies have also shown that there is a considerable variability between women in the response to estrogen treatment. Since several other menopause and estrogen related phenotypes, including postmenopausal bone loss, have been demonstrated to be influenced by genetic factors (4-11), it has also been speculated that the response to hormone replacement therapy (HRT) could be partly determined by heritable factors.
From a scientific point of view investigating this further would be of interest, since knowledge about the potential influence of genetic variants on response to hormone replacement treatment could lead to a deeper understanding of the importance of the genes and the functional importance of the genetic variants. From the patients point of view knowledge about the influence of genetic variants would be desirable. If genetic variants interfere with proper response to treatment, the patient might want a different treatment or no treatment. Furthermore, if genetic variants also increase the risk of side effects, the balance between positive and negative effects might change significantly and the patient may choose not to be treated. In the following some examples of interaction between response to HRT and genetic variants will be mentioned.

Hormones and their receptors

Estrogen receptors (ER) and aromatases

In the promoter of the *ER-α* gene a TA repeat polymorphism has been demonstrated (rs3138774) (12). The polymorphism is located 1174 base pairs upstream from the first exon. Short TA repeat alleles have been found to be associated with reduced BMD and a BMD-independent predictor of osteoporotic fractures (13, 14). In a study comprising 284 postmenopausal Korean women treated with equine estrogen alone or in combination with medroxypregesterone for one year, Yim et al. found that women with short TA repeat alleles responded with greater increases in BMD at the lumbar spine but not at the femoral neck (15). Furthermore, women that lost bone mineral density, despite being compliant with the hormone replacement therapy, had significantly longer TA repeat alleles.
In the first intron 2 polymorphisms have been demonstrated, T to C 397 base pairs upstream from exon 2 identified by RFLP assay using *PvuII* (rs2234693) (16) and A to G 351 base pairs upstream from exon 2, identified by RFLP using *XbaI* (rs9340799) (17).These two polymorphisms are in strong linkage disequilibrium with each other, but also with the TA repeat polymorphism in the promoter. These polymorphisms have been examined separately and in combination and have in some studies been demonstrated to be associated with bone mass in men and women (14, 18, 19). However, the largest metaanalysis to date comprising 18,917 women and men, found no effect of the polymorphisms on BMD, but women car-

rying the XX genotype had reduced risk of fractures in general and vertebral fractures in particular (20).
Several studies have found associations between these polymorphisms and postmenopausal bone loss, however, not always with the same genotype (19, 21-25). The possible influence of the *PvuII* and *XbaI* polymorphisms on changes in bone mass during hormone replacement therapy in postmenopausal women has been examined in several studies. In an American study comprising 79 women treated with hormone replacement therapy for 3 years, Rapuri et al. found that women with the PP and the XX genotypes responded with higher increases in BMD at the lumbar spine, the femoral neck and total body, however, the increase was only significantly different at the total body for women with the XX genotype (24). In a study comprising 124 Thai women, it was demonstrated that after one year of treatment with low dose equine estrogen, women carrying the P allele responded with greater increase in BMD at the lumbar spine, compared with women with the PP genotype. No genotype dependent differences were found in women treated with higher doses or at the hip for any dose (26). Several other investigators found no association between these genotypes and bone mass response to hormone replacement therapy (21, 22, 25, 27-31). In a Finnish study, comprising 151 early postmenopausal women who were treated with estradiol for 5 years, Salmen et al. found that women carrying the P-allele sustained significantly fewer fractures compared with women with the pp genotype (32). Weel et al. have found that *PvuII* alleles are associated with early natural menopause and the risk of hysterectomy due to metrorraghia and uterus myomatosis (33).
A tetranucleotide repeat polymorphism has been demonstrated in intron 4 of the aromatase CYP-19 gene. The effect of this polymorphism on BMD has been examined in several studies. Some studies found that few repeats were associated with reduced bone mass and increased risk of osteoporotic fractures (34, 35). In a prospective study, the Danish Osteoporosis Prevention Study (DOPS) comprising approximately 1800 early postmenopausal women of whom 440 received continuous treatment with estradiol for 5 years, Tofteng et al. found that response to hormone replacement therapy revealed an allele dose effect of the long allele on BMD at the lumbar spine, the hip and the forearm (36).

Vitamin D receptor (VDR)

The most studied genetic variants in the field of bone mass and osteoporotic fractures are located in the 3' end of the VDR. They comprise two variants in intron 8: G to A (*BsmI*) (rs1544410) and G to T (ApaI) (rs17879735), T^{1055}-C in exon 9 (TaqI) (rs17880009) and a poly-A microsatelite along with many other SNPs further down the 3'UTR of the gene (37-40). The b, a and T alleles are in strong linkage disequilibrium forming a baT haplotype. This baT haplotype is also strongly coupled to the long polyA repeat sequence. Two meta-analyses from 1996 and 1999 found that the 3' polymorphisms were weakly associated with bone mass, but non-genetic factors and genetic heterogeneity may interfere with the detection of the effects (41, 42). The largest meta-analysis comprising 26,242 women and men was performed using individual data and found no association between the $C^{2}T$ or the 3' polymorphisms and BMD or fracture risk, however, an association between BMD and the Cdx2 polymorphism (rs17883966) was found (43).
Kurabayashi et al. followed 82 postmenopausal women treated with equine estrogen for at least 3 years. Initially women with the TT genotype responded with significantly greater increases in lumbar spine BMD (27), however, after 3 years no difference between the two groups of women could be demonstrated (30). In a small American study comprising 54 women treated with low-dose equine estrogen for 3.5 years, Deng et al. found that women with the BB genotype responded better (22). Giguere et al. found no effect of the BsmI polymorphism on changes in QUS (quantitative ultrasound) during hormone replacement therapy (29). However, a subgroup of women with the bb genotype and the PP genotype of the *PvuII* polymorphisms in the *ER-*α gene had a more pronounced response to long-term hormone placement therapy than women with the other possible combinations of these two genotypes.

Collagen, matrix proteins and other genes that influence bone structure

Collagen type Iα*1 and methyltetrahydrofolate reductase (MTHFR)*

The variant T-allele of the Sp1 polymorphism has been found to be associated with reduced BMD and increased fracture risk (44-46). Meta-analyses of which the largest comprised 20,786 women and men have confirmed the association of the T-allele with reduced bone mass and increased risk of osteoporotic fractures (47-49). In a Scottish study comprising 239 early postmenopausal women treated with estradiol for 5-7 years, no difference in the effect of the treatment on BMD was found between women with different Sp1 genotypes (50). In a small Turkish study comprising 111 early postmenopausal women, women carrying the normal genotype responded better to 18 months of hormone replacement therapy (51). In the Danish Osteoporosis Prevention Study, the promoter polymorphism G^{-1997}-T (rs1107946) was associated with reduced bone mass, but none of the investigated polymorphisms affected the response to HRT (52).
A point mutation in the MTHFR gene, causing an alanine to valine substitution, the $C^{677}T$ mutation (rs1801133), gives rise to a thermolabile variant of MTHFR with reduced activity (53), resulting in elevated levels of circulating homocysteine. Elevated plasma levels of homocysteine interfere with the cross-linking of collagen, a process that is essential in the formation of the triplehelix-structure of collagen type I during bone formation (54). The effect of this polymorphism on BMD has been examined in several studies and most studies have found that the TT genotype is associated with reduced BMD (55, 56) and increased risk of osteoporotic fractures (56, 57). However, in a study comprising 473 early postmenopausal Danish women, the BMD response to 5 years of hormone replacement therapy was not influenced by this polymorphism (56).

Other genes

Transforming Growth Factor-β *(TGF-*β*), osteoprotegerin (OPG), LDL receptor-related peptide (LRP)-5 and -6 and Sclerostin (SOST)*

Several polymorphisms have been identified in the *TGF-*β*1* gene (58-61). Some of these polymorphisms have been found to be associated with bone mass or fracture risk: C^{-1348}-T (60, 62), T^{29}-C (63, 64) and $T^{861\text{-}20}$-C (60, 61). In a large-scale metaanalysis no effect of these polymorphisms on either BMD or risk of osteoporotic fractures could be demonstrated. Furthermore, no interaction between these polymorphisms and postmenopausal hormone replacement therapy was found (65). However, in the Danish Osteoporosis Prevention Study,

the C^{-1348}-T and T^{29}-C polymorphisms were associated with early postmenopausal bone loss and response to hormone replacement therapy (66).
Osteoprotegerin (OPG) is a decoy receptor for RANKL and a competitive inhibitor of osteoclast recruitment and activity (67). Several polymorphisms have been demonstrated in the *OPG* gene, some of these have been associated with changes in bone mass and fracture risk (68-71). In the Danish Osteoporosis Prevention Study, we found that two of the promoter polymorphisms; A^{163}-G and T^{245}-G were associated with perimenopausal bone mass, but not with postmenopausal bone loss or response to hormone replacement therapy. Another polymorphism: G^{1181}-C located in the first exon interacted significantly with the response to hormone replacement therapy over 5 and 10 years (72).
Several disease associated mutations (73, 74), but also polymorphisms have been identified in the LDL receptor-related peptide (LRP)-5 and -6. Two polymorphisms in the *LRP-5* gene: Val ^{667}-Met and Ala^{1330}-Val have consistently been associated with reduced bone mass and increased fracture risk (75). The effect on BMD was confirmed in the Danish Osteoporosis Prevention Study. However, no effect of the polymorphisms on early postmenopausal bone loss or response to hormone replacement therapy could be demonstrated (76).
The *SOST* gene encodes sclerostin that is produced by the osteocytes and is a strong inhibitor of bone formation. Loss-of-function mutations cause sclerosteosis (77) and van Buchem's disease (78). Common polymorphisms in the *SOST* gene are associated with changes in bone mass (79). These effects on bone mass were confirmed in perimenopausal Danish women. Furthermore, the SRP9 polymorphism was also associated with early postmenopausal bone loss (80).

Discussion and future aspects

The interests in unraveling the genetics behind the variable response to hormone replacement therapy with respect to changes in bone mass, reduction in fracture risk and increase in risk of breast cancer and cardiovascular disease are several. For the patient and the treating physician optimal treatment is the goal. Optimal treatment is a combination of many things, but first of all efficacy and minimal side effects. It would be helpful in choosing the right treatment for the individual patient if efficacy and side effects could, at least partly, be predicted by known genetic variants. For the scientist the interesting thing about the genetic background of response to hormone replacement therapy or other treatments is that it may provide information about the importance of a specific genes for bone health and about the functionality of the genetic variants.
Although some evidence suggests that the X allele of the *XbaI* polymorphism and the P allele of the *PvuII* polymorphism in the estrogen receptor a gene, the long TTTA repeat allele of the CYP19 gene along with polymorphisms in the TGF-β1, OPG and SOST genes influence the response to postmenopausal hormone replacement therapy, we are still far from achieving these goals. The vast majority of the studies on the interaction between hormone replacement therapy and genetic variants are performed in too small populations resulting in severely underpowered statistical analyses with high risk of overseeing a true association or reporting chance findings. Most studies have less than 100 participants and the largest studies have less than 500 participants on long term hormone replacement therapy. Even 500 participants do not provide statistical power to perform complex analyses of gene – gene or gene – environmental interactions. Furthermore, the primary aims of many of the studies were not to investigate the interaction between hormone replacement therapy and genetic variants and some of the studies therefore lack important information that could help understand the underlying mechanisms for example information about dietary intake, smoking and serum samples that would make it possible to perform additional analyses. A serious obstacle to change this is, that is has become impossible to conduct randomized trials on hormone replacement therapy with the knowledge we have on serious side effects to long-term estrogen treatment. There will always be women that choose hormone replacement treatment due to menopausal symptoms and it would therefore be possible to investigate the interaction between hormone replacement therapy and genetic variants in this group of women. However, women who choose estrogen treatment with all the information available about risk of breast cancer and cardiovascular disease are predominantly women with severe menopausal symptoms and we have to bear in mind that the severity of menopausal symptoms may be influenced by the same set of genetic variants as the ones we want to investigate or by other genetic variants or environmental factors. One way or the other this may lead to that any group of women, who have chosen hormone replacement therapy, constitutes a group of women with a specific genetic background and the results obtained may therefore not be applicable to the entire population of postmenopausal women. A better approach would therefore be for researchers who already have detailed information about hormone replacement therapy, bone phenotypes, covariates and genotypes from randomized study or high quality cohort studies to combine these data for further analyses.

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