Author’s response to reviews

Title: Associations between ERα/β Gene Polymorphisms and Osteoporosis Susceptibility and Bone Mineral Density in Postmenopausal Women: A systematic review and meta-analysis

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Author’s response to reviews:

Dear Prof. Stavros Liatis,

We appreciate very much your encouraging decision and reviewers’ constructive and positive comments for our original manuscript entitled 'Associations between ERα/β Gene Polymorphisms and Osteoporosis Susceptibility and Bone Mineral Density in Postmenopausal Women: A systematic review and meta-analysis'. Based on the reviewers’ comments, we have further revised our manuscript and made itemized responses to the comments as below. The changes in the revised manuscript are highlighted in red. We would like to re-submit this revised manuscript to BMC Endocrine Disorders, and hope that it is acceptable for publication in the journal. Please do not hesitate to contact us with additional questions or concerns.

Looking forward to hearing from you soon.

Below are our point-by-point responses to the reviewers’ comments.

Responses:

Reviewer #1 (Patricia Canto):
1. Although the majority of the articles report the ERα and ERβ polymorphisms using the nomenclature according to the restriction enzymes such (PvuII and XbaI for ERα, AluI and Rsal for ERβ), the authors should clarify in the introduction what is the nomenclature of these polymorphisms according to NCBI (National Center for Biotechnology Information); since, currently, the nomenclature used for polymorphisms should be according to the National Center for Biotechnology Information (refSNP number).

Answer: Thank you for your comments. We have added refSNP number of ERα and ERβ gene polymorphisms in our revised manuscript.

WANG et al showed that the ERα XbaI (rs9340799) polymorphism was associated with BMD at diverse skeletal sites, and ERα PvuII (rs2234693) PP genotype played a role in protecting the lumbar spine but on the other hand might be a risk factor for the femoral neck fracture. However, to the best of our knowledge, no meta-analysis has been performed to explore the relationships between ER gene [ERα XbaI (rs9340799), ERα PvuII (rs2234693) and ERα G2014A (rs2228480)] and ERβ gene [ERβ AluI (rs4986938) and ERβ Rsal (rs1256049)] polymorphisms and PMOP susceptibility and BMD of the lumbar spine and femoral neck in postmenopausal women. To address these issues, we performed a meta-analysis of all currently available studies relating ER gene [ERα XbaI (rs9340799), ERα PvuII (rs2234693) and ERα G2014A (rs2228480)] and ERβ gene [ERβ AluI (rs4986938) and ERβ Rsal (rs1256049)] polymorphisms with PMOP risk and BMD.

2. In inclusion criteria, the authors refer that were as follow: "ERα XbaI, ERα PvuII, ERα G2014A, Erβ AluI and ERβ Rsal" With this nomenclature might be not take into consideration, the articles that used the nomenclature of the National Center for Biotechnology Information (refSNP number).

Answer: Thank you for your comments. We have revised it.

'We searched PubMed, EMBASE, Web of Science, the Cochrane Library and China WeiPu Library to identify case-control studies that investigated the associations between ERα gene polymorphisms [ERα XbaI (rs9340799), ERα PvuII (rs2234693) and ERα G2014A (rs2228480)] ERβ gene polymorphisms [ERβ AluI (rs4986938) and ERβ Rsal (rs1256049)] and osteoporosis susceptibility and BMD in postmenopausal women by using the following search terms (‘PMOP’ OR ‘Postmenopausal osteoporosis’ OR ‘Postmenopausal’) AND (‘Estrogen Receptor’ OR ‘ER’) AND (‘polymorphism’ OR ‘single nucleotide polymorphism’ OR ‘SNP’ OR ‘variation’).’

'studies reporting alleles and genotypes of at least one of the ER gene polymorphisms in women with or without PMOP: ERα XbaI (rs9340799), ERα PvuII (rs2234693), ERα G2014A (rs2228480), ERβ AluI (rs4986938) and ERβ Rsal (rs1256049)'

3. How the authors take into consideration the several confounding factors for osteoporosis (like age, years since menopause, estrogen therapy, etc) reported in the articles analyzed?

Answer: Thank you for your comments. We are totally agree with you. Indeed, there are several confounding factors for osteoporosis other than gene polymorphisms. We had planned to explore
the interactions between ER gene polymorphisms and these confounding factors (like age, years since menopause, estrogen therapy, etc) in PMOP patients in our meta-analysis; however, no available data could be used in all the recruited studies. PMOP is a disease, whose etiology might be involved in several confounding factors, and all these confounding factors might interact with each other and play a key role in etiology and progression of PMOP. Therefore, we should take all these confounding factors into consideration in our study rather than studying them separately, which is a limitation of our meta-analysis. We have discussed it in our Limitation Section.

'Second, PMOP is a disease whose etiology might be involved in several confounding factors, and other confounding factors such as age, years since menopause and estrogen therapy might interact with each other and play a key role in the etiology and progression of PMOP. However, no data available could be used in all recruited studies to detect the interactions between these confounding factors in PMOP patients. We should take all these confounding factors into consideration in our study rather than studying them separately, which is also a limitation of our meta-analysis.'

4. The authors take into consideration the differences of the frequencies of the alleles of the different polymorphisms of the both ethnic groups (Caucasian and Asian), since the frequencies of the alleles are different in both ethnic groups (HapMap).

Answer: Thank you for your comments. We are totally agree with you.

PMOP is a disease with multiple related risk factors. Heredity and gene polymorphisms have been found to be significantly associated with etiology and progression of PMOP. It has been verified that gene polymorphisms play different roles in many diseases such as idiopathic scoliosis, diabetes and et al. in various populations, which has also been observed in PMOP patients. In Shang et al.'s study, ERα PvuII and XbaI might be associated with PMOP, and could be used as markers to screen those with high risks to postmenopausal OP in Chinese women; however, Harslof et al. did not find any evidence of interaction between polymorphisms and PMOP risk in Danish populations. Therefore, we performed a sub-group analysis to detect the associations between gene polymorphisms and PMOP in various ethnicities to explore whether ethnicity play an important role in etiology of PMOP.

Overall, we did not find any significant association between ERα XbaI and ERα PvuII polymorphism and risk of PMOP in either overall, Caucasian or Asian populations. ERα G2014A polymorphism played a protective role in developing PMOP in Caucasian populations; while no significant association was observed in overall and Asian populations. With regard to ERβ polymorphism, ERβ AluI was significantly associated with risk of developing PMOP in Asian postmenopausal populations; however, we did not observe any significant association between ERβ AluI and PMOP risk in overall and Caucasian populations. Furthermore, we also found that there was remarkable relationship between ERβ Rsal polymorphism and decreased PMOP risk in overall and Asian populations.
All these results of our subgroup analysis demonstrated the different role of gene polymorphisms in various ethnicities, suggesting that we should consider the difference between various ethnicities when we treat PMOP patients.

5. The authors take into consideration the Hardy-Weinberg equilibrium for the polymorphisms reported in the articles analyzed.

Answer: Thank you for your comments. We have added the Hardy-Weinberg equilibrium for the polymorphisms in Methods and Results Section in our revised manuscript.

1) In the study of associations between ER gene polymorphisms and PMOP risk, the following data were collected: author list, year of publication, ethnicity, sample size, alleles, genotype of each gene polymorphism and Hardy-Weinberg equilibrium (HWE).

2) HWE was calculated in the control population to evaluate the quality of the data by using chisquare test.

3) In addition, all these studies had complied with HWE.

4) Table 1

6. The authors take into consideration the power of the different studies analyzed.

Answer: Thank you for your comments. We have added the power of the different studies in our revised manuscript.

1) Power analysis was performed using the Power and Precision V4 software (Biostat Inc, Englewood, USA).

2) Power analysis

Before initiation of the meta-analysis, a power analysis was conducted by using the Power and Precision V4 software to verify whether the included studies could offer adequate power (>80%). The result showed that the statistical power in our study was sufficient to detect the associations between ER gene polymorphisms and PMOP risk.

7. The redaction of the results is confusing, perhaps they should reduce the writing and put only the most important, since in the tables are all the information.

Answer: Thank you for your comments. We have revised it.

Associations between ER gene polymorphisms and PMOP risk

Overall, we did not find any significant association between ERα XbaI and ERα PvuII polymorphisms and risk of PMOP in either overall, Caucasian or Asian populations (all P>0.05) (Table 2). ERα G2014A polymorphism played a protective role in developing PMOP in
Caucasian populations, while no significant association was observed in overall and Asian populations (both P>0.05). All the data are shown in Table 2 and Figure 2.

With regard to ERβ polymorphism, ERβ AluI was significantly associated with the risk of developing PMOP in Asian postmenopausal women under the recessive model; however, we did not observe any significant association between ERβ AluI and PMOP risk in overall and Caucasian populations (both P>0.05) (Table 2 and Figure 3). Furthermore, we also found that there was a remarkable association between ERβ RsaI polymorphism and decreased PMOP risk in overall and Asian populations (Table 2).

Associations between ER gene polymorphisms and BMD in PMOP women

ERα XbaI and lumbar spine bone mineral density (BMD g/cm² and BMD Z value)

In our meta-analysis, no significant difference in lumbar spine BMD (g/cm²) was observed between PMOP women with ERα XbaI XX, ERα XbaI Xx and ERα XbaI xx genotype in either overall, Caucasian or Asian populations (all P>0.05) (Table 5). The lumbar spine BMD Z value in Caucasian PMOP women carrying ERα XbaI XX genotype was greater than that in those carrying xx genotype, while no significant difference was observed in overall and Asian populations (both P>0.05). ERα XbaI Xx genotype was found to be significantly associated with high lumbar spine BMD Z value in either overall or Caucasian populations but not in Asian populations.

ERα XbaI and femoral neck bone mineral density (BMD g/cm² and BMD Z value). Our pooled analyses indicated that the ERα XbaI XX genotype was significantly associated with increased femoral neck BMD in overall and Caucasian populations. In contrast, ERα XbaI XX genotype did not play a key role in femoral neck BMD in Asian populations (Table 5 and Figure 4). Interestingly, compared with PMOP women with xx genotype, XX genotype was significantly associated with decreased femoral neck Z value in Caucasians, and increased femoral neck Z value in Asians (Table 5). However, no significant association was observed between XX genotype and the femoral neck Z value in overall populations. In addition, Caucasians and Asians carrying the ERα XbaI Xx genotype were at risk of a high femoral neck Z value, while no significant association was found in overall populations. We did not observe remarkable relationships between ERα XbaI Xx genotype and femoral neck BMD in either overall, Caucasian or Asian populations (all P>0.05). All data are shown in Table 5.

ERα PvuII and lumbar spine bone mineral density (BMD g/cm² and BMD Z value)

With regard to ERα PvuII, the difference in the lumbar spine Z value between the PP and pp genotypes was -0.07 (95% CI=-0.03 to -0.01, P=0.031) in Caucasian PMOP women; however, no significant difference was observed in overall and Asian populations. For the Pp versus pp genotype, the difference in lumbar spine BMD was -0.01 (95% CI=-0.02 to -0.00, P=0.036) in overall populations, and the difference in the lumbar spine Z value was -0.16 (95% CI=-0.20 to -0.12, P<0.001) in Caucasian populations; however, we did not find any significant difference in lumbar spine BMD in either Caucasians or Asians, and in the lumbar spine Z value in overall and Asian populations (Table 5 and Figure 5). In addition, no significant difference in lumbar spine BMD was observed between PP and pp genotypes (P>0.05) (Table 5).
ERα PvuII and femoral neck bone mineral density (BMD g/cm2 and BMD Z value)

We further found that the ERα PvuII PP genotype was associated with decreased femoral neck BMD and Z value compared with the pp genotype in Asians, while no significant difference in femoral neck BMD and Z value was observed in either overall and Caucasian populations (both P>0.05) (Table 5). Furthermore, PMOP women carrying the Pp genotype were at risk of a low femoral neck Z value, which was found in overall, Caucasian and Asian populations. Our study showed that there was no significant difference in femoral neck BMD between PMOP women with the Pp genotype and those with the pp genotype (P>0.05). All the data are shown in Table 5.

8. It is unclear whether the authors together analyzed studies reporting BMD as a Z-value with those reporting BMD in g/cm2.

Answer: Thank you for your comments. We apologize for confusing you about this. We described the results of associations between BMD (BMD g/cm2 and BMD Z value) and ER gene polymorphisms separately, which was also reported separately in original studies recruited in our meta-analysis. We have added subtitle in Methods Section and rewritten it in our revised manuscript.

9. It is too long, and it does not focus on something new of the mechanisms involved in these receptors in osteoporosis.

Answer: Thank you for your comments. We have rewritten the Discussion Section in our revised manuscript, and added something new of the mechanisms involved in these receptors in osteoporosis.

Reviewer #2 (Torben Harslof):

1. First sentence is not true. Postmenopausal women may very well have secondary or glucocorticoid-induced osteoporosis.

Answer: Thank you for your comments. We have revised it.

Postmenopausal osteoporosis (PMOP) is a common metabolic bone disorder characterized by low bone mineral density (BMD) and increased fracture risks.

2. Introduction line 5. "It was estimated that 10 million individuals older than 50 years-old suffered from osteoporosis." Where??

Answer: Thank you for your comments. We have revised it.

It is estimated that there are approximately 2400 million people with osteoporosis in the United States alone, with about 1.5 million osteoporotic fractures each year.
3. The introduction is too long and bears too much resemblance to a review of osteoporosis pathophysiology and discusses previous finding too thoroughly.

Answer: Thank you for your comments. We have rewritten the Introduction Section.

Postmenopausal osteoporosis (PMOP) is a common metabolic bone disorder characterized by low bone mineral density (BMD) and increased fracture risks [1-3]. It is estimated that there are approximately 2400 million people with osteoporosis in the United States alone, with about 1.5 million osteoporotic fractures each year [3, 4].

The pathophysiology of PMOP is considered as a disorder or negative imbalance of bone metabolism and remodeling, with bone resorption outpacing bone formation [3], suggesting that vitamin D and parathyroid hormone (PTH) and other factors related to bone resorption and formation may play a key role in the underlying mechanism and pathophysiology of PMOP [5-8]. Furthermore, genetic factors including genes and gene polymorphisms may also play an important role in the development of PMOP [9].

Estrogen is another important hormone that plays an important role in the pathogenesis of PMOP, knowing that reduced ovarian production of estrogen after menopause is a cause for the initial phase of rapid bone loss and osteoporosis in women [3]. Estrogen is known as an important regulator of bone metabolism, and estrogen deficiency is believed to be the cause of BMD loss, increased mechanical loading-induced bone remodeling, and the development of PMOP [10]. Knowing that the action of estrogen is predominantly mediated by estrogen receptor (ER), including ERα and ERβ by binding to different ligands to mediate various biological effects [3, 10], more attention has been paid to the relationship between ERs and PMOP risk and BMD in postmenopausal women [4, 11-37]. However, the results of studies currently available about this issue are controversial.

Previous meta-analyses have been performed to assess the pooled effects of ER gene polymorphisms on BMD and fracture risk [38-40]. WANG et al [38] showed that the ERα XbaI (rs9340799) polymorphism was associated with BMD at diverse skeletal sites, and ERα PvulI (rs2234693) PP genotype played a role in protecting the lumbar spine but on the other hand might be a risk factor for the femoral neck fracture. However, to the best of our knowledge, no meta-analysis has been performed to explore the relationships between ER gene [ERα XbaI (rs9340799), ERα PvulI (rs2234693) and ERα G2014A (rs2228480)] and ERβ gene [ERβ AluI (rs4986938) and ERβ RsaI (rs1256049)] polymorphisms and PMOP susceptibility and BMD of the lumbar spine and femoral neck in postmenopausal women. To address these issues, we performed a meta-analysis of all currently available studies relating ER gene [ERα XbaI (rs9340799), ERα PvulI (rs2234693) and ERα G2014A (rs2228480)] and ERβ gene [ERβ AluI (rs4986938) and ERβ RsaI (rs1256049)] polymorphisms with PMOP risk and BMD.

4. Page 7, line 14: "Although there existed meta-analyses that explored the relationships between ER gene polymorphisms and BMD or fracture risk, none of these studies were performed to discuss these issues in postmenopausal women. In addition, to the best of knowledge, no meta-analysis has been conducted to determinate the associations between
ER gene polymorphisms and PMOP susceptibility in postmenopausal women." The sentence is unclear. Please rephrase.

Answer: Thank you for your comments. We have revised it.

However, to the best of our knowledge, no meta-analysis has been performed to explore the relationships between ER gene [ERα XbaI (rs9340799), ERα PvuII (rs2234693) and ERα G2014A (rs2228480)] and ERβ gene [ERβ AluI (rs4986938) and ERβ RsaI (rs1256049)] polymorphisms and PMOP susceptibility and BMD of the lumbar spine and femoral neck in postmenopausal women. To address these issues, we performed a meta-analysis of all currently available studies relating ER gene [ERα XbaI (rs9340799), ERα PvuII (rs2234693) and ERα G2014A (rs2228480)] and ERβ gene [ERβ AluI (rs4986938) and ERβ RsaI (rs1256049)] polymorphisms with PMOP risk and BMD.

5. Page 10, line 4: "We calculated odds ratios (OR) and 95 % confidence interval (CI) to evaluate the association between ER gene polymorphisms and PMOP risk." Please state the applied definition of PMOP.

Answer: Thank you for your comments. We have added the applied definition of PMOP in Inclusion and exclusion criteria Section.

1) Definition: osteoporosis occurred in postmenopausal women due to the estrogen deficiency and is characterized by low BMD and increased fracture risks

2) We calculated odds ratios (OR) and 95 % confidence interval (CI) to evaluate the association between ER gene polymorphisms and PMOP risk (osteoporosis occurred in postmenopausal women due to estrogen deficiency as represented by low BMD and increased fracture risks).

6. Fig. 1. The text in the two upper boxes concerns VDR-polymorphisms??

Answer: Thank you for your comments. We have revised it.

7. The language needs a general revision by someone fluent in English.

Answer: Thank you for your comments. Our revised manuscript has been polished by a native English speaker.

8. The discussion is way too long and extends 10 pages. This should at least be halved. E.g. ref 14 is discussed both on the first and third page of the discussion (for the same polymorphism XbaI). In addition it is pointless to discuss all studies included in a meta-analysis. Some of the studies have to differ from each other and the conclusion of the meta-analysis. Otherwise there would be no point in making the meta-analysis. Therefore the discussion should be of a more general character.
Answer: Thank you for your comments. We have rewritten the Discussion Section in our revised manuscript.