

# The Association of Bone Mineral Density and G2014A Polymorphism in the Estrogen Receptor Alpha Gene in Osteoporotic Hip Fracture in Thai Population

Wiwat Wajanavisit MD\*,  
Sakda Suppachokmongkorn MD\*, Patarawan Woratanarat MD, PhD\*,  
Boonsong Ongphiphadhanakul MD\*\*, Tulyapruerk Tawonsawatruk MD, PhD\*

\* Department of Orthopedic, Faculty of Medicine, Ramathibodi Hospital Mahidol University, Bangkok, Thailand

\*\* Department of Medicine, Faculty of Medicine, Ramathibodi Hospital Mahidol University, Bangkok, Thailand

---

**Objective:** This study aimed to determine the association of a recent identified G2014A single nucleotide polymorphism (SNP) genotype distribution in exon 8 of the estrogen receptor in postmenopausal Thai women.

**Material and Method:** A prospective study was conducted at Ramathibodi Hospital between July 2005 and July 2006. Postmenopausal Thai women, aged more than 55 years and had sustained osteoporotic hip fracture, were included. Exclusion criteria were renal and metabolic bone diseases. Age, body mass index (BMI), blood tests for metabolic bone disease, and G2014A SNP genotype, bone mineral density (BMD) were collected. The relationship between the degree of osteoporosis (normal, osteopenia, and osteoporosis) and SNP genotype was analyzed by Fisher's exact test.

**Results:** Sixty-five postmenopausal women with osteoporosis were included. The average age was  $76.2 \pm 10.9$  years old, and the average BMI was  $21.3 \pm 3.5$  kg/m<sup>2</sup>. The data expressing the genotype distribution of gene G2014A SNP were G/G 23.1%, G/A 29.2% and A/A 47.7%. There was no statistical difference between age and BMI in each genotype. Gene G2014A was associated with osteoporosis of lumbar spine, femoral neck, ward triangle, and femoral neck.

**Conclusion:** It could be concluded that a G2014A SNP genotype in exon 8 of the estrogen receptor was associated with postmenopausal women who had osteoporotic hip fracture.

**Keywords:** Osteoporosis, Hip fracture, Estrogen receptor, G2014A, Thai

**J Med Assoc Thai 2015; 98 (Suppl. 8): S82-S87**

**Full text. e-Journal:** <http://www.jmatonline.com>

---

Osteoporotic hip fracture is one of catastrophic incidences in elder population and accounts for significant mobility and mortality<sup>(1)</sup>. It has been reported that the estimated cumulative incidence of hip fracture in Thai population is 181 per 100,000 populations<sup>(2)</sup> and the burden of the disease has become a major concern for healthcare system in Thailand. There are severe risk factors of the osteoporotic fracture including smoking, taking steroid, caffeine consumption or a metabolic condition, etc<sup>(3)</sup>. One of those is a genetic factor. The first evidence of heritability of osteoporosis was from the twin and family studies demonstrated by Bone Mineral Density (BMD)<sup>(4,5)</sup>. However, there was no report on the specific

gene involvement. Over the last decade, several candidate genes, which might associate with osteoporosis, were identified involving collagen type 1, alpha 1 (COL1A1), estrogen receptor alpha (ER-alpha), lipoprotein receptor related protein 5 (LRP5), bone morphogenesis proteins (BMPs), RUNX2 and sclerostin (SOST)<sup>(6)</sup>.

In Thailand, it was found that the G2014A polymorphism in the estrogen receptor alpha (ER-alpha) gene was related to the presence of osteoporosis<sup>(7)</sup> and it was also demonstrated as the tool for prediction of osteoporosis<sup>(8)</sup>. However, this polymorphism has not been studied in a specific group of osteoporotic hip fracture, which is an adverse consequence of osteoporosis condition. Thus, the purposes of this study are to demonstrate the genotype of the G2014A polymorphism in the estrogen receptor alpha (ER-alpha) gene and to determine the association between BMD and this polymorphism in Thai patients who had been suffering from osteoporotic hip fracture.

---

**Correspondence to:**

Tawonsawatruk T, Department of Orthopedics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

Phone & Fax: +66-2-2011589

E-mail: [Tulyapruerk@gmail.com](mailto:Tulyapruerk@gmail.com)

## Material and Method

Sixty-six postmenopausal women who were at least 55 years old with osteoporotic hip fracture were enrolled in this study. The subjects underwent hip surgery during July 2005-July 2006 at the Department of Orthopedics, Ramathibodi Hospital, Bangkok, Thailand. Prior to the recruitment, the patients were informed, and the consent was obtained. This study was approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. The subjects who had secondary causes of osteoporosis including glucocorticoid excess, hyperthyroidism, hyperparathyroidism and a metastasis bone tumor were excluded. In addition, all were non-smokers, non-alcoholic consumers and did not take estrogen or other antiresorptive agents. Before conducting the study, signed informed consent was submitted. The baseline data of the patients were reviewed and blood tests were taken for evaluation of possible causes of secondary osteoporosis including CBC, electrolytes, creatinine, calcium, TSH and free T4. 10 ml peripheral blood sample, which was collected from the patients using ethylenediaminetetraacetic acid as an anticoagulant and processed for SNP analysis. Genomic DNA of the patients was extracted from peripheral leukocytes. The technique used in this study was reported in a previous study by Ongphiphadhanakul et al<sup>(7)</sup>. In brief, DNA segment containing the G2014A SNP site was amplified by polymerase chain reaction (PCR) with the following primers: forward, 5'-GACGGACCAAAGCCACTTGG; reverse, 5'-CGTG TGGGAGCCAGGGAGCT. The final reaction contained 0.5 mg DNA, 1 unit of Taq polymerase, 10 mM TrisHCl, pH 8.3, 50 mM KCl, 2 mM MgCl<sub>2</sub>. The reaction was run for 30 cycles with denaturation at 95°C for 30s, annealing at 67°C for 60s and extension at 72°C for 60s. The 124 base-pair (bp) DNA fragments were then subjected to digestion with BtgI restriction endonuclease. The genotypes were thus identified as AA, GA and GG. Bone mineral density (BMD) was measured by dual energy x-ray absorp-

tiometry (DXA; Lunar Expert XL, Lunar, Madison WI) in which calibration and quality control were done routinely according to the manufacturer's recommendation. BMD at the anteroposterior lumbar spine (L2-L4) and contralateral hip were measured in each subject. The subjects were classified: normal (BMD, T-score >1.0), osteopenia (BMD, T-score between 1.0 and -2.5) and osteoporosis (BMD, T-score <-2.5).

## Statistical analysis

The baseline characteristics were described as the mean for continuous data and percentage for categorical data. The difference in genotype distribution and BMD was assessed by Fisher's exact test. Analysis of variance (ANOVA) was used to determine the association of clinical parameters and genotype. All statistical analysis was performed by using the STATA 9.0 programme (STATA Corp, Texas, and USA). A *p*-value <0.05 was considered to be of statistical significance.

## Sample size estimation

Sample size was calculated by using Power and Sample Size Program (version 3.02, Vanderbilt) based on alpha error 0.05, power of the study 0.8, A2014 allele frequency in the normal group 0.15, A2014 allele frequency in the osteoporosis group 0.6, the sample size was 17 cases per group.

## Results

Sixty-five patients were included in the study. One patient was excluded due to renal insufficiency. The distribution of the genotype in G2014A SNP and the baseline characteristics of the patients are shown in Table 1. The percentages of AA, AG and GG were 47.4 (n = 31), 29.2 (n = 19) and 23.1 (n = 15), respectively. Calculated allele frequency for A was 73.97% and for G was 26.03%. There was no significant difference in the average age of patients and BMI among these three genotypes or between alleles.

There was significant difference in the BMD

**Table 1.** The distribution between genotypes and demographic data

Factors	AA (n = 31)	AG (n = 19)	GG (n = 15)	<i>p</i> -value
Age (year), mean (SD)	76.5±11.9	76.3±10.8	75.9±9.6	0.9863
Weight (kg), mean (SD)	46.9±8.9	49.5±11.3	52.4±8.0	0.1831
Height (cm), mean (SD)	150.4±8.4	150.0±5.7	154.3±6.7	0.1738
BMI (kg/m <sup>2</sup> ), mean (SD)	20.6±2.9	21.8±4.1	22.0±3.5	0.3310

from lumbar (average) and hip (femoral neck, ward and intertrochanteric area) of the patients with AA, AG and GG. The genotype was statistically associated with osteoporotic status of subjects (T-score >1.0 as normal, T-score between 1.0 and -2.5 as osteopenia and, T-score <-2.5 as osteoporosis) ( $p < 0.05$ ). The distribution of the genotype and the BMD in the particular area was demonstrated in Table 2. However, the majority of patients with osteoporotic hip fracture in this study presented with their BMD by T-score >-2.5, which could not be diagnosed with osteoporosis by using BMD only.

### Discussion

It has been reported that several genes are associated with BMD and osteoporosis in several cohorts<sup>(6,7,9-11)</sup>. These evidences represent the role of

genetic factor in osteoporosis. This study demonstrated the genetic distribution of G2014A SNP in Thai postmenopausal women having osteoporotic hip fracture, which was strongly, represented phenotype of osteoporosis. It has been reported in some previous studies<sup>(7,12,13)</sup> that this polymorphism is actually associated with the osteoporosis diagnosed by using BMD. This study revealed that the allele A frequency was common in the population. The finding was different from previous research<sup>(7)</sup> reporting about Thai postmenopausal osteoporosis shown in Table 3. The allele A in G2014A was a common genotype in the osteoporotic fracture of hip. According to this study's results, it may be postulated that this allele could represent a susceptibility to osteoporotic hip fracture. In addition, the association between BMD and the polymorphism was found to be statistical significant.

**Table 2.** The association between genotypes and BMD in each regions

BMD	Genotype (%)			p-value
	AA (n = 31)	AG (n = 19)	GG (n = 15)	
Lumbar				0.003
Normal	21 (67.7)	11 (57.9)	4 (26.7)	
Osteopenia	7 (22.6)	1 (5.3)	2 (13.3)	
Osteoporosis	3 (9.7)	7 (36.8)	9 (60)	
Femoral neck				0.015
Normal	17 (58.8)	8 (42.1)	1 (6.6)	
Osteopenia	4 (12.9)	4 (21.1)	7 (46.7)	
Osteoporosis	10 (32.3)	7 (36.8)	7 (46.7)	
Ward				0.002
Normal	16 (51.6)	7 (36.8)	0	
Osteopenia	3 (9.7)	2 (10.5)	1 (6.7)	
Osteoporosis	12 (38.7)	10 (52.6)	14 (93.3)	
Intertrochanter				0.009
Normal	18 (58.1)	9 (47.3)	1 (6.7)	
Osteopenia	5 (16.1)	6 (31.6)	6 (40)	
Osteoporosis	8 (25.8)	4 (21.1)	8 (53.3)	

**Table 3.** Genotype distributions based on the G2014A SNP in Osteoporotic hip fracture, osteoporotic and non-osteoporotic subjects

	Osteoporosis hip fracture (%)	Osteoporosis by BMD (%)*	Non-osteoporosis (%)*
G/G	23.1	54.7	71.3
G/A	29.2	37.7	27.0
A/A	47.7	7.5	1.6
A2014 allele frequency	62.3	26.4	15.2

From the findings, the study suggested the importance of the polymorphism as the hereditary risk factor of osteoporosis in Thai population.

Estrogen deficiency has been reported to be associated with the postmenopausal osteoporosis<sup>(14)</sup>. The estrogen receptor alpha (ER $\alpha$ ) is expressed in bone cells and its function is the importance regulator in osteogenesis pathway. Although G2014A SNP in the human gene is located on the exon region of chromosome 6p25.1, it may contribute via the epigenetic level for the efficiency of translation or receptor protein expression<sup>(15)</sup>. It may be a potential susceptibility to determine risk of osteoporosis fracture when combining with others environmental factors or other surrogate parameters.

BMD has been used as one of the parameters including in the Fracture Risk Assessment Tool (FRAX) to determine the possibility of a ten-year risk in osteoporosis fracture<sup>(16)</sup>. However, in this study, the majority of patients who had osteoporotic hip fracture had normal BMD. The results of this study suggested that it may still be controversial to consider BMD as one standard parameter to predict the osteoporotic hip fracture. Therefore, other risk factors should be concerned and included when prescribing osteoporotic drugs. It is believed that the low calcium intake, lifestyle habits, smoking, poor diet such as tea or coffee are considered as risk factors of osteoporosis. In addition, frequent uses of certain medications such as thyroid, lithium, furosemide, steroid and chemotherapy may cause and increase the risk of osteoporosis. The previous osteoporotic fracture is also included as a substantial risk for osteoporosis. With respect to heredity, family history has also been used in FRAX. This supports the important role of hereditary risk in osteoporosis. However, the genetic risk should be more specific. According to the study findings, the ESR $\alpha$  polymorphism may be a potential candidate genetic as a marker in osteoporotic hip fracture.

Several limitations in this study could be addressed. Despite assessing 65 individuals, it might be considered that this investigation was relatively small. However, the population was diagnosed with osteoporosis by using definite phenotype of hip fracture as eligible criteria. It was also found that more than 50% of the patients presenting consideration of osteoporotic status carried the polymorphism, which suggested a strong clinical relevance of this particular factor. The researchers of this study assumed that the results could be generalized to the wider population;

the primary limitation of this study was the potential selection bias as the sample data were obtained from the patients presenting to a single referral hospital. This limitation is mitigated by the additional bone marrow density measurement for not only lumbar, but also hip region that we were able to perform with this study design.

### Conclusion and clinical significance

This study provides a strong evidence of the hereditary risk factor in osteoporosis. The results support the association of the genetic risk of G2014A SNP in Thai postmenopausal women and osteoporosis hip fracture. Further investigation should be conducted in larger sample size with multicentre collaboration. The defined polygenic risks or other polymorphisms should be included to determine or predict a fracture risk of osteoporosis.

### Potential conflicts of interest

None.

### References

1. Marks R. Hip fracture epidemiological trends, outcomes, and risk factors, 1970-2009. *Int J Gen Med* 2010; 3: 1-17.
2. Wongtriratanachai P, Luevitoonvechkij S, Songpatanasilp T, Sribunditkul S, Leerapun T, Phadungkiat S, et al. Increasing incidence of hip fracture in Chiang Mai, Thailand. *J Clin Densitom* 2013; 16: 347-52.
3. Duntas IA, Yiannakopoulos CK. Risk factors and prevention of osteoporosis-related fractures. *J Musculoskelet Neuronal Interact* 2007; 7: 268-72.
4. Seeman E, Hopper JL, Bach LA, Cooper ME, Parkinson E, McKay J, et al. Reduced bone mass in daughters of women with osteoporosis. *N Engl J Med* 1989; 320: 554-8.
5. Smith DM, Nance WE, Kang KW, Christian JC, Johnston CC Jr. Genetic factors in determining bone mass. *J Clin Invest* 1973; 52: 2800-8.
6. Richards JB, Kavvoura FK, Rivadeneira F, Styrkarsdottir U, Estrada K, Halldorsson BV, et al. Collaborative meta-analysis: associations of 150 candidate genes with osteoporosis and osteoporotic fracture. *Ann Intern Med* 2009; 151: 528-37.
7. Ongphiphadhanakul B, Chanprasertyothin S, Payattikul P, Saetung S, Piaseu N, Chailurkit L, et al. Association of a G2014A transition in exon 8 of the estrogen receptor-alpha gene with

- postmenopausal osteoporosis. *Osteoporos Int* 2001; 12: 1015-9.
8. Ongphiphadhanakul B, Chanprasertyothin S, Saetung S, Rajatanavin R. A specific haplotype in the 3' end of estrogen-receptor alpha gene is associated with low bone mineral density in premenopausal women and increased risk of postmenopausal osteoporosis. *Osteoporos Int* 2005; 16: 1233-8.
  9. Zhang C, Ma J, Chen G, Fu D, Li L, Li M. Evaluation of common variants in CNR2 gene for bone mineral density and osteoporosis susceptibility in postmenopausal women of Han Chinese. *Osteoporos Int* 2015 Jun 9. [Epub ahead of print]
  10. He W, Liu M, Huang X, Qing Z, Gao W. The influence of vitamin D receptor genetic variants on bone mineral density and osteoporosis in Chinese postmenopausal women. *Dis Markers* 2015; 2015:760313
  11. Herlin M, McGuigan FE, Luthman H, Akesson K. Polymorphisms in inflammation associated genes ALOX15 and IL-6 are associated with bone properties in young women and fracture in elderly. *Bone* 2015; 79:105-9.
  12. Rojano-Mejia D, Coral-Vazquez RM, Coronel A, Cortes-Espinosa L, Carmen Aguirre-Garcia M, Valencia-Villalvazo EY, et al. Relation of the estrogen receptor and vitamin D receptor polymorphisms with bone mineral density in postmenopausal Mexican-mestizo women. *Gene* 2014; 537: 10-4.
  13. Wang KJ, Shi DQ, Sun LS, Jiang X, Lu YY, Dai J, et al. Association of estrogen receptor alpha gene polymorphisms with bone mineral density: a meta-analysis. *Chin Med J (Engl)* 2012; 125: 2589-97.
  14. Syed F, Khosla S. Mechanisms of sex steroid effects on bone. *Biochem Biophys Res Commun* 2005; 328: 688-96.
  15. Keaveney M, Parker MG, Gannon F. Identification of a functional role for the 3' region of the human oestrogen receptor gene. *J Mol Endocrinol* 1993; 10: 143-52.
  16. Watts NB. The Fracture Risk Assessment Tool (FRAX(R)): applications in clinical practice. *J Womens Health (Larchmt)* 2011; 20: 525-31.

---

การศึกษาการกระจายตัวของ *gene G2014A polymorphism* ใน *estrogen receptor* ในคนไทยที่มีกระดูกข้อสะโพกหักจากโรคกระดูกพรุน

วิวัฒน์ วจนะวิศิษฐ์, ศักดา สุโขทัยมงคล, ภทรวินัย วรรณรัตน์, บุญส่ง องค์พัฒน์กุล, ตฤยพุกษ์ ถาวรสวัสดิ์รักษ์

**วัตถุประสงค์:** ศึกษาความเกี่ยวข้องในการกระจายของ *gene G2014A SNP* ภายใน *exon* ที่ 8 ของ *estrogen receptor* ในหญิงไทยวัยหมดประจำเดือนที่มีภาวะโรคกระดูกพรุนร่วมกับภาวะกระดูกข้อสะโพกหัก

**วัสดุและวิธีการ:** โดยศึกษาในกลุ่มหญิงไทยวัยหมดประจำเดือนที่มีภาวะโรคกระดูกพรุนร่วมกับกระดูกข้อสะโพกหักที่ไม่มีโรคประจำตัวอื่นจำนวน 65 คน ซึ่งส่วนใหญ่อายุมากกว่า 55 ปี ที่เข้ารับการรักษาในโรงพยาบาลรามธิบดี ระหว่างเดือนกรกฎาคม พ.ศ. 2548 ถึงกรกฎาคม พ.ศ. 2549 วิจัยใช้การเก็บข้อมูลพื้นฐานต่างๆ ความหนาแน่นของมวลกระดูก น้ำหนัก ส่วนสูง ดัชนีมวลกายและตรวจเลือดเพื่อคัดแยกภาวะโรคกระดูกเมตาบอลิซึมร่วมกับการกระจายของ *gene G2014A SNP* โดยตรวจวินิจฉัยความหนาแน่นมวลกระดูกว่าปกติอยู่ในเกณฑ์กระดูกบางหรือมีภาวะกระดูกพรุนและศึกษาความสัมพันธ์ของภาวะกระดูกพรุนกับการกระจายของ *gene G2014A SNP*

**ผลการศึกษา:** ได้พบว่ามีการกระจาย *genotype G/G* ร้อยละ 23.1, *G/A* ร้อยละ 29.2, *A/A* ร้อยละ 47.7 และเมื่อนำการกระจาย *genotype* มาวิเคราะห์ไม่พบว่ามีความเกี่ยวข้องกัน อายุ น้ำหนัก ส่วนสูง อย่างมีนัยสำคัญทางสถิติ ( $p > 0.05$ ) โดยอายุเฉลี่ยเท่ากับ  $76.2 \pm 10.9$  ปี  $BMI$   $21.3 \pm 3.5$   $kg/m^2$  เมื่อดูความหนาแน่นของกระดูกบริเวณกระดูกสันหลังส่วนเอวและกระดูกข้อสะโพกข้างปกติพบว่ามีการกระจายของ *gene G2014A SNP* มีความเกี่ยวข้องกับภาวะกระดูกพรุนอย่างมีนัยสำคัญทางสถิติ ( $p > 0.05$ )

**สรุป:** *G2014A SNP genotype* ภายใน *exon 8 estrogen receptor* มีความเกี่ยวข้องกันภาวะกระดูกพรุนและกระดูกข้อสะโพกหักในผู้หญิงไทยวัยหมดประจำเดือน

---