# Association of MTHFR C677T Polymorphism with Bone Mineral Density of Osteoporosis in Postmenopausal Thai Women

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***Background:*** *Osteoporosis and osteopenia is rising with the increase in numbers of postmenopausal women. Methylenetetrahydrofolate reductase (MTHFR), a homocysteine catabolizing enzyme, is involved in the regulation of bone mineral density (BMD). The association between MTHFR C677T polymorphism with osteoporosis in postmenopausal Thai women is hitherto unclear.*
***Objective:*** *To investigate the association between MTHFR C677T and BMD in postmenopausal Thai women.*
***Material and Method:*** *The study subjects consisted of 346 postmenopausal Thai women volunteers. Standard dual-energy X-ray absorptiometry (DXA) was used for measurement of BMD T-score. Restriction fragment length polymorphism (RFLP) analysis was used for measurement of MTHFR C677T polymorphism.*
***Results:*** *In the evaluation of 346 postmenopausal Thai women heterozygous (CT) genotype had a risk of osteopenia than normal control (odds ratio (OR) = 5.66, p<0.001).BMD T-scores at each bone position revealed that heterozygous (CT) genotype had increased risk of osteopenic bones than normal controls at lumbar spines 1, 2, and 4 (OR = 2.48, p<0.001, OR = 1.98, p = 0.008 and OR = 1.83, p = 0.016 respectively), ward's triangle (OR = 2.08, p = 0.008), and head of radius (OR = 2.95, p = 0.008).*
***Conclusion:*** *These results indicate the possibility of using MTHFR C677T polymorphism to identify postmenopausal Thai women at high risk of osteopenia.*

***Keywords:*** *Postmenopausal women, MTHFR (C677T) polymorphism, Osteopenia, Osteoporosis, Bone mineral density*

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The incidence of osteoporosis and osteopenia increases with the rise in number of elderly people[1-5]. In the United States, 13 to 18% of 50 years old women have osteoporosis[6], in England and Wales, 22% of women aged more than 50 years have femoral neck osteoporosis[7,8], and in Japan, 24% of elderly women are afflicted with osteoporosis[9]. The prevalence of lumbar spines (L1-L4) and femoral neck osteoporosis in Thai women over 40 years old were reported between 19 and 21% and 11 and 13% respectively[10,11].

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Both moderate and hyperhomocysteinemia are highly prevalent and involved in many diseases of old age[12]. Furthermore, plasma homocysteine (HCy) concentration is higher in postmenopausal than in premenopausal women and homocystinuria patients are often diagnosed with osteoporosis[13]. Early onset osteoporosis associated with homocystinuria is thought to be due to the effect of HCy or other metabolites interfering with cross-linking of collagen[14].

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5, 10 methylene-tetrahydrofolate (MTHF) to 5-MTHF, which provides the methyl group in the conversion of homocysteine to methionine by B12 dependent methionine synthase[15]. MTHFR gene is located on chromosome 1p36[15]. The study of MTHFR gene polymorphism has revealed that the variant TT genotype C677T is

associated with reduction of bone mineral density (BMD) in postmenopausal Japanese women[16]. MTHFR TT and CT genotypes are significantly correlated with decreased MTHFR activity[17] and TT genotype is associated with raised plasma HCy level that could affect collagen maturation[14].

The present study investigated the association between MTHFR C677T and BMD in postmenopausal Thai women.

## Material and Method

### *Subjects*

The study subjects consisted of 346 post-menopausal Thai women volunteers, who attended the menopause post-operation follow-up clinic, Department of Obstetrics and Gynecology, Faculty of Medicine Ramathibodi Hospital, Mahidol University Bangkok, Thailand for physical examination and bone scan (DEXA). All subjects were apparently good in health. Exclusion criteria were subjects with disorders that affect bone metabolism, such as diabetes mellitus, hypertension, and cardiovascular diseases. Age, life style, and anthropometric measurements were assessed through standardized questionnaires. Informed consent from the participants was obtained before participation in the present study, which was approved by the Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Mahidol University.

### *BMD measurement*

Standard dual-energy x-ray absorptiometry (DXA) (Lunar Prodigy®; GE Healthcare, Lunar, USA) was used for measurement of BMD T-score at lumbar spine (L1-4), neck of femur, trochanter (greater trochanter), ward's triangle, hip (total), head of radius, 33% radius (shaft of radius) and total radius. Women with a T-score ≤ -2.5 SD below normal are designated as being osteoporotic and T-score ≥ -1.0 SD is considered normal[18].

### *Polymerase chain reaction (PCR) for amplification of MTHFR*

Venous blood (5 ml) was drawn from each subject into heparinized tube and centrifuged at 3,000 g for 10 minutes at room temperature to obtain buffy coat. Flexi gene DNA kit (Qiagen, Hilden, Germany) was used to extract genomic DNA from buffy coat.

DNA amplification was performed using Gene Amp PCR system 9700 (Applied Biosystem, USA). PCR reactions were performed in a total volume of 50 µl containing 20 mM each primer, 1.0 U *Taq* DNA polymerase, 1X PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM dNTPs and 50 ng of genomic DNA. Primers specific for each variant sequence were forward primer (5'-TGAAGGA GAAGGTGTCTGCGGGA-3', Reverse primer: 5'-AGGACGGTGCGGTGAGAGTG-3'. Thermal cycling conditions were as follows: at 93°C; 30 cycles of 60 seconds at 95°C, 60 seconds at 58°C, 60 seconds at 72°C and a final heating for seven minutes at 72°C. PCR amplicons were separated by 2% agarose gel electrophoresis in 1X TBE (10.8 g Tris and 5.5 g Boric acid in 900 ml distilled water. Add 4 ml 0.5 M $Na_2EDTA$ (pH 8.0) adjust volume to 1,000 ml), stained with ethidium bromide and photographed under UV illumination.

### *Restriction fragment length polymorphism (RFLP) analysis*

HinfI (New England BioLabs Inc., USA) was used to digest PCR amplicons. Reaction volume of 50 µl contained 20 µl of PCR amplicon, 4 µl of 10X buffer (Bio Basic Inc., Canada) and 8 U HinfI and was incubated at 37°C for 4 hours. The digested fragments were analyzed by 4% agarose gel electrophoresis. Homozygous genotype (TT) is characterized by fragments of 175 and 23 bp, heterozygotes (CT) by fragments of 198 and 175, and wild type (CC) by fragment of 198 bp.

### *Statistical analysis*

Mann-Whitney U-test (two-tailed) was used to calculate difference among osteoporosis, osteopenia, and control subjects employing SPSS 10.0 for Windows (SPSS, Chicago, IL). Chi-square was used to analyze statistical differences among genotypic frequencies and groups as calculated by Epi Info™ 3.5.3 free edition software package (Centers for Disease Control and Prevention, USA). $P<0.05$ was set for statistically significant.

## Results

The majority of osteoporosis subjects (56%) were found in subjects of 61 to 70 years of age and 57% of osteopenia and 53.3% of control subjects were of 51 to 60 years of age. Most of the subjects had BMI ranging from 18.5 to 25 kg/m$^2$ and had waist/hip ratio >0.77 (Table 1).

Median age of osteoporosis is significantly higher than control subjects ($p<0.001$), but median of weight, BMI, waist circumference, and hip circumference in controls are significantly higher than

**Table 1.** Demographic and anthropometric variables in osteopenia, osteoporosis and control subjects

| Parameter | Control, n/total (%) | Osteopenia, n/total (%) | Osteoporosis, n/total (%) |
|---|---|---|---|
| Age distribution (years) | | | |
| 35-40 | 3/75 (4) | - | - |
| 41-50 | 20/75 (27) | 18/163 (11) | 1/108 (1) |
| 51-60 | 40/75 (53) | 93/163 (57) | 39/108 (36) |
| 61-70 | 12/75 (16) | 48/163 (29) | 60/108 (56) |
| 71-80 | - | 4/163 (2) | 8/108 (7) |
| BMI ($kg/m^2$) distribution | | | |
| <16.00 (severe thinness) | - | - | 3/108 (3) |
| 16.00-16.99 (moderate thinness) | - | - | 1/108 (1) |
| 17.00-18.49 (mild thinness) | - | 3/163 (2) | 8/108 (7) |
| 18.50-24.99 (normal) | 40/75 (53) | 110/163 (67) | 83/108 (77) |
| 25.00-29.99 (pre-obese) | 25/75 (33) | 42/163 (26) | 12/108 (11) |
| 30.00-34.99 (obese class I) | 9/75 (12) | 8/163 (5) | 1/108 (1) |
| 35.00-39.99 (obese class II) | 1/75 (1) | - | - |
| Waist/hip ratio distribution | | | |
| <0.77 | 16/75 (21) | 34/163 (21) | 20/108 (18) |
| ≥0.77 | 59/75 (79) | 129/163 (79) | 88/108 (82) |

BMI = body mass index

**Table 2.** Median and 95% CI of anthropometric parameters in osteoporosis and control subjects

| Parameter | Osteoporosis (n = 108) median (range) | 95% CI | Control (n = 75) median (range) | 95% CI | p-value* |
|---|---|---|---|---|---|
| Age (years) | 62.0 (48.0-75.0) | 60.3-62.4 | 53.0 (36.0-67.0) | 51.0-54.3 | 0.000 |
| Weight (kg) | 51.3 (37.5-78.0) | 50.6-53.6 | 59.0 (43.6-95.0) | 59.1-64.2 | 0.000 |
| Height (cm) | 155.0 (138.0-166.0) | 153.1-155.2 | 155.0 (146.0-173.0) | 154.5-157.3 | 0.090 |
| BMI ($kg/m^2$) | 21.5 (15.4-31.2) | 21.3-22.4 | 24.8 (19.3-39.5) | 24.4-26.3 | 0.000 |
| Waist (cm) | 76.0 (60.0-97.0) | 74.5-77.4 | 81.0 (64.5-111.0) | 79.2-83.6 | 0.000 |
| Hip (cm) | 94.0 (81.5-117.0) | 92.7-95.0 | 99.0 (86.0-125.0) | 97.9-101.6 | 0.000 |
| Waist/hip ratio | 0.8 (0.6-0.9) | 0.7-0.8 | 0.8 (0.6-0.9) | 0.8-0.82 | 0.367 |

BMI = body mass index

* Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed). Statistical significant difference between osteoporosis and control subject is when $p<0.05$.

osteoporosis subjects ($p<0.001$) (Table 2). Median age of osteopenia is lower than that of osteoporosis, but significantly higher than control subjects ($p<0.001$). Only median of weight, BMI and hip circumference in osteopenia subjects are significantly lower than control subjects ($p<0.001$) (Table 3).

The numbers of mutant MTHFR genotype are higher in subjects with low BMD than in the control subjects. Frequency of heterozygous CT genotype in osteopenia (37%) is statistically higher than control subjects (9%) (odds ratio (OR) = 5.66 (2.32-14.48), $p<0.001$). The frequency of heterozygous CT genotype in osteoporosis (25%) is statistically higher than control subjects (9%) (OR = 3.39, p = 0.009). There was no statistically significant difference between homozygous genotype TT of both osteopenia and osteoporosis with control subjects (Table 4).

When BMD T-scores of all subjects were grouped into each position of bone heterozygous (CT) MTHFR genotype has increased risk of osteopenic bones at lumbar spines 1, 2 and 4 (OR = 2.48, $p<0.001$; OR = 1.98, p = 0.008; OR = 1.83, p = 0.016, respectively), ward's triangle (OR = 2.08, p = 0.008) and head of radius (OR = 2.95, $p<0.001$) than normal controls (Table 5).

## Discussion

Methylenetetrahydrofolate reductase (MTHFR) is involved in the pathway of Hcy removal from blood circulation[13]. A polymorphism (CaT) at

**Table 3.** Median and 95% CI of anthropometric parameters in osteopenia and control subjects

| Parameter | Osteopenia (n = 163) median (range) | 95% CI | Control (n = 75) median (range) | 95% CI | p-value* |
|---|---|---|---|---|---|
| Age (years) | 57.0 (41.0-73.0) | 55.7-57.6 | 53.0 (36.0-67.0) | 51.0-54.3 | 0.000 |
| Weight (kg) | 56.0 (42.1-95.0) | 56.0-58.7 | 59.0 (43.6-95.0) | 59.1-64.2 | 0.004 |
| Height (cm) | 155.0 (142.0-170.0) | 154.5-156.0 | 155.0 (146.0-173.0) | 154.5-157.3 | 0.529 |
| BMI ($kg/m^2$) | 23.0 (17.9-33.4) | 23.2-24.2 | 24.8 (19.3-39.5) | 24.4-26.3 | 0.004 |
| Waist (cm) | 77.5 (26.0-108.0) | 77.2-80.2 | 81.0 (64.5-111.0) | 79.2-83.6 | 0.071 |
| Hip (cm) | 96.0 (35.0-119.0) | 95.3-97.8 | 99.0 (86.0-125.0) | 97.9-101.6 | 0.012 |
| Waist/hip ratio | 0.8 (0.6-1.0) | 0.80-0.82 | 0.8 (0.6-0.9) | 0.8-0.82 | 0.684 |

BMI = body mass index
* Mann-Whitney U-Wilcoxon rank sum W-test (two-tailed). Statistical significant difference between osteopenia and control subjects is when $p<0.05$.

**Table 4.** Association of heterozygous (CT) and homozygous variant (TT) and wild type (CC) of MTHFR gene polymorphism in osteopenia, osteoporosis and control subjects

| MTHFR genotype | Osteopenia, n (%) | Control, n (%) | Odd ratio (95% CI) | p-value* |
|---|---|---|---|---|
| CT | 61/163 (37) | 7/75 (9) | 5.66 (2.32-14.48) | 0.000 |
| CC | 100/163 (61) | 65/75 (87) | | |
| TT | 2/163 (1) | 3/75 (4) | 0.43 | 0.641 |
| | Osteoporosis, n (%) | | | |
| CT | 27/108 (25.0) | 7/75 (9) | 3.39 (1.30-9.20) | 0.009 |
| CC | 74/108 (68.5) | 65/75 (87) | | |
| TT | 7/108 (6.5) | 3/75 (4) | 2.05 | 0.484 |

MTHFR genotype: CC = wild type; CT = heterozygous variant; TT = homozygous variant
* Based on Chi-square test, $p<0.05$ is considered statistically significant.

codon 677 in exon 4 of MTHFR has recently been identified as a candidate gene for osteoporosis[16]. The present study showed that the frequency of heterozygous genotype (CT) of MTHFR is higher in osteopenia and osteoporosis than control subjects ($p<0.001$ and $p = 0.009$ respectively). Subjects with CT genotype have a 6-fold and 3-fold risk for osteopenia (only at L1, L2, L4, ward's triangle, and head of radius) and osteoporosis, respectively.

Study of postmenopausal Japanese women has shown an association of T allele with low BMD[16]. Meta-analysis of studies concerning the association between MTHFR C677T polymorphism and BMD found similar results[19]. However, the homozygous variant genotype (TT) is not significantly associated with low BMD status[20].

In summary, the heterozygous genotype C677T of MTHFR affected bone homeostasis in postmenopausal Thai women. Using MTHFR C677T genotype as a potential tool to identify Thai women at risk of osteoporosis is feasible.

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**Potential conflicts of interest**

None.

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**Table 5.** Association between heterozygous (CT), homozygous (TT) and normal (CC) genotypes of MTHFR with osteoporosis, osteopenia and normal BMD T-scores in different positions bone

| Bone position | MTHFR genotype | Osteopenia n/total (%) | Normal n/total (%) | Odd ratio (95% CI) | p-value* | MTHFR genotype | Osteoporosis n/total (%) | Normal n/total (%) | Odd ratio (95% CI) | p-value* |
|---|---|---|---|---|---|---|---|---|---|---|
| Lumbar spine (L1) | CT | 50/130 (38) | 39/188 (21) | 2.48 (1.45-4.23) | <0.001 | CT | 6/28 (21) | 39/188 (21) | 1.00 (0.34-2.84) | >0.05 |
| | CC | 74/130 (57) | 143/188 (76) | | | CC | 22/28 (79) | 143/188 (76) | | |
| | TT | 6/130 (5) | 6/188 (3) | 1.93 (0.53-7.06) | 0.260 | TT | 0 | 6/188 (3) | 0.00 (0.00-6.62) | 0.337 |
| Lumbar spine (L2) | CT | 46/133 (35) | 38/178 (21) | 1.98 (1.50-3.40) | 0.008 | CT | 11/35 (31) | 38/178 (21) | 1.69 (0.70-4.03) | 0.199 |
| | CC | 82/133 (62) | 134/178 (75) | 1.36 (0.35-5.24) | | CC | 23/35 (66) | 134/178 (75) | | |
| | TT | 5/133 (4) | 6/178 (3) | | 0.618 | TT | 1/35 (3) | 6/178 (3) | 0.97 (0.15-6.22) | 0.978 |
| Lumbar spine (L3) | CT | 33/106 (31) | 59/229 (26) | 1.27 (0.74-2.18) | 0.359 | CT | 3/11 (27) | 59/229 (26) | 1.17 (0.23-5.25) | 0.824 |
| | CC | 71/106 (67) | 161/229 (70) | | | CC | 7/11 (64) | 161/229 (70) | | |
| | TT | 2/106 (2) | 9/229 (4) | 0.50 (0.07-2.79) | 0.379 | TT | 1/11 (9) | 9/229 (4) | 2.56 (0.33-17.66) | 0.387 |
| Lumbar spine (L4) | CT | 41/119 (34) | 50/217 (23) | 1.83 (1.08-3.11) | 0.016 | CT | 4/10 (40) | 50/217 (23) | 2.15 (0.49-9.05) | 0.241 |
| | CC | 72/119 (60) | 161/217 (74) | | | CC | 6/10 (60) | 161/217 (74) | | |
| | TT | 6/119 (5) | 6/217 (3) | 2.24 (0.61-8.16) | 0.166 | TT | 0 | 6/217 (3) | 0.00 (0.00-32.79) | 0.636 |
| Neck of femur | CT | 38/131 (29) | 56/210 (27) | 1.18 (0.70-1.98) | 0.517 | CT | 1/5 (20) | 56/210 (27) | 0.67 (0.03-6.52) | 0.716 |
| | CC | 86/131 (66) | 149/210 (71) | | | CC | 4/5 (80) | 149/210 (71) | | |
| | TT | 7/131 (5) | 5/210 (2) | 2.43 (0.67-9.12) | 0.129 | TT | 0 | 5/210 (2) | 0.00 (0.00-63.79) | 0.714 |
| Ward's triangle | CT | 63/180 (35) | 23/111 (21) | 2.08 (1.15-3.76) | 0.008 | CT | 9/55 (16) | 23/111 (21) | 0.79 (0.31-2.00) | 0.592 |
| | CC | 112/180 (62) | 85/111 (77) | | | CC | 42/55 (76) | 85/111 (77) | | |
| | TT | 5/180 (3) | 3/111 (3) | 1.26 (0.25-6.89) | 0.751 | TT | 4/55 (7) | 3/111 (3) | 2.70 (0.48-16.07) | 0.191 |
| Trochanter (greater trochanter) | CT | 27/83 (32) | 68/257 (26) | 1.35 (0.76-2.40) | 0.277 | CT | 0 | 68/257 (26) | 0.00 (0.00-2.56) | 0.133 |
| | CC | 53/83 (64) | 180/257 (70) | | | CC | 6/6 (100) | 180/257 (70) | | |
| | TT | 3/83 (4) | 9/257 (4) | 1.13 (0.23-4.79) | 0.856 | TT | 0 | 9/257 (4) | 0.00 (0.00-22.55) | 0.584 |
| Hip (total) | CT | 22/75 (29) | 73/266 (27) | 1.08 (0.59-1.98) | 0.787 | CT | 0 | 73/266 (27) | 0.00 (0.00-3.00) | 0.159 |
| | CC | 51/75 (68) | 183/266 (69) | | | CC | 5/5 (100) | 183/266 (69) | | |
| | TT | 2/75 (3) | 10/266 (4) | 0.72 (0.10-3.65) | 0.673 | TT | 0 | 10/266 (4) | 0.00 (0.00-25.25) | 0.601 |
| Head of radius | CT | 55/141 (39) | 22/126 (17) | 2.95 (1.60-5.45) | <0.001 | CT | 18/79 (23) | 22/126 (17) | 1.45 (0.68-3.09) | 0.302 |
| | CC | 84/141 (60) | 99/126 (79) | | | CC | 56/79 (71) | 99/126 (79) | | |
| | TT | 2/141 (1) | 5/126 (4) | 0.16 (0.02-1.04) | 0.020 | TT | 5/79 (6) | 5/126 (4) | 1.77 (0.42-7.44) | 0.378 |
| Radius 33% (shaft of radius) | CT | 39/122 (32) | 49/196 (25) | 1.41 (0.83-2.41) | 0.179 | CT | 7/28 (25) | 49/196 (25) | 1.00 (0.36-2.70) | >0.05 |
| | CC | 79/122 (65) | 140/196 (71) | | | CC | 20/28 (71) | 140/196 (71) | | |
| | TT | 4/122 (3) | 7/196 (4) | 1.01 (0.24-4.01) | 0.984 | TT | 1/28 (4) | 7/196 (4) | 1.00 (0.15-6.54) | >0.05 |
| Radius (total) | CT | 47/143 (33) | 40/164 (24) | 1.54 (0.90-2.62) | 0.092 | CT | 8/39 (20) | 40/164 (24) | 0.82 (0.32-2.07) | 0.652 |
| | CC | 91/143 (64) | 119/164 (73) | | | CC | 29/39 (74) | 119/164 (73) | | |
| | TT | 5/143 (3) | 5/164 (3) | 1.31 (0.32-5.40) | 0.677 | TT | 2/39 (3) | 5/164 (3) | 1.64 (0.21-10.30) | 0.561 |

MTHFR genotype: CC = wild type; CT = heterozygous variant; TT = homozygous variant

* Based on Chi-square test, $p<0.05$ is considered statistically significant.

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***ความสัมพันธ์ระหว่าง MTHFR C677T polymorphism กับมวลกระดูกในผู้หญิงวัยหมดประจำเดือน***

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***ภูมิหลัง:*** *อัตราความชุกของโรคกระดูกพรุนเพิ่มขึ้นมากในกลุ่มผู้หญิงวัยหมดประจำเดือน และ methylenetetrahydrofolate reductase (MTHFR) เป็นเอนไซม์ที่เกี่ยวข้องกับการขับและกำจัด homocysteine ซึ่งสัมพันธ์กับการควบคุมมวลกระดูก อย่างไรก็ตามการควบคุมเอนไซม์ MTHFR โดยความผิดปกติของจีนที่ควบคุมที่ตำแหน่ง C677T ยังไม่มีการศึกษากันมากนักในประเทศไทย*

***วัตถุประสงค์:*** *เพื่อทำการศึกษาความสัมพันธ์ของการเปลี่ยนแปลงของจีน C677T ที่ควบคุมเอนไซม์ MTHFR กับมวลกระดูกในกลุ่มผู้หญิงวัยหมดประจำเดือน*

***วัสดุและวิธีการ:*** *ผู้เข้าร่วมการศึกษาคือผู้หญิงวัยหมดประจำเดือนจำนวน 346 ราย, ใช้เครื่องมือ dual-energy X-ray absorptiometry (DXA) เพื่อวัดค่าความหนาแน่นกระดูก (BMD T-score) และ restriction fragment length polymorphism (RFLP) วิธี เพื่อตรวจหาการเปลี่ยนแปลงของจีน C667T ที่ควบคุมเอนไซม์ MTHFR*

***ผลการศึกษา:*** *การเปลี่ยนแปลงของจีน C677T ที่ควบคุมเอนไซม์ MTHFR กับมวลกระดูกในกลุ่มผู้หญิงวัยหมดประจำเดือนจำนวน 346 ราย พบว่า heterozygous (CT) สัมพันธ์กับการมีกระดูกบาง (odds ratio (OR) = 5.66, p<0.001) และเมื่อวิเคราะห์ลงไปในรายละเอียดพบว่าสัมพันธ์กับการบางของกระดูกส่วน lumbar spines ที่ 1, 2 และ 4 (OR = 2.48, p<0.001, OR = 1.98, p = 0.008 และ OR = 1.83, p = 0.016 ตามลำดับ), ward's triangle (OR = 2.08, p = 0.008), และ head of radius (OR = 2.95, p = 0.008)*

***สรุป:*** *ผลการศึกษานี้แสดงความเป็นไปได้ในการใช้ MTHFR C677T เพื่อตรวจหาภาวะความเสี่ยงกระดูกบางในผู้หญิงไทยวัยหมดประจำเดือน*