Genetics and Molecular Research

Meta-analysis of association between IL-6 -634C/G polymorphism and osteoporosis

L. Yan, R. Hu, S. Tu, W.J. Cheng, Q. Zheng, J.W. Wang, W.S. Kan and Y.J. Ren

Department of Reparative and Reconstructive Surgery of Orthopedics, Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Corresponding author: Y.J. Ren
E-mail: yijunr818@126.com

Genet. Mol. Res. 14 (4): 19225-19232 (2015)
Received August 26, 2015
Accepted October 28, 2015
Published December 29, 2015
DOI http://dx.doi.org/10.4238/2015.December.29.32

ABSTRACT. Osteoporosis is a common disease in the aging population and studies have shown that interleukin-6 (IL-6) is potentially implicated in its pathogenesis. This study was designed to assess the association between the IL-6 gene -634C/G polymorphism and osteoporosis. PubMed, Embase, China National Knowledge Infrastructure, and Wanfang databases were searched for eligible studies published up to and including December 2014 in English or Chinese. Meta-analysis was conducted by the RevMan5.2 software. Weighted mean difference and 95% confidence interval (95%CI) were calculated by a fixed-effect or random-effect model. Bone mineral density (BMD) was regarded as the assessment index. As a result, a total of four articles with 3068 subjects were included. Differences in BMD between the CC and GG genotypes were 0.03 g/cm^2 (95%CI = 0.01 to 0.05) at total body, 0.01 g/cm^2 (95%CI = 0.00 to 0.03) at femoral neck, and 0.03 g/cm^2 (95%CI = 0.00 to 0.06) at the lumbar spine ($P < 0.05$). For the CG versus GG genotypes, the differences in BMD were 0.03 g/cm^2 (95%CI = 0.02 to 0.05) at total body and 0.02 g/cm^2 (95%CI = 0.00 to 0.03 at the femoral neck ($P < 0.05$). For the CC versus CG genotypes, the differences in BMD were not significant ($P > 0.05$). In conclusion, the GG genotype of

the -634C/G polymorphism in IL-6 appears to play a role in reducing BMD, which affects normal bone metabolism and leads to osteoporosis.

Key words: Osteoporosis; IL-6 polymorphism; -634C/G; Meta-analysis

INTRODUCTION

Osteoporosis (OP) is a metabolic bone disease characterized by increased bone fragility and susceptibility to fracture, which is due to bone mineral content loss and microstructure disorder (Adams, 2013). OP is the outcome of the combined action of multiple factors, including heredity, nutrition, lifestyle, and hormones (Willson et al., 2015). However, the molecular pathogenesis has not been fully elucidated.

Seventy percent of OP cases are determined by genetic factors that affect bone turnover and bone mineral density (BMD) (Pocock et al., 1987). These factors include the estradiol receptor, the calcitonin receptor, interleukin-6 (IL-6) and parathyroid hormone (PTH) (Al-Daghri et al., 2014). Therefore, determining the relationship between these cytokines and OP is necessary to explore the pathogenesis of OP. Recent studies have shown that IL-6 can regulate the proliferation, differentiation, and apoptosis of osteoblasts through various pathways (Kaneshiro et al., 2014). For postmenopausal women, the decrease in the level of estrogen may trigger the expression of IL-6, which has been shown to result in bone resorption. When bone resorption exceeds bone formation, OP can commence (Erices et al., 2002). Scheidt et al. (2001) found that IL-6 was one of the main factors to predict bone loss. IL-6 is a multifunctional cytokine regulating immune reactions (Kosa et al., 2009), bone resorption (Nakamura et al., 2014), and osteoarthritis (Valdes et al., 2010). The -174G/C and -634C/G (rs1800796) single nucleotide polymorphisms (SNPs) in the IL-6 gene were found to be associated with IL-6 promoter activity and were significantly associated with BMD (Oishi et al., 2012). Since OP is commonly characterized by lower BMD (Senn et al., 2014), the purpose of this study was to evaluate the correlation between the IL-6 -634C/G polymorphism and risk of OP by meta-analysis.

MATERIAL AND METHODS

Literature retrieval

The PubMed, Embase, China National Knowledge Infrastructure (CNKI), and Wanfang databases were searched for eligible studies published from January 2000 to December 2014, with no language restrictions and the subject terms as “bone”, “bone mineral density”, “interleukin-6”, “polymorphism”, and “-634C/G”. The potential relevant articles were screened by reading titles and abstracts. A full-text review was undertaken to filter for subsequent studies.

Inclusion and exclusion criteria

Inclusion criteria included the following: 1) sufficient information that the -634C/G genotype was exhibited in OP patients; and 2) BMD value at total body, femoral neck, lumbar spine, and distal radius was shown by genotype. Exclusion criteria were as follows: 1) studies that did not include the -634C/G polymorphism; 2) study that did not concern OP; 3) studies with subjects under 15 years of age; and 4) studies with unclear genotype data.

Data extraction

Two investigators (L. Yan and R. Hu) independently extracted the data, including authors, journal, publication year, population location, sample size, age, gender, menopausal status, and genotype information. The mean BMD values and standard deviation for each genotype at total body, femoral neck, lumbar spine, and distal radius were recorded. When there were conflicting evaluations, an agreement was reached after a discussion. If data were missing in the paper, the corresponding authors were contacted in order to obtain missing data. The articles with unavailable data were excluded from the study.

Literature quality assessment

The same two investigators (L. Yan and R. Hu) independently assessed the quality of the studies. Using grading standards modified from Thakkinstian et al. (2004) (Table 1), the quality scores were calculated. Total scores ranged from 0, being the worst, to 9, being the best. When scores were less than 6, the study was considered to be of low quality; studies with a score higher than 6 were ranked as high quality.

Table 1. Criteria of methodological quality assessment for eligible studies.

Items	Score
A. Representativeness of subjects	
Consecutive/randomly selected from population with clearly defined sampling frame	2
Consecutive/randomly selected from population without clearly defined sampling frame	1
Not described	0
B. Ascertainment of BMD measurement	
Clearly described standard method of measuring BMD (e.g., DXA) with details about calibration	2
Described standard method of measuring BMD (e.g., DXA) with details about calibration	1
Not described	0
C. Ascertainment of IL-6 genotype	
Genotyping done under blind conditions	1
Genotype unblinded or not mentioned	0
D. Test for HWE	
HWE in study group	2
Hardy-Weinberg disequilibrium in study group	1
Insufficient data for test	0
E. Assessment of association	
Assessment association between genotypes and BMD with appropriate statistics and adjusting confounders	2
Assessment association between genotypes and BMD with appropriate statistics without adjusting confounders	1
Inappropriate statistics used	0
Total	9

IL-6, interleukin-6; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; HWE, Hardy-Weinberg equilibrium. The scores are used to assess the methodological quality of the included studies.

Statistical analysis

The main analysis addressed differences in BMD between different genotypes. Genotypes were assessed as CC *vs* CG, CC *vs* GG, and CG *vs* GG. Heterogeneity between studies was evaluated by the χ^2 test and $P < 0.05$ was considered to be significant (Bowden et al., 2011). Heterogeneity was also assessed by the I^2 metric, which was considered to be significant for $I^2 > 50\%$. When heterogeneity was absent, a fixed-effect model was used to pool results from individual studies; otherwise, a random-effect model was used. The Z-test was used to assess the significance of the pooled weighted mean difference (WMD) and $P < 0.05$ was considered to be significant.

RESULTS

Characteristics of eligible studies

A total of four articles (Ota et al., 2001; Yamada et al., 2003; Li et al., 2008; Oishi et al., 2012) with 3068 subjects were included in this study (Figure 1) and the characteristics are summarized in Table 2. All the subjects were Asian and 1942 were women and 1126 were men. Of the women, 176 subjects were premenarche, 379 subjects were premenopausal and 1387 subjects were postmenopausal. The BMD values of total body, femoral neck, lumbar spine, and distal radius in relation to the IL-6 gene -634C/G polymorphism were analyzed.

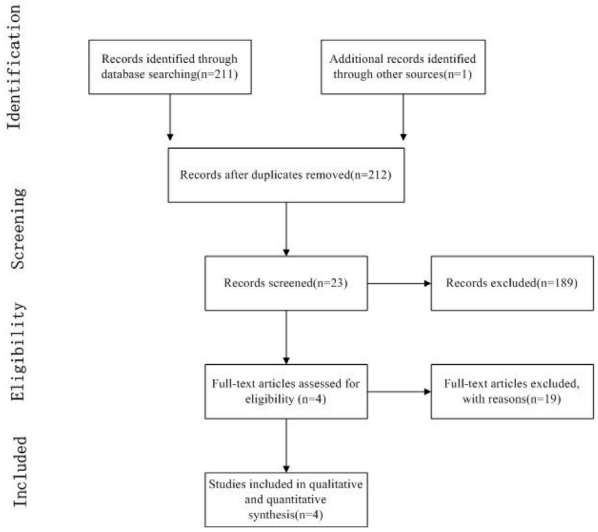

Figure 1. Flow diagram of the study selection process.

Table 2. Characteristics of the studies included.

First Author	Country	Year	Gender	Menopausal status	No. of subjects	Covariates	Quality score
Ota N	Japan	2001	Female	Postmenopausal	470	Age, height, weight	5
Yamada Y-a	Japan	2003	Female	Premenopausal	279	Age, BMI	8
Yamada Y-b	Japan	2003	Female	Postmenopausal	817	Age, BMI	8
Yamada Y-c	Japan	2003	Male	None	1126	Age, BMI	8
Li X	China	2008	Female	Premenarche	176	None	7
Oishi Y-a	Japan	2012	Female	Premenopausal	100	None	4
Oishi Y-b	Japan	2012	Female	Postmenopausal	100	Age, height, weight	6

Quality score was obtained according to the criteria of methodological quality assessment for eligible studies, and it represents the quality of the study included.

Association between the -634C/G IL-6 polymorphism and BMD

For CC *vs* CG genotypes, there was no difference in BMD of the total body (Z = 0.48, P = 0.63), femoral neck (Z = 0.13, P = 0.90), lumbar spine (Z = 0.82, P = 0.41), and distal radius (Z

= 1.62, P = 0.11). For CC *vs* GG genotypes, there was an obvious difference in BMD of the total body (Z = 3.26, P = 0.001), femoral neck (Z = 2.08, P = 0.04), and lumbar spine (Z = 2.00, P = 0.05) (Figure 2). However, there was no significant difference in the distal radius (Z = 1.09, P = 0.28). The differences in BMD between the CC and GG genotypes were 0.03 g/cm^2 (95%CI = 0.01 to 0.05) of the total body, 0.01 g/cm^2 (95%CI = 0.00 to 0.03) the femoral neck, and 0.03 g/cm^2 (95%CI = 0.00 to 0.06) the lumbar spine. For CG *vs* GG genotypes, there was also a difference in BMD of the total body (Z = 3.89, P = 0.0001) and femoral neck (Z = 2.45, P = 0.01) (Figure 3), but no significant difference for the lumbar spine (Z = 1.81, P = 0.07) or distal radius (Z = 0.64, P = 0.52). The differences in BMD were 0.03 g/cm^2 (95%CI = 0.02 to 0.05) for the total body and 0.02 g/cm^2 (95%CI = 0.00 to 0.03) for the femoral neck (P < 0.05).

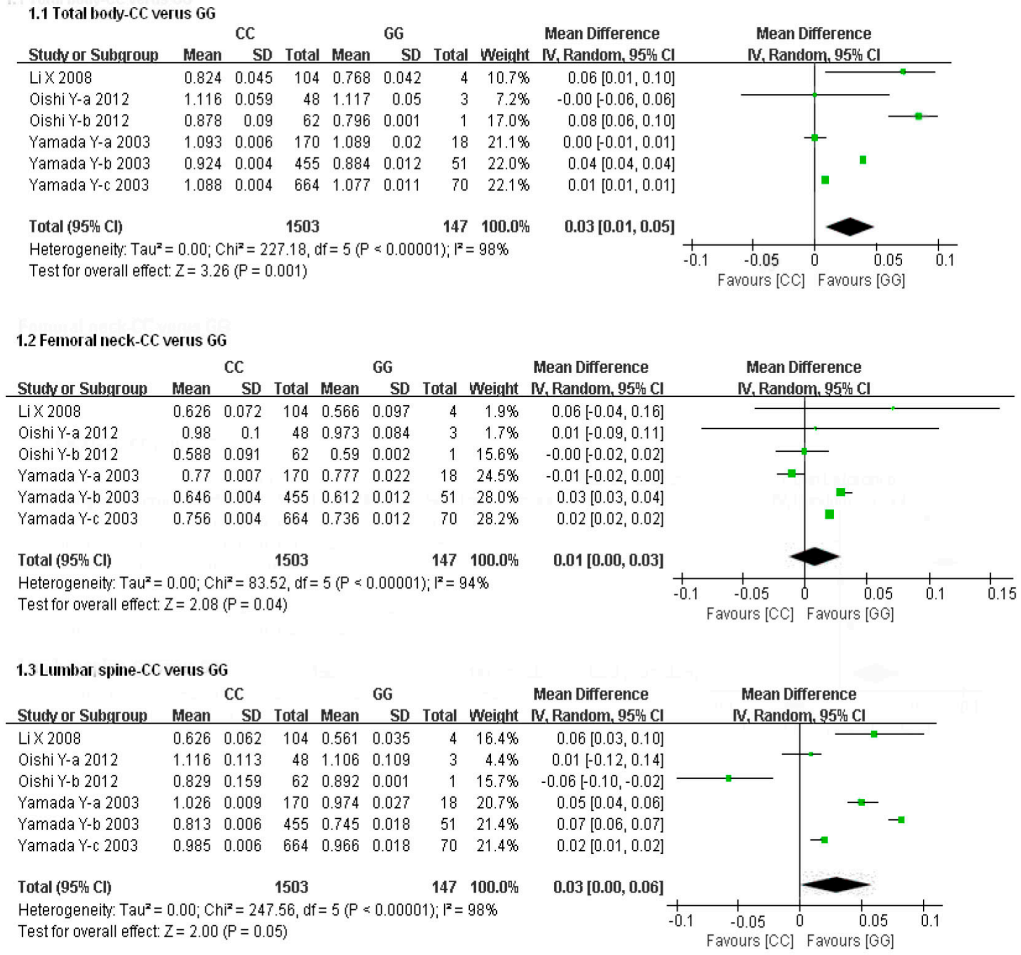

Figure 2. Association between the -634C/G SNP in IL-6 and BMD for CC *vs* GG genotypes. Green square represents the position of MD value, the size of square represents the weight of corresponding study. Black diamond represents the position of total MD value and the 95% confidence interval, the size of diamond represents the weight of all the corresponding studies.

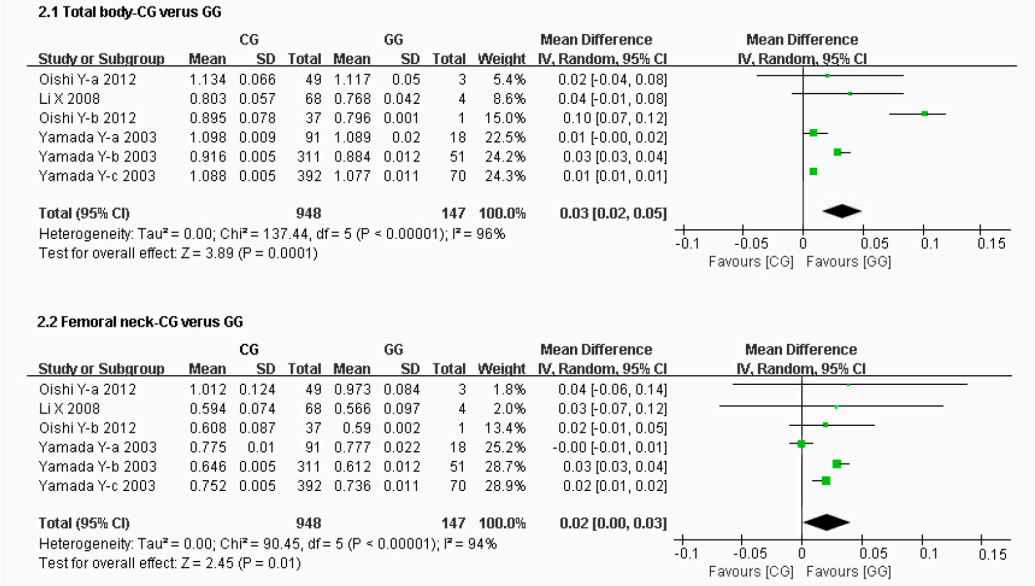

Figure 3. Association between the -634C/G SNP in IL-6 and BMD for CG *vs* GG genotypes. Green square represents the position of MD value, the size of square represents the weight of corresponding study. Black diamond represents the position of total MD value and the 95% confidence interval, the size of diamond represents the weight of all the corresponding studies.

Sensitivity and publication bias diagnosis

Sensitivity analysis confirmed the stability of the association between the -634C/G polymorphism in IL-6 and BMD at the total body, femoral neck, and lumbar spine and no publication bias was detected in the studies included.

DISCUSSION

OP has been shown to be a multifactorial disease with strong genetic influence. Studies on the candidate genes of OP mainly focus on the vitamin D receptor (VDR), calcitonin receptor, estrogen receptor, IL-6 and type I collagen (COLIA1) genes (Clark and Duncan, 2015). Among the various cytokines affecting bone metabolism, IL-6 plays an important role in osteoclast differentiation and maturation.

This study conducted a comprehensive analysis of literature to assess the association between the IL-6 gene -634C/G polymorphism and BMD in different areas of the body (total body, femoral neck, lumbar spine, and distal radius). The results indicate that the differences in BMD between the CC and GG genotypes of the total body, femoral neck, and lumbar spine were of statistical significance. For the CG versus GG genotypes, the differences in BMD at the total body and femoral neck were significant. For the CC versus CG genotypes, the differences in BMD were not significant. The G allele increased the risk of osteoporosis and many other studies have shown similar data (Ota et al., 2001; Yamada et al., 2003; Li et al., 2008; Oishi et al., 2012; Wang et al., 2013). The differences in BMD among different genotypes for the distal radius had

no statistical significance. This was not similar to the results from Ni et al. (2014), who found a significant association between the -634C/G polymorphism and distal radius BMD in an Asian population. All the data showed that the genetic influence of the -634C/G SNP on BMD may have site-specific skeletal character. However, there are some limitations to our research. Firstly, the eligible articles we retrieved were all from Asian countries, which may result in publication bias. Therefore, further studies should be conducted that include other populations to further confirm the conclusions from this study. Second, every included study has its own design characteristics and other influencing factors including diet, lifestyle, environment and genetic factors were not taken into account. Finally, the number of eligible studies and sample size were small and the reliability of the conclusions remains to be further optimized.

Until now, the involvement of IL-6 in the occurrence of OP has remained unclear. IL-6 expression increases in patients with OP, which has been regarded as a candidate gene regulating bone mineral density (Inada and Miyaura, 2010). IL-6 is synthesized from osteoblasts and is a bone restorative cytokine. It can promote the proliferation and differentiation of precursor cells, stimulate the aggregation of osteoclasts, and inhibit the apoptosis of osteoclasts (Bakker et al., 2014). In addition, estrogen can inhibit the synthesis of IL-6 in osteoblasts and affect the intracellular signal transduction of IL-6, which regulates IL-6 bone resorption activity (Garnero et al., 2002). Therefore, the role of IL-6 in OP mainly depends on mediating estrogen pathways to cause bone loss.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Adams JE (2013). Advances in bone imaging for osteoporosis. *Nat. Rev. Endocrinol.* 9: 28-42.

Al-Daghri NM, Yakout S, Al-Shehri E, Al-Fawaz H, et al. (2014). Inflammatory and bone turnover markers in relation to PTH and vitamin D status among Saudi postmenopausal women with and without osteoporosis. *Int. J. Clin. Exp. Med.* 7: 2812-2819.

Bakker AD, Kulkarni RN, Klein-Nulend J and Lems WF (2014). IL-6 alters osteocyte signaling toward osteoblasts but not osteoclasts. *J. Dent. Res.* 93: 394-399.

Bowden J, Tierney JF, Copas AJ and Burdett S (2011). Quantifying, displaying and accounting for heterogeneity in the meta-analysis of RCTs using standard and generalised Q statistics. *BMC Med. Res. Methodol.* 11: 41.

Clark GR and Duncan EL (2015). The genetics of osteoporosis. *Br. Med. Bull.* 113: 73-81.

Erices A, CongetP, Rojas C and Minguell JJ (2002). Gp130 activation by soluble interleukin-6 receptor/interleukin-6 enhances osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells. *Exp. Cell Res.* 280: 24-32.

Garnero P, Borel O, Sornay-Rendu E, Duboeuf F, et al. (2002). Association between a functional interleukin-6 gene polymorphism and peak bone mineral density and postmenopausal bone loss in women: the OFELY study. *Bone* 31: 43-50.

Inada M and Miyaura C (2010). [Cytokines in bone diseases. Cytokine and postmenopausal osteoporosis]. *Clin. Calcium* 20: 1467-1472.

Kaneshiro S, Ebina K, Shi K, Higuchi C, et al. (2014). IL-6 negatively regulates osteoblast differentiation through the SHP2/MEK2 and SHP2/Akt2 pathways *in vitro*. *J. Bone Miner. Metab.* 32: 378-392.

Kosa JP, Balla B, Kiss J, Podani J, et al. (2009). Postmenopausal expression changes of immune system-related genes in human bone tissue. *J. Clin. Immunol.* 29: 761-768.

Li X, He GP, Zhang B, Chen YM, et al. (2008). Interactions of interleukin-6 gene polymorphisms with calcium intake and physical activity on bone mass in pre-menarche Chinese girls. *Osteoporos. Int.* 19: 1629-1637.

Nakamura M, Uehara S, Nakamura H and Udagawa N (2014). [Cytokine-mediated bone resorption]. *Clin. Calcium* 24: 837-844.

NiY, Li H, Zhang Y, Zhang H, et al. (2014). Association of IL-6 G-174C polymorphism with bone mineral density. *J. Bone Miner. Metab.* 32: 167-173.

Oishi Y, Watanabe Y, Shinoda S, Naka M, et al. (2012). The IL6 gene polymorphism -634C>G and IL17F gene polymorphism 7488T>C influence bone mineral density in young and elderly Japanese women. *Gene* 504: 75-83.

Ota N, Nakajima T, Nakazawa I, Suzuki T, et al. (2001). A nucleotide variant in the promoter region of the interleukin-6 gene associated with decreased bone mineral density. *J. Hum. Genet.* 46: 267-272.

Pocock NA, Eisman JA, Hopper JL, Yeates MG, et al. (1987). Genetic determinants of bone mass in adults. A twin study. *J. Clin. Invest.* 80: 706-710.

Scheidt-Nave C, Bismar H, Leidig-Bruckner G, Woitge H, et al. (2001). Serum interleukin 6 is a major predictor of bone loss in women specific to the first decade past menopause. *J. Clin. Endocrinol. Metab.* 86: 2032-2042.

Senn C, Gunther B, Popp AW, Perrelet R, et al. (2014). Comparative effects of teriparatide and ibandronate on spine bone mineral density (BMD) and microarchitecture (TBS) in postmenopausal women with osteoporosis: a 2-year open-label study. *Osteoporos. Int.* 25: 1945-1951.

Thakkinstian A, D'Este C, Eisman J, Nguyen T, et al. (2004). Meta-analysis of molecular association studies: vitamin D receptor gene polymorphisms and BMD as a case study. *J. Bone Miner. Res.* 19: 419-428.

Valdes AM, Arden NK, Tamm A, Kisand K, et al. (2010). A meta-analysis of interleukin-6 promoter polymorphisms on risk of hip and knee osteoarthritis. *Osteoarthritis Cartilage* 18: 699-704.

Wang Z, Yang Y, He M, Wang R, et al. (2013). Association between interleukin-6 gene polymorphisms and bone mineral density: a meta-analysis. *Genet. Test Mol. Biomarkers* 17: 898-909.

Willson T, Nelson SD, Newbold J, Nelson RE, et al. (2015). The clinical epidemiology of male osteoporosis: a review of the recent literature. *Clin. Epidemiol.* 7: 65-76.

Yamada Y, Ando F, Niino N and Shimokata H (2003). Association of polymorphisms of interleukin-6, osteocalcin, and vitamin D receptor genes, alone or in combination, with bone mineral density in community-dwelling Japanese women and men. *J. Clin. Endocrinol. Metab.* 88: 3372-3378.