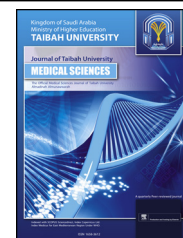




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Original Article

## The association between interleukin 6 –174 G/C gene polymorphism and the risk of osteoporosis: A meta-analysis

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### المخلص

**أهداف البحث:** تهدف هذه الدراسة للتحقيق في العلاقة بين تعدد الأشكال الجينية للجين IL 6-174 G/C وخطر هشاشة العظام باستخدام التحليل التلوي.

**طرق البحث:** تم البحث عن المقالات المنشورة في قاعدة المعلومات PubMed و EMBASE المؤهلة للنشر. وأستخرجت المعلومات التالية من كل بحث: اسم الباحث الأول، سنة النشر، بلد المنشأ، حجم عينة الحالات والضوابط، وحجم كل أليل. كما تم تقييم نسبة الأرجحية المشتركة وفترة الثقة ٩٥٪ للعلاقة بين تعدد الأشكال الجينية للجين IL 6-174 G/C مع خطر هشاشة العظام باستخدام نموذج الأثر العشوائي أو الثابت. واستخدام التحليل التلوي الشامل لتحليل البيانات.

**النتائج:** شملت الدراسة ١٢ بحثاً (٤٩٢٣ حالة / ٣٤٣١ ضابطة) في التحليل التلوي. وأظهرت النتائج أن الجين IL 6-174 G/C متعدد الأشكال الجينية كان مرتبطاً بزيادة (G vs C) وانخفاض خطورة حدوث هشاشة العظام.

**الاستنتاجات:** أظهر الجين متعدد الأشكال IL 6-174 G/C ارتباطاً إيجابياً مع خطورة حدوث هشاشة العظام.

**الكلمات المفتاحية:** IL 6-174 G/C؛ هشاشة العظام؛ تعدد الأشكال الجيني

### Abstract

**Objectives:** This study aimed to investigate the association between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis by performing a meta-analysis.

**Methods:** Published literature from PubMed and Embase databases was searched for eligible publications. The following information was extracted from each study: Name of first author, year of publication, country of origin, sample size of cases and controls, and size of each allele. The combined odds ratio (ORs) and 95% confidence intervals (95% CIs) for the association between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis were assessed using a random or fixed effects model. A comprehensive meta-analysis (CMA) 2.0 was used to analyse the data.

**Results:** Twelve studies (4923 cases/3431 controls) were included in this meta-analysis. The results indicated that IL6 –174 G/C gene polymorphism was associated with an increased (G vs C, OR 95%CI = 1.29 [1.03–1.62], p = 0.029) and decreased risk of osteoporosis (C vs G, OR 95%CI = 0.77 [0.62–0.97], p = 0.029; CC vs GG + GC, OR 95%CI = 0.58 [0.39–0.88], p = 0.010).

**Conclusion:** The IL6 –174 G/C gene polymorphism was shown to be positively correlated with osteoporosis risk.

**Keywords:** Genetic polymorphism; IL6 –174 G/C gene polymorphism; Osteoporosis

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### Introduction

Osteoporosis, an important public health problem, is a very common multifactorial progressive skeletal or

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metabolic bone disorder in the elderly that is characterized by low bone density and microarchitectural deterioration of bony tissue or bone mineral density (BMD) more than 2.5 SDs below the young normal mean.<sup>1–3</sup> It results in loss of bone mass, decreased bone strength,<sup>4</sup> increased bone fragility, and increased risk of developing spontaneous and traumatic fractures.<sup>5,6</sup> Osteoporosis is considered a silent disease because it progresses without symptoms until a fracture occurs.<sup>6</sup> Osteoporosis affects 200 million individuals worldwide,<sup>2</sup> 2.1 million females in England and Wales,<sup>3</sup> 13 million in Japan,<sup>4</sup> 3.5 million in Italy,<sup>5</sup> and 26 million in India.<sup>6</sup> The frequency of osteoporosis varies among studies. In the United States, the frequency of osteoporosis was 4% of 50- to 59-year-old Caucasian women and 52% of women aged 80 years or more,<sup>1</sup> whereas in India, the frequency of osteoporosis was 20% of women and 10–15% of men.<sup>6</sup> Osteoporosis is estimated to cause 1.5 million fractures annually in the United States, including 300,000 hip fractures, approximately 700,000 vertebral fractures, 250,000 wrist fractures, and more than 300,000 fractures at other sites.<sup>7</sup> The mortality associated with osteoporotic fractures ranges from 15 to 30%, a rate similar to that of breast cancer and stroke.<sup>5</sup> The annual cost expenditure for osteoporotic fractures is approximately £1.7 billion in England and Wales,<sup>1</sup> £2100 million in England,<sup>3</sup> over \$14 billion in the United States in 2003<sup>1</sup> and \$16 billion in 2008,<sup>8</sup> and it is predicted to reach approximately \$25.3 billion in 2025.<sup>9</sup> Osteoporosis is a common disease with a strong genetic component.<sup>10</sup> Stewart and Ralston<sup>11</sup> revealed that genetic factors play an important role in regulating bone mineral density and other determinants of osteoporotic fracture risk. IL6 is one of the candidate genes that regulate bone density because IL6 has some effect on the stimulation of osteoclast resorption and has been implicated in the pathogenesis of bone loss associated with oestrogen deficiency.<sup>12</sup>

The IL6 gene is located at chromosome 7p21 and 5 in the human genome. The gene contains four introns and five exons.<sup>13</sup> IL6 is a cytokine involved not only in inflammation and infection responses but also in the regulation of metabolic, regenerative, and neural processes.<sup>14</sup> Overexpression of IL6 has been implicated in the pathology of a number of diseases, including osteoporosis.<sup>13</sup> Several studies have reported the role of IL6 in the pathogenesis of osteoporosis. Bellido et al.<sup>15</sup> revealed that IL6 mediates the upregulation of osteoclastogenesis. Kudo et al.<sup>16</sup> reported that IL6, which is thought to play a role in several osteolytic bone disorders, is directly capable of inducing osteoclast formation by a receptor activator of nuclear factor-kappa B ligand (RANKL)-independent mechanism. Yoshitake et al.<sup>17</sup> reported that IL6 directly acts on osteoclast progenitors and suppresses their differentiation by regulating the transcription of specific genes related to mitogen-activated protein kinase (MAPK) phosphatases and the ubiquitin pathway. Scheidt-Nave et al.<sup>18</sup> found that serum IL6 was elevated in postmenopausal osteoporosis. Gaber et al.<sup>19</sup> showed that the G/C promoter polymorphism at –174 of the IL6 gene had an effect on basal IL6 levels, and the C allele of the IL6 –174 G/C gene was associated with reduced gene expression and reduced plasma levels of IL6.

Several polymorphism studies regarding the association between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis have shown inconsistent results. The studies conducted by Magana et al.,<sup>20</sup> Mendez et al.,<sup>21</sup> and Ferrari et al.<sup>22</sup> showed that the IL6 –174 G/C gene polymorphism was associated with an increased risk of osteoporosis. In contrast, the studies conducted by Czerny et al.,<sup>23</sup> Garnero et al.,<sup>24</sup> Deveci et al.,<sup>25</sup> Maedebib26,<sup>26</sup> Nordstrom et al.,<sup>27</sup> Lee et al.,<sup>28</sup> Korvala et al.,<sup>29</sup> Dincel et al.,<sup>30</sup> and Moffet et al.<sup>31</sup> showed that the IL6 –174 G/C gene polymorphism had no significant association with an increased risk of osteoporosis. A meta-analysis study is the solution to determine the actual association in the many studies.

This study aimed to investigate the association between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis by performing a meta-analysis. The results of this study are expected to be useful for the future treatment and prevention of osteoporosis. In addition, this study is expected to be useful as a comparison to other studies of the IL6 –174 G/C gene polymorphism and osteoporosis.

## Materials and Methods

### Study design

A meta-analysis was conducted to assess the association between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis. To achieve this goal, several studies regarding this association were collected to calculate a combined OR 95%CI, and the data were assessed using a fixed or random effects model. An article search was conducted in PubMed and Embase. The study was conducted from January to April 2016.

### Study procedures

The procedures of this study were to (1) identify the potentially relevant studies in PubMed and Embase up to March 20th, 2016; (2) determine the eligibility of each study using an exclusion process involving the following several steps: (a) reading the title and abstract, (b) ensuring that the study design complied with the inclusion criteria, and (c) ensuring that the study provided sufficient data to calculate OR 95%CI; (3) collect the abstract and full-text data from the studies; (4) collect the data for calculating OR 95%CI; and (5) analyse the data statistically.

### Eligibility criteria and data extraction

The eligibility criteria consisted of predefined inclusion and exclusion criteria. Studies were included in the analysis if they met the following inclusion criteria: (1) case-control; (2) cohort; (3) cross-sectional studies; (4) randomized-controlled trials (RCTs); (5) controlled before-and-after studies; (6) cross-over studies; (7) evaluation of the association between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis; and (8) sufficient data for calculation of OR 95% CI. Some of the required data were extracted from each study for calculating OR 95%CI. The following information was extracted from each study: (1) name of first author; (2) year

of publication; (3) country of origin; (4) sample size of cases and controls; and (5) size of each allele.

### Search strategy and literature

PubMed and Embase were searched with no language restrictions using specified search terms to identify studies published up to March 20th, 2016. The search strategy involved the use of a combination of the following key words: (IL 6 –174 G/C gene) and (variant or variation or polymorphism) and (osteoporosis). The publication languages were restricted to English. The reference lists of retrieved articles were hand-searched. If more than one article was published using the same study data, only the study with the largest sample size was included. We used a scoring system to evaluate the quality of the studies. We used a quality assessment score modified from previous meta-analysis for observational studies. The total scores ranged from 0 (worst) to 9 (best). A study was considered low quality if the score was <6 and high quality if the score was  $\geq 6$ .<sup>32</sup>

### Study variables

#### 1. Interleukin 6 –174 G/C

Interleukin-6 is a cytokine involved not only in inflammation and infection responses but also in the regulation of metabolic, regenerative, and neural processes.<sup>14</sup> The measurement results of this variable were the G and C alleles. Data were obtained by a search strategy. A nominal scale was used to assess this variable.

#### 2. Risk of osteoporosis

Osteoporosis is a very common multifactorial progressive skeletal or metabolic bone disorder in the elderly that is characterized by low bone density and microarchitectural deterioration of bony tissue or a bone mineral density (BMD) more than 2.5 SDs below the young normal mean.<sup>1–3</sup> The measurement results of this variable were an increased or decreased risk of osteoporosis. The data were obtained by a search strategy. A nominal scale was used to assess this variable.

### Statistical analysis

The correlation between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis was estimated by calculating pooled ORs and 95% CIs. The significance of pooled ORs was determined by Z tests ( $p < 0.05$  was considered statistically significant). A Q test was performed to evaluate whether heterogeneity existed. A random effects model was used to calculate OR 95%CI if heterogeneity existed ( $p < 0.10$ ). A fixed effect model was used to calculate OR 95%CI if no heterogeneity existed. Publication bias was assessed by Egger's test ( $p < 0.05$  was considered statistically significant). Subgroup analyses based on continent (Europe, America, and Asia), gender (female, male, and mixed), and sample size (small < 400, large  $\geq 400$  samples) were also performed. A comprehensive meta-analysis (CMA) 2.0 was used to analyse the data.

## Results

### Characteristics of the studies

A total of 1798 potentially relevant papers were identified based on the search strategy. Of these, 1769 papers were excluded because of obvious irrelevance by reading their titles and abstracts. After the full texts were read, six papers were excluded because they did not provide sufficient data for calculation of OR with 95%CI; two papers were excluded because they were family-based studies. In addition, nine reviews were excluded. A flow chart demonstrating the inclusion or exclusion of studies is displayed in Figure 1. A total of 12 studies were included in the meta-analysis. Seven studies were from Europe, four studies were from America, and one study was from Asia. Table 1 describes the characteristics of the studies included in the meta-analysis.

### Quantitative data synthesis

A total of 4923 cases and 3431 controls were identified. Overall, the results showed that allele G vs C, C vs G, and CC vs GG + GC had significant associations with the risk of osteoporosis. In contrast, GG vs GC + CC and GC vs GG + CC had no significant associations with the risk of osteoporosis. The results indicated that the IL6 –174 G/C gene polymorphism was associated with an increased (G vs C, OR 95%CI = 1.29 [1.03–1.62],  $p = 0.029$ ) and a decreased risk of osteoporosis (C vs G, OR 95%CI = 0.77 [0.62–0.97],  $p = 0.029$ ; CC vs GG + GC, OR 95%CI = 0.58 [0.39–0.88],  $p = 0.010$ ). A forest plot showing the correlation between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis is shown in Figure 2 for G vs C. A summary of ORs and 95% CIs regarding the correlation between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis is described in Table 2. In the subgroup analysis, the IL6 –174 G/C gene polymorphism was associated with the risk of osteoporosis in two genetic models of the European continent subgroup (GC vs GG + CC  $p = 0.005$ ; CC vs GG + GC  $p = 0.017$ ), two genetic models of the American continent subgroup (G vs C  $p = 0.019$ ; C vs G  $p = 0.019$ ), three genetic models of the female subgroup (G vs C  $p = 0.047$ ; C vs G  $p = 0.047$ ; CC vs GG + GC  $p = 0.028$ ), four genetic models of the mixed gender subgroup (G vs C  $p = 0.007$ ; GG vs GC + CC  $p = 0.024$ ; GC vs GG + CC  $p < 0.001$ ; C vs G  $p = 0.007$ ), and one genetic model of the small-sample-size subgroup (CC vs GG + GC  $p = 0.024$ ). In contrast, the IL6 –174 G/C gene polymorphism had no significant association with the risk of osteoporosis in three genetic models of the European continent subgroup (G vs C  $p = 0.497$ ; GG vs GC + CC  $p = 0.333$ ; C vs G  $p = 0.497$ ), three genetic models of the American continent subgroup (GG vs GC + C  $p = 0.100$ ; GC vs GG + CC  $p = 0.433$ ; CC vs GG + GC  $p = 0.054$ ), all genetic models of the Asia continent subgroup (G vs C  $p = 0.723$ ; GG vs GC + CC  $p = 0.722$ ; GC vs GG + CC  $p = 0.722$ ; C vs G  $p = 0.723$ ; CC vs GG + GC  $p = NA$ ), two genetic models of the female subgroup (GG vs GC + CC  $p = 0.163$ ; GC vs GG + CC  $p = 0.535$ ), all genetic models of the male subgroup (G vs C  $p = 0.635$ ; GG vs GC + CC  $p = 0.205$ ; GC vs GG + CC  $p = 0.118$ ; C vs G  $p = 0.635$ ; CC vs GG + GC  $p = 0.595$ ), one

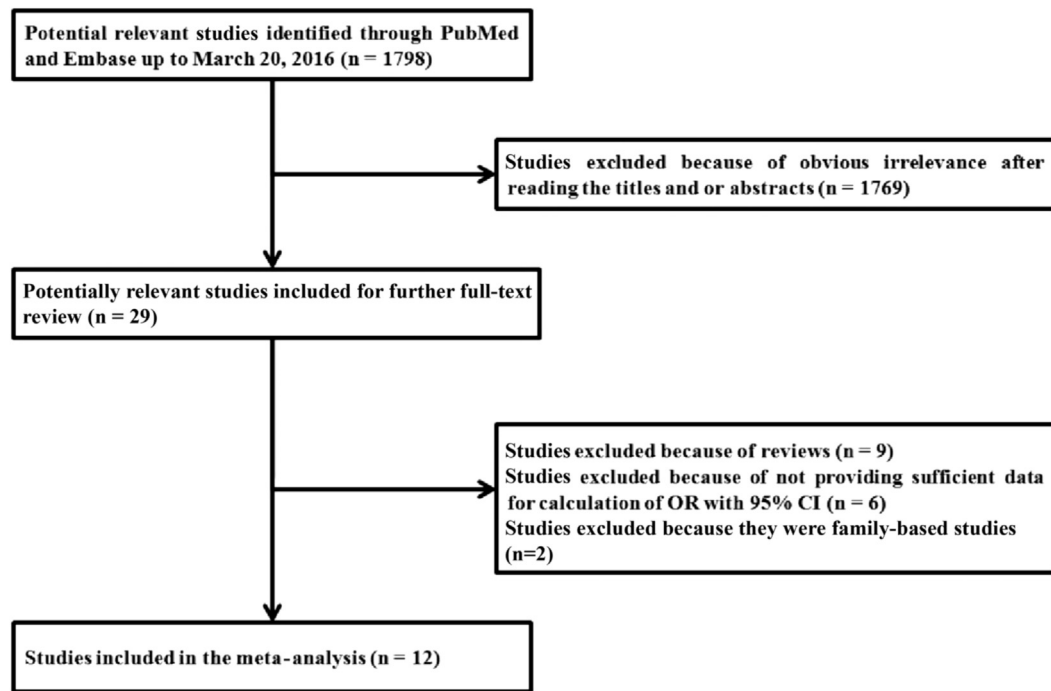


Figure 1: Selection of articles for inclusion in the meta-analysis.

Table 1: Characteristics of studies included in the meta-analysis.

Author & Year	Country	Continent	Mean age $\pm$ SD	Gender	SS	NS		Ost genotype			Cont genotype		
						Ost	Cont	GG	GC	CC	GG	GC	CC
Czerny et al., 2010	Poland	Europe	63.3 $\pm$ 5.1	Female	L	226	224	67	126	33	76	103	45
Magana et al., 2008	Mexico	America	34.3 $\pm$ 10.2	Female	S	70	70	56	13	1	42	25	3
Garnero et al., 2002	France	Europe	64.6 $\pm$ 8.8	Female	L	372	255	134	180	58	93	126	36
Deveci et al., 2012	Turkey	Europe	57.0 $\pm$ 7.0	Female	S	201	155	127	50	24	93	31	31
Mendez et al., 2013	Mexico	America	58.3 $\pm$ 6.8	Female	S	180	180	138	37	5	53	95	32
Maedeb 2009	France	Europe	70.0 $\pm$ 7.4	Female	S	92	69	34	47	11	30	30	9
Nordstrom et al., 2004	Sweden	Europe	75.0 $\pm$ 0.0	Female	L	232	544	68	121	43	167	246	131
Lee et al., 2010	South Korea	Asia	12.5 $\pm$ 1.4	Female	S	198	120	197	1	0	119	1	0
Korvala et al., 2010	Finland	Europe	20.3 $\pm$ 1.6	Male	S	72	120	15	42	15	35	56	29
Dincel et al., 2008	Turkey	Europe	74.5 $\pm$ 8.9	Mixed	S	20	17	0	10	10	0	7	10
Ferrari et al., 2004	USA	America	60.1 $\pm$ 9.5	Mixed	L	626	935	206	390	30	360	390	185
Moffett et al., 2004	USA	America	73.0 $\pm$ 5.0	Female	L	2634	742	869	1272	493	245	354	143

Notes USA = United States of America, SD = standard deviation, SS = sample size, L = large ( $\geq 400$  samples), S = small ( $< 400$  samples), NS = number of samples, Ost = osteoporosis, cont = control.

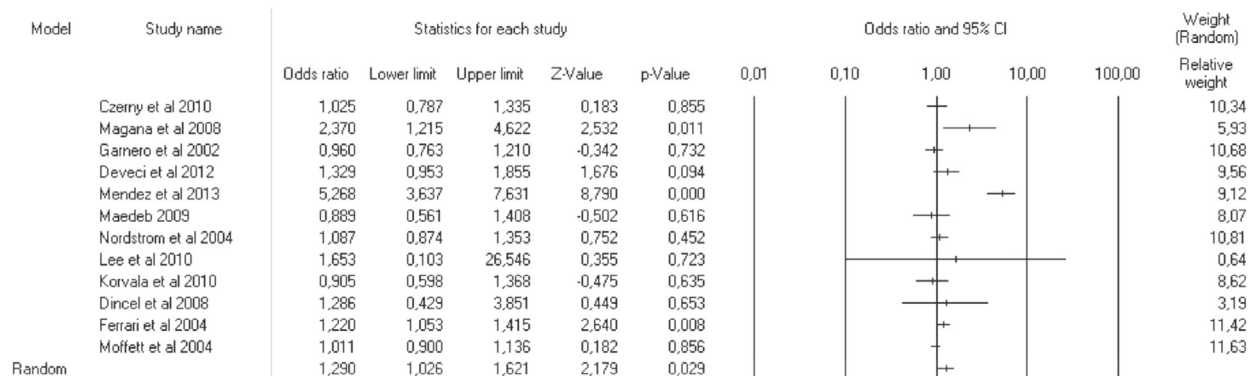


Figure 2: Meta-analysis of the association between the IL6 -174 G/C gene polymorphism and the risk of osteoporosis (G vs C).

**Table 2: Summary of ORs and 95% CIs in the association between the IL6 -174 G/C gene polymorphism and the risk of osteoporosis.**

No.	Genetic model	Parameter	All	Continent			Gender			Sample size	
				Europe	America	Asia	Female	Male	Mixed	S	L
1	G vs C	OR	1.29	1.04	1.90	1.65	1.37	0.90	1.22	1.62	1.07
		95%CI	1.03–1.62	0.93–1.17	1.11–3.23	0.10–26.54	1.00–1.87	0.60–1.37	1.05–1.41	0.87–3.03	1.00–1.15
		p	0.029	0.497	0.019	0.723	0.047	0.635	0.007	0.129	0.094
		p <sub>H</sub>	<0.001	0.711	<0.001	1.000	<0.001	1.000	<0.001	<0.001	0.296
2	GG vs GC + CC	OR	1.20	0.92	1.93	1.65	1.36	0.64	0.78	1.65	0.91
		95%CI	0.84–1.70	0.77–1.09	0.88–4.24	0.10–26.71	0.88–2.10	0.32–1.28	0.63–0.97	0.65–4.18	0.81–1.02
		p	0.313	0.333	0.100	0.722	0.163	0.205	0.024	0.291	0.102
		p <sub>H</sub>	<0.001	0.722	<0.001	1.000	<0.001	1.000	1.000	<0.001	0.478
3	GC vs GG + CC	OR	1.06	1.26	0.72	1.60	0.89	1.60	2.28	0.82	1.35
		95%CI	0.74–1.50	1.07–1.49	0.37–1.64	0.04–9.75	0.63–1.27	0.89–1.89	1.86–2.80	0.40–1.69	0.94–1.95
		p	0.762	0.005	0.433	0.722	0.535	0.118	<0.001	0.591	0.108
		p <sub>H</sub>	<0.001	0.627	<0.001	1.000	<0.001	1.000	0.476	<0.001	<0.001
4	C vs G	OR	0.77	0.96	0.53	0.60	0.73	1.10	0.82	0.62	0.94
		95%CI	0.62–0.97	0.86–1.08	0.31–1.90	0.04–9.72	0.53–0.10	0.73–1.67	0.70–0.95	0.33–1.15	0.87–1.01
		p	0.029	0.497	0.019	0.723	0.047	0.635	0.007	0.129	0.094
		p <sub>H</sub>	<0.001	0.711	<0.001	1.000	<0.001	1.000	0.926	<0.001	0.296
5	CC vs GG + GC	OR	0.58	0.77	0.31	NA	0.69	0.83	0.32	0.51	0.64
		95%CI	0.39–0.88	0.63–0.95	0.09–1.02	NA	0.49–0.96	0.41–1.67	0.10–1.03	0.29–0.92	0.36–1.14
		p	0.010	0.017	0.054	NA	0.028	0.595	0.056	0.024	0.131
		p <sub>H</sub>	<0.001	0.589	<0.001	NA	0.003	1.000	0.077	0.051	<0.001
		p <sub>E</sub>	0.584	<0.001	1.087	NA	0.355	<0.001	0.719	0.512	0.622

Notes OR = odds ratio, CI = confidence interval, p = p value based on a between-study Z test, p<sub>H</sub> = p value based on Q test for heterogeneity between studies, p<sub>E</sub> = p value based on Egger's test between studies, NA = not available, L = large (≥400 samples), S = small (<400 samples).

genetic model of the mixed gender subgroup (CC vs GG + GC p = 0.056), four genetic models of the small-sample-size subgroup (G vs C p = 0.129; GG vs GC + CC p = 0.291; GC vs GG + CC p = 0.591; C vs G p = 0.129), and all genetic models of the large-sample-size subgroup (G vs C p = 0.094; GG vs GC + CC p = 0.102; GC vs GG + CC p = 0.108; C vs G p = 0.094; CC vs GG + GC p = 0.131).

#### Source of heterogeneity

Evidence for heterogeneity (p < 0.10) between studies was found in all multiplicative models (G vs C p<sub>H</sub> < 0.001; GG vs GC + CC p<sub>H</sub> < 0.001; GC vs GG + CC p<sub>H</sub> < 0.001; C vs G p<sub>H</sub> < 0.001; CC vs GG + GC p<sub>H</sub> < 0.001). Therefore, the data in this study were assessed using a random effects model. A summary of the evidence of heterogeneity regarding the correlation between the IL6 -174 G/C gene polymorphism and the risk of osteoporosis is described in Table 2. In the subgroup analysis, evidence for heterogeneity was found in the American continent subgroup, female subgroup, mixed gender subgroup, small-sample-size subgroup, and large-sample-size subgroup. Therefore, a random effects model was used to calculate OR 95%CI in these subgroups. In contrast, no evidence of heterogeneity was found in the European continent subgroup, Asian continent subgroup, male subgroup, mixed gender subgroup, and large-sample-size subgroup. Therefore, a fixed effect model was used to calculate OR 95%CI in these subgroups.

#### Potential publication bias

Using Egger's test, no publication bias could be detected (G vs C p<sub>E</sub> = 0.337; GG vs GC + CC p<sub>E</sub> = 0.517; GC vs GG + CC p<sub>E</sub> = 0.534; C vs G p<sub>E</sub> = 0.337; CC vs GG + GC p<sub>E</sub> = 0.584). A summary of Egger's test regarding the correlation between the IL6 -174 G/C gene polymorphism and the risk of osteoporosis is described in Table 2. In the subgroup analysis, no publication bias was detected in the American continent subgroup (G vs C p<sub>E</sub> = 0.511; GG vs GC + CC p<sub>E</sub> = 0.768; GC vs GG + CC p<sub>E</sub> = 0.808; C vs G p<sub>E</sub> = 0.511; CC vs GG + GC p<sub>E</sub> = 1.087), female subgroup (G vs C p<sub>E</sub> = 0.415; GG vs GC + CC p<sub>E</sub> = 0.588; GC vs GG + CC p<sub>E</sub> = 0.456; C vs G p<sub>E</sub> = 0.415; CC vs GG + GC p<sub>E</sub> = 0.355), mixed gender subgroup (CC vs GG + GC p<sub>E</sub> = 0.719), small-sample-size subgroup (G vs C p<sub>E</sub> = 0.741; GG vs GC + CC p<sub>E</sub> = 1.056; GC vs GG + CC p<sub>E</sub> = 0.855; C vs G p<sub>E</sub> = 0.741; CC vs GG + GC p<sub>E</sub> = 0.512), and large-sample-size subgroup (GC vs GG + CC p<sub>E</sub> = 0.392; CC vs GG + GC p<sub>E</sub> = 0.622). In contrast, publication bias was detected in the European continent subgroup (G vs C p<sub>E</sub> < 0.001; GG vs GC + C p<sub>E</sub> < 0.001; GC vs GG + CC p<sub>E</sub> < 0.001; C vs G p<sub>E</sub> < 0.001; CC vs GG + GC p<sub>E</sub> < 0.001), Asian continent subgroup (G vs C p<sub>E</sub> < 0.001; GG vs GC + C p<sub>E</sub> < 0.001; GC vs GG + CC p<sub>E</sub> < 0.001; C vs G p<sub>E</sub> < 0.001), male subgroup (G vs C p<sub>E</sub> < 0.001; GG vs GC + C p<sub>E</sub> < 0.001; GC vs GG + CC p<sub>E</sub> < 0.001; C vs G p<sub>E</sub> < 0.001; CC vs GG + GC p<sub>E</sub> < 0.001), mixed gender subgroup (G vs C p<sub>E</sub> < 0.001; GG

vs GC + C  $p_E < 0.001$ ; GC vs GG + CC  $p_E < 0.001$ ; C vs G  $p_E < 0.001$ ), and large-sample-size subgroup (G vs C  $p_E = 0.044$ ; C vs G  $p_E = 0.044$ ).

## Discussion

IL6 is a cytokine involved not only in inflammation and infection responses but also in the regulation of metabolic, regenerative, and neural processes.<sup>14</sup> Overexpression of IL6 has been implicated in the pathology of a number of diseases including osteoporosis.<sup>13</sup> Several studies have reported the role of IL6 in the pathogenesis of osteoporosis. Scheidt-Nave et al.<sup>18</sup> found that serum IL6 was elevated in postmenopausal osteoporosis. Gaber et al.<sup>19</sup> showed that G/C promoter polymorphism at -174 of the IL-6 gene affected basal IL6 levels, and the C allele of the IL6 -174 G/C gene was associated with reduced gene expression and reduced plasma levels of IL6. Because of the effects of IL6 on the inflammatory response, a series of studies have focused on the contribution of polymorphisms within IL6 cluster genes to the osteoporosis risk. However, the results have been contradictory. This study reported the association between the IL6 -174 G/C gene polymorphism and the risk of osteoporosis, although the meta-analysis was limited due to the size and heterogeneity of the studies.

The results indicated that the IL6 -174 G/C gene polymorphism was associated with an increased (G vs C, OR 95%CI = 1.29 [1.03–1.62],  $p = 0.029$ ) and decreased risk of osteoporosis (C vs G, OR 95%CI = 0.77 [0.62–0.97],  $p = 0.029$ ; CC vs GG + GC, OR 95%CI = 0.58 [0.39–0.88],  $p = 0.010$ ). A summary of the ORs 95%CIs, correlation, heterogeneity, and Egger's test regarding the correlation between the IL6 -174 G/C gene polymorphism and the risk of osteoporosis is described in Table 2, and the study characteristics are described in Table 1. A forest plot showing the correlation between the IL6 -174 G/C gene polymorphism and the risk of osteoporosis is described in Figure 2 for G vs C. Previous similar meta-analyses have reported the correlation between IL6 gene polymorphism and the risk of osteoporosis and fracture. Wang et al.<sup>33</sup> studied the association between the IL6 -634 C/G and -174 G/C polymorphisms with bone mineral density (BMD). They showed that the -634 C/G and -174 G/C polymorphisms had modest effects on BMD. However, they did not include an analysis of each genetic model and did not focus on the risk of osteoporosis; they only correlated the polymorphisms to BMD score. Our study only focused on the risk of osteoporosis and provided the results of an analysis of several genetic models. Therefore, these results are expected to be complementary to several gene studies regarding the risk of osteoporosis. Another study by Wang et al.<sup>34</sup> evaluated the association between the IL6 -174 G/C gene polymorphism and the risk of fracture. They showed that the IL6 -174 C/G gene polymorphism was associated with an increased risk of wrist and osteoporotic fracture (OR 95%CI = 1.60 [1.12–2.28]). A study by Zhang et al.<sup>35</sup> analysed the association between IL6 -174 G/C and fracture risk. They found that the IL6 -174 G/C polymorphism contributed to the development of fracture (OR 95%CI = 1.32 [1.10–1.58]). However, the study characteristics were not included, and

only six studies were included in the Wang et al.<sup>34</sup> and Zhang et al.<sup>35</sup> studies. Thus, a larger sample size was needed to determine a better association. Our study used a larger sample size. Therefore, the results of our study are expected to provide superior results. Furthermore, in the subgroup analysis, the IL6 -174 G/C gene polymorphism was associated with the risk of osteoporosis in two genetic models of the European continent subgroup (GC vs GG + CC; CC vs GG + GC), two genetic models of the American continent subgroup (G vs C; C vs G), three genetic models of the female subgroup (G vs C; C vs G; CC vs GG + GC), four genetic models of the mixed gender subgroup (G vs C; GG vs GC + CC; GC vs GG + CC; C vs G), and one genetic model of the small-sample-size subgroup (CC vs GG + GC). In contrast, the IL6 -174 G/C gene polymorphism had no significant association with the risk of osteoporosis in three genetic models of the European continent subgroup (G vs C; GG vs GC + CC; C vs G), three genetic models of the American continent subgroup (GG vs GC + C; GC vs GG + CC; CC vs GG + GC), all genetic models of the Asian continent subgroup, two genetic models of the female subgroup (GG vs GC + CC; GC vs GG + CC), all genetic models of the male subgroup, one genetic model of the mixed gender subgroup (CC vs GG + GC), four genetic model of the small-sample-size subgroup (G vs C; GG vs GC + CC; GC vs GG + CC; C vs G), and all genetic models of the large-sample-size subgroup. However, these results should be interpreted with caution considering that the relatively small sample size or multiple testing could have resulted in false-positive findings.

These results also indicated that the G allele of the IL6 -174 G/C gene was correlated with susceptibility to osteoporosis, whereas the C allele was correlated with a reduced risk of osteoporosis. See Table 2 for a detailed summary of the ORs 95%CIs regarding the correlation between the IL6 -174 G/C gene polymorphism and the risk of osteoporosis. Theoretically, these results are clearly unexplainable. However, several studies have supported these results. Scheidt-Nave et al.<sup>18</sup> conducted a study regarding the role of serum IL6 as a predictor of bone loss in 137 postmenopausal German women. They found that serum IL6 was a predictor of postmenopausal bone loss and that the effect appears to be most relevant through the first postmenopausal decade. A study by Ershler and Keller<sup>36</sup> revealed that after menopause, IL6 levels are elevated, even in the absence of infection, trauma, or stress, and this condition was associated with low BMD levels. Li et al.<sup>37</sup> conducted a study regarding the contribution of IL6 to the osteogenesis of osteoporotic mice. They showed that IL6 over-secretion impaired osteogenesis in osteoporotic mice. They also showed that in vivo administration of IL6 neutralizing antibody was helpful to rescue the osteoporotic phenotype in the mouse vertebral body. De Benedetti et al.<sup>38</sup> conducted a study regarding in vivo neutralization of IL6 by human IL-6 (hIL-6) receptor antagonist in osteoporotic mice. They found that immunization with hIL-6 receptor antagonist could represent a novel and simple therapeutic approach for the specific neutralization of IL6. A study by Theoharides et al.<sup>39</sup> also found that serum IL6 was elevated in osteoporosis patients and was correlated with severity of symptoms. Based on these studies, it can be concluded that serum IL6 levels have a correlation with osteoporosis. Serum IL6 levels are

determined by –174 G/C promoter polymorphism. No study has reported the association of serum IL levels with –174 G/C promoter polymorphism in osteoporotic models. However, several studies have reported this association in other disease models. Gaber et al.<sup>19</sup> conducted a study regarding the correlation between serum IL6 and –174 G/C promoter polymorphism in rheumatoid arthritis patients. They found that the G allele of the IL6 –174 gene had a significant positive correlation with serum IL6 levels, whereas the C allele had a significant negative correlation with serum IL6 levels. Belluco et al.<sup>40</sup> conducted a study regarding the association between serum IL6 levels and the –174 G/C gene polymorphism in patients with colorectal cancer. They found that C– subjects produced higher IL6 levels than did C+ subjects. Tonet et al.<sup>41</sup> conducted a study regarding the correlation between serum IL6 levels and the –174 G/C gene polymorphism in patients with cardiovascular disease. They found that serum IL6 levels were markedly lower in the C allele, whereas the G allele displayed a trend towards higher levels of circulating IL6. Fishman et al.<sup>42</sup> conducted a study regarding the association between serum IL6 levels and the –174 G/C gene polymorphism in patients with systemic-onset juvenile chronic arthritis. They found that the C allele was associated with significantly lower levels of plasma IL6. Burzotta et al.<sup>43</sup> conducted a study regarding the association between serum IL6 levels and the –174 G/C gene polymorphism in patients with cardiovascular disease. They showed that the G allele was associated with higher levels of serum IL6. Therefore, the results of this study showing that the G allele of the IL6 –174 G/C gene polymorphism correlated with a susceptibility to osteoporosis are understandable because the G allele of the IL6 –174 G/C gene polymorphism has an association with increased levels of serum IL6.

Osteoporosis is a very common multifactorial progressive skeletal or metabolic bone disorder in the elderly characterized by low bone density and microarchitectural deterioration of bony tissue or BMD more than 2.5 SDs below the young normal mean.<sup>1–3</sup> The role of IL6 in the osteoporosis process is complex. The balance between bone resorption caused by osteoclasts and bone formation caused by osteoblasts plays an important role in bone metabolism. The lower serum oestrogen levels in postmenopausal women cause an imbalance between osteoblasts and osteoclasts.<sup>44</sup> IL6, a multifunctional cytokine involved in osteoclast differentiation, is secreted by osteoblasts and appears to be a key molecule in the osteoporotic process.<sup>45</sup> The expression of the IL6 gene in osteoblasts and bone marrow stromal cells is down-regulated by oestrogen.<sup>44</sup> IL6 is widely recognized as a potent stimulator of osteoclast-driven bone absorption in the context of chronic inflammation and oestrogen deficiency.<sup>46</sup> The mechanism of IL6 in the process of osteoporosis is twofold, occurring both inside and outside the osteoclast cells. In osteoclast cells, IL6 has an important role in bone resorption by activating immature osteoclasts. Under normal conditions, oestrogen inhibits the IL6 promoter in the absence of a functional oestrogen receptor (ER) binding site. This process is mediated by nuclear factor – kappa B (NF- $\kappa$ B) and CCAAT-enhancer-binding proteins (C/EBP)  $\beta$ .<sup>44</sup> The ER impairs IL6 induction by preventing c-rel and, to a lesser extent, RelA proteins from binding to the NF- $\kappa$ B site of the IL6

promoter.<sup>47</sup> ER directly interacts with the nuclear factor interleukin-6 (NF-IL6) and NF- $\kappa$ B and inhibits their ability to bind deoxyribonucleic acid (DNA), which might be the molecular basis for repression of IL6 gene expression by oestrogens.<sup>48</sup> The physical and functional interaction depends on the DNA binding domain and region D of the ER and on the Rel homology domain of NF- $\kappa$ B and the basic leucine zipper (bZIP) region of C/EBP  $\beta$ . The cross-coupling among ER, NF- $\kappa$ B, and C/EBP  $\beta$  also results in reduced activity of promoters with the ER binding sites.<sup>44</sup> This leads to a balance between osteoblast and osteoclast activity. However, in pathological states or oestrogen deficiency, IL6 acts to activate immature osteoclasts without restriction, mediated by NF- $\kappa$ B, C/EBP  $\beta$ , and nuclear factors of activated T-cells cytoplasmic 1 (NFATc1).<sup>44</sup> This leads to an imbalance between osteoblast and osteoclast activity, where osteoclast activity is more dominant than osteoblast activity, causing bone loss and osteoporosis. Outside osteoclast cells, after release from osteoblast-lineage cells,<sup>49</sup> IL6 first binds to IL6R and forms a complex with gp130 to stimulate intracellular signalling machinery. IL6 then stimulates osteoblastic downstream production of signalling molecules, especially RANKL, which subsequently enhances osteoclast formation and activity.<sup>46</sup> IL6 induces the expression of RANKL on the surface of osteoblasts. RANKL then interacts with receptor activator of nuclear factor – kappa B (RANK) expressed on osteoclast progenitors, inducing osteoclast differentiation via the RANK signalling pathway, which involves NF- $\kappa$ B, c-Jun N-terminal kinase (JNK), and p38. IL6 also directly acts on osteoclast progenitors to suppress their differentiation via an inhibition of RANK signalling pathways. IL6 specifically suppresses RANK-mediated I kappa B (I $\kappa$ B) degradation and JNK activation. IL6 and RANKL up-regulate the transcription of MKP1 and MKP7, which encode enzymes that dephosphorylate JNK, and down-regulate the transcription of sentrin-specific protease 2 (Senp2) and cullin 4A (Cul4A), which are related to the ubiquitin pathway.<sup>17</sup> Furthermore, the activity of IL6 on osteoclasts frequently interplays with IL1 and tumour necrosis factor (TNF), and IL6 increases the stimulatory effect of IL1 and TNF on bone resorption by increasing the osteoclastic progenitor pool.<sup>46</sup> These mechanisms are thought to underlie the results of this study that showed a correlation between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis.

There were several limitations to the meta-analysis. First, our analysis was primarily based on unadjusted effect estimates. Therefore, the potential covariates, including age, gender, menopausal factors, history of trauma, and smoking habits, were not controlled for. Second, the possibility of a false negative exists due to the small size of the studies, even when combined. Thus, further studies with larger sample sizes are required to investigate the associations.

## Conclusions and suggestions

In summary, our meta-analysis suggested that the G allele of the IL6 –174 G/C gene polymorphism was associated with an increased risk of osteoporosis, whereas the C allele of the IL6 –174 G/C gene polymorphism was associated with a

decreased risk of osteoporosis. Further studies considering gene–environment interactions should be conducted to investigate the associations between the IL6 –174 G/C gene polymorphism and the risk of osteoporosis.

### Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

### Author contributions

Conceived and designed the experiments = Jonny Karunia Fajar (JKF), Azharuddin Azharuddin (AA). Performed the experiments = JKF. Analysed the data = JKF. Contributed reagents/materials/analysis tools = JKF. Wrote the manuscript = JKF, AA. Reference collection and data management = JKF, AA. Statistical analyses and paper writing = JKF. Study design = JKF, AA.

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