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Original Article

Association of *COL1A1* polymorphisms with osteoporosis: a meta-analysis of clinical studies

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Abstract: Objective: To conduct a meta-analysis of all association studies on two of the collagen 1 alpha 1 (*COL1A1*) gene polymorphisms, the -1997G/T (rs1107946) and the -1663indelT (rs2412298) polymorphisms and osteoporosis/BMD and fracture. Methods: PubMed/Medline and Web of Knowledge were searched for relevant association studies published in English. Pooled OR and its corresponding 95% CI or pooled MD and its corresponding 95% CI was calculated with the Cochrane Review Manager (Revman, version 5.2) using a random-effect or a fixed effect model. Results: No significant association between the -1997G/T polymorphism and Lumbar Spine (LS) and Femoral Neck (FN) BMD except for the Caucasian subpopulation wherein subjects with the T allele of the -1997G/T polymorphism was associated with significantly higher LS BMD. Our analysis did reveal that women, especially postmenopausal or perimenopausal women with the GG genotype, had significantly higher Total Hip (TH) BMD than those with the GT. Additionally, our meta-analysis did not show significant association between the -1997G/T polymorphism and risk of fracture, between the -1663indelT polymorphism and LS BMD in postmenopausal or perimenopausal women, or between the -1663indelT polymorphism and the risk of fracture. Conclusions: Our results suggested the possibility of the *COL1A1* -1997G/T and the -1663indelT polymorphisms individually playing very little role in osteoporosis and fracture, although more studies are needed especially for the analysis of association between these two polymorphisms and fracture. Haplotype studies may become one important future direction of study to further elucidate whether and how various *COL1A1* polymorphisms affect bone health, osteoporosis and fracture.

Keywords: *COL1A1,* -1997G/T, -1663indelT, polymorphism, osteoporosis, bone mineral density, fracture

Introduction

Osteoporosis is a common systemic bone disease, it is characterized by low bone mass, reduced bone mineral density (BMD) and deterioration of bone microarchitecture, which leads to increased bone fragility and increased risk of fracture [1]. It commonly occurs in elderly people and affects approximately 200 million adults worldwide, approximately 30% of all postmenopausal women in Europe and USA have osteoporosis [2]. Studies have shown that osteoporosis is a polygenic disease with a strong genetic component, although there have been many reports on numerous genes being associated with reduced BMD and increased risk of osteoporosis and fracture, different studies often generated inconsistent or conflicting results [3-5].

Type I collagen is the most abundant protein constituent of bone matrix and genes encoding Type I collagen are considered strong candidate genes that could be important in BMD regulation, therefore, potential associations between polymorphisms within collagen 1 alpha 1 (*COL1A1*) gene and osteoporosis has been subjects of numerous studies, however, results from different studies were often conflicting [6-14]. Among various polymorphisms within the *COL1A1* gene, the most frequently studied polymorphism has been the +1245G/T polymorphism (rs1800012, Sp1), it is a G to T polymorphism in the first intron of *COL1A1* at the base of it binding site for the transcription factor Sp1, and it has been found that collagen produced from osteoblasts with heterozygotes for this polymorphism has an increased ratio of *COL1A1* mRNA compared to *COL1A2* [6, 7], it is cur-

rently the prevailing view that the T allele of the Sp1 polymorphism is associated with reduced BMD and increased risk of osteoporosis and fracture, especially in postmenopausal women [8-12]. In 2002, Garcia-Giralt et al. identified two new single nucleotide polymorphisms in the *COL1A1* promoter region, the -1997G/T (rs1107946) and the -1663indelT (rs2412298) polymorphisms and found that the T allele of the 1997G/T polymorphism has significant association with reduced lumbar spine and femoral neck BMD although the -1663indelT polymorphism did not show such significant association [13]. Since then, studies on these two new polymorphisms and their association with BMD, osteoporosis and risk of fracture have yielded conflicting and inconclusive results [10-23]. The only meta-analysis on these two newer *COL1A1* polymorphisms was done in 2011 by Jin et al [8] which found that the GG genotype of the 1997G/T polymorphism was associated with significantly higher lumber spine BMD than the GT genotype in female (P=0.02), whereas in the whole population, there was only a borderline significant association between this polymorphism and BMD using a fixed-effect model. Jin et al. further found significant association between -1663indelT polymorphism and lumbar spine BMD using a fixed effect model (P= 0.03), but there was no significant association between either of these two polymorphisms and risk of fracture. Since there was very limited number of studies included in the meta-analysis, Jin et al. concluded that "further studies are required to fully evaluate the contribution of the -1997G/T and -1663in/delT sites to these phenotypes" [8]. Since then, there have been several more relevant studies published [10, 15, 16, 19], further, Jin et al. only included studies on subjects with fracture in its meta-analysis, it did not include studies such as Garcia-Giralt et al. [13], Lau et al. [21] and Jin et al. [23] which focused on the association between the -1997G/T or the -1663indelT polymorphism and BMD in subjects without regard to whether they had fracture or not. In this article, we performed an updated meta-analysis on associations between the -1997G/T and the -1663indelT polymorphisms and BMD and risk of fracture incorporating the later published studies and also studies on subjects without regards to whether they had fractures, such a meta-analysis would provide more information on whether the -1997G/T and -1663indelT polymorphisms play any role in osteoporosis and fracture.

Methods

Search strategy

PubMed/Medline and Web of Science (Web of Knowledge) were searched up to August 2014 to identify all association studies on the -1997G/T or the -1663indelT polymorphism and osteoporosis or fracture using the following search terms: "COL1A1", "COLIA1", "collagen", "polymorphism", "SNP", "genetic variant", "bone mineral density" "polymorphic", "BMD", "osteoporosis", "osteoporotic", "fracture", "bone loss", "bone mass".

Inclusion and exclusion criteria

All association studies published in English reporting association between the -1997G/T or the -1663indelT polymorphism and osteoporosis/BMD or fracture were considered potentially eligible for inclusion into our meta-analysis. Reviews and meeting abstracts were excluded.

Two contributing authors (PX and BL) independently considered and selected potentially eligible studies by reviewing titles and abstracts of the studies identified by the search and then reviewing their full text. Any disagreement regarding whether a particular study should be included was resolved by discussions among all of the authors to reach a consensus.

Outcome measures

Data on the following outcome measures were analyzed: 1) For the -1997G/T polymorphism, Means ± standard deviations (SD) values of the Lumbar Spine (LS), Femoral Neck (FN) and Total Hip (TH) BMD of the individuals in each genotype group (GG, GT and TT) were compared (GG vs GT, GG vs TT and GT vs TT); 2) For the -1663indelT polymorphism, Means ± SD values of the LS BMD of the individuals in each genotype group (InsIns [II], InsDel [ID] and DelDel [DD]) were compared (II vs ID, II vs DD and ID vs DD); and 3) For the -1997G/T and the -1663-indelT polymorphisms, the number of individuals in each genotype group with or without fracture.

Data extraction

Two authors (PX and BL) independently extracted data from the included studies and they resolved any disagreement by discussion. For

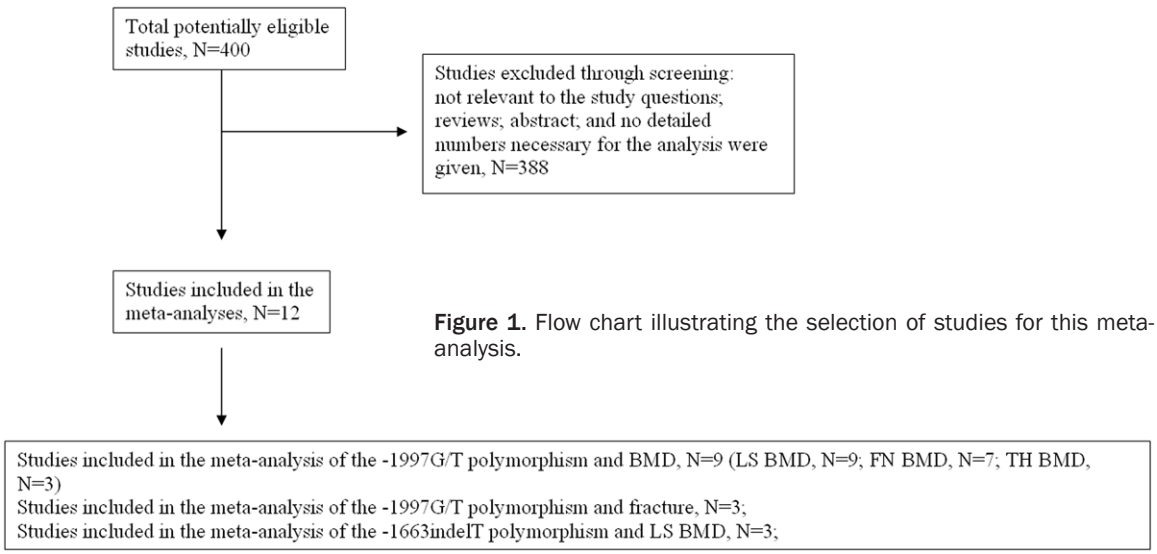

Figure 1. Flow chart illustrating the selection of studies for this meta-analysis.

outcome measure Numbers 1 and 2, relevant group means ± standard deviations (SD) were extracted from each included study whenever possible. When relevant values were presented only as means ± standard errors (SE), SD was extracted as SE x square root of sample size [24]. For outcome measure Number 3, the numbers of individuals in each genotype group with or without fracture were extracted.

Quality of the studies

Two contributing authors (PX and BL) independently assessed included studies' adequacy in four key areas (methodological, genetic, clinical and statistical). All of the studies were then screened with the Newcastle-Ottawa Scale [25].

Statistical analysis

Statistical analysis was performed with the Cochrane Review Manager (RevMan, version 5.2). Continuous outcomes (our outcome measure Numbers 1 and 2) was analyzed using Mean Difference (MD) while Dichotomous outcomes (our outcome measure Number 3) were analyzed using pooled Odds Ratio (OR). In addition, for the dichotomous outcomes, since the type of inheritance of the -1997G/T and the -1663indelT polymorphisms were unknown, we used three biallelic analysis models: the biallelic frequency model (BFM), the biallelic dominant model (BDM) and the biallelic recessive model (BRM). Pooled OR with its corresponding 95% CI and MD with its corresponding 95% CI were calculated first with a random-effect model because it assumes a genuine diversity in the results of the included studies due to between-studies heterogeneity and thus incorporates a between-studies variance into the calculation [26]. We used the *Z* test to evaluate the statistical significance of the pooled OR and MD. We assessed between-studies heterogeneity with chi-square (x^2) test based on Cochran Q statistic [27] and we further used I^2 index to assess the heterogeneity, wherein an I^2 value around 25%, 50% and 75% represented low, moderate and large heterogeneity, respectively [28]. When no heterogeneity was found using the random-effect model, data was then analyzed using a fixed-effect model and result from the fixed-effect model was used. A *P* value of <0.05 was considered to be statistically significant except for the Q statistics wherein a *P* value of <0.10 was considered to be statistically significant.

For analysis on association between the -1997G/T polymorphism and BMD, Sub-population analysis was performed according to ethnicity and gender of the individuals studied whenever there were enough studies. Additional sub-population analysis was also performed according to whether the female subjects were premenopausal or postmenopausal whenever possible. For analysis on association between the -1997G/T and the -1663indelT polymorphisms and risk of fracture, sub-population analysis based on fracture types was also performed. In addition, publication bias in the analysis was assessed using funnel plots.

Table 1. Characteristics of studies included in the meta-analysis of *COL1A1* gene polymorphisms andosteoporosis or risk of fracture

First author	Study design	Population ethnicity and relevant characteristics	Number	Male/Female	Mean age
Garcia-Giralt 2002 [13]	Cross-sectional	Spanish postmenopausal	256	0/256	51.1±5.6
Lau 2005 [21]	Population based cohort association study	Chinese	531 (262 premenopausal and 269 postmenopausal)	0/531	48.7±17.3
Yamada 2005 [20]	Population based prospective cohort study	Japanese	2236	1126/1110	Women *COL1A1* -1997G/T GG 60.0±0.5, GT 58.9±0.5, TT 58.4±0.8; Men GG 58.5±0.5, GT 59.7±0.5, TT 59.2±0.9
Stewart 2006 [14]	Population-based association study	-	5119	0/5119	48.4±2.3
Bustamante 2007 [22]	Cross-sectional	Spanish postmenopausal women	719	0/719	54.7±8.5
Yazdanpanah 2007 [12]	Population based	Elderly Caucasian	5826	2452/3374	Women 68.3±8.2; Men 67.6±7.7
Husted 2009 [11]	Case-control study	-	291 vertebral fracture patients and 283 normal controls	Fracture patients 63/228; Normal controls: 57/226	Fracture women 65.1±8.2; fracture men 60.2±14.1; normal control women 65.3±8.2; normal men 59.3±14.3
Jin 2009 [23]	Case-control	Caucasian	98 cases (hip fracture) and 3418 controls (perimenopausal 3275 and postmenopausal 143)	Case 23/75; Controls 0/3418	Cases 81.6±8.9; Perimenopausal controls 48.5±2.4; Postmenopausal controls 71.1±5.6
Gonzalez-Bofill 2011 [15]	Cross-sectional study	Danish perimenopausal	1717	0/1717 (on hormone therapy [HT]): randomized 435, by choice 191; no HT: randomized 437, by choice 651)	On HT: randomized 50.0±2.7, by choice 50.6±2.7; No HT: randomized 50.6±2.8, by choice 51.1±2.9
Urreizti 2012 [1]	Case-control	Spanish	203 cases (hip fracture) and 397 Control	Cases 37/166; Control 0/397	Cases (Barcelona) 85.0±7.8, Cases (Cantabria) 77.4±6.8; Controls 67.9±6.9
Rojano-Mejia 2013 [10]	Population-based observational study	Mexican-Mextizo postmenopausal	750	0/750	60.0±7.55
Singh 2013 [16]	Cross-sectional study	Asian (Indian), postmenopausal	349 (145 osteoporotic, 87 osteopenic and 117 normal)	0/349	58.8±9.0 (osteoporotic 58.5±8.4; osteopenic 59.1±7.6; normal 58.3±8.2)

Table 2. Studies included in the meta-analysis of *COL1A1* -1997G/T (rs1107946) and bone mineral density (BMD) (g/cm^2)

First author	*GG*		*GT*		*TT*		*P* value
	N	BMD	N	BMD	N	BMD	
Garcia-Giralt 2002 [13]	194	Lumbar (LS) 0.890±0.129	56	LS 0.918±0.157	6	LS 0.823±0.100	Analysis of variance (ANOVA) 0.014
Lau 2005 [21]	Pre/postmenopausal 128/141	Pre/Post-menopausal LS 0.983±0.102/0.766±0.131; Femoral neck (FN) 0.749±0.091/0.591±0.095; Total hip (TH) 0.838±0.091/0.684±0.107	Pre/postmenopausal 109/99	Pre/Post-menopausal LS 0.975±0.104/0.781±0.129; FN 0.744±0.094/0.602±0.090; TH 0.830±0.084/0.684±0.109	Pre/postmenopausal 25/29	Pre/postmenopausal LS 0.957±0.105/0.781±0.124; FN 0.714±0.090/0.608±0.092; TH 0.813±0.085/0.703±0.108	
Yamada 2005 [20]	Women 407 (pre/postmenopausal 94/306); Men 457	Women LS 0.855±0.121 (pre/postmenopausal 1.018±0.116/0.798±0.122), FN 0.672±0.081 (pre/postmenopausal 0.782±0.087/0.634±0.087); Men LS 0.990±0.150, FN 0.754±0.107	Women 526 (pre/postmenopausal 140/377); Men 511	WomenLS 0.870±0.138 (pre/postmenopausal 1.026±0.118/0.813±0.136), FN 0.681±0.092 (pre/postmenopausal 0.767±0.095/0.650±0.078); Men LS 0.975±0.158, FN 0.754±0.090	Women 177 (pre/postmenopausal 44/132); Men 158	Women LS 0.878±0.133 (pre/postmenopausal 1.044±0.119/0.821±0.127), FN 0.680±0.093 (pre/postmenopausal 0.771±0.093/0.647±0.080); Men LS 0.983±0.151, FN 0.744±0.101	Women LS 0.039 *GT+TT* vs. *GG*; FN postmenopausal 0.*034 GT vs. GG*; FN postmenopausal 0.011 *GT+TT vs. GG*
Stewart 2006 [14]	2247	Baseline LS 1.053±0.190, FN 0.882±0.143; 6-year follow-up LS 1.037±0.476, FN 0.844±0.333	699	Baseline LS 1.064±0.158, FN 0.890±0.132; 6-year follow-up LS 1.048±0.290, FN 0.852±0.211	65	Baseline LS 1.111±0.145, FN 0.894±0.112; 6-year follow-up LS 1.109±0.185, FN 0.878±0.128	ANOVA Baseline LS 0.003, FN 0.196; Follow-up LS 0.003, FN 0.039
Bustamante 2007 [22]	459	Adjusted LS 0.86±0.214	134	Adjust LS 0.88±0.116	12	Adjust LS 0.84±0.139	General model 0.06; Dominant model 0.04
Yazdanpanah 2007 [12]	1663 Men 2157 Women	Men LS 1.16±0.19; Men FN 0.92±0.13; Women LS 1.03±0.18; Women FN 0.83±0.14	494 Men 710 Women	Men LS 1.17±0.20; Men FN 0.92±0.14; Women LS 1.04±0.18; Women FN 0.84±0.13	37 Men 53 Women	Men LS 1.15±0.22; Men FN 0.90±0.14; Women LS 1.01±0.18; Women FN 0.85±0.13	ANOVA Men LS 0.96; Men FN 1.00; Women 0.69; Women 0.22
Gonzalez-Bofill 2011 [15]	1252	LS 1.030±0.137; FN 0.800±0.113; TH 0.921±0.116	420	LS 1.016±0.147; FN 0.786±0.118; TH 0.904±0.123	44	LS 0.988±0.124; FN 0.765±0.109; TH 0.887±0.109	ANOVA, test for trend: LS 0.04; FN 0.03; TH 0.01
Rojano-Mejia 2013 [10]	380	LS 0.856±0.136; FN 0.745±0.126; TH 0.917±0.135	298	LS 0.841±0.143; FN 0.727±0.053; Total hip (TH) 0.91±0.134	72	LS 0.848±0.139; FN 0.724±0.104; Total hip (TH) 0.908±0.147	ANOVA LS 0.489; FN 0.305; TH 0.683
Singh 2013 [16]	247	Lumbar (LS) 0.86±0.16; Femoral neck (FN) 0.81±0.16	84	LS 0.91±0.23; FN 0.87±0.17	18	LS 0.93±0.18; FN 0.85±0.19	ANOVA LS 0.04; FN 0.01

Table 3. Studies included in the meta-analysis of *COL1A1* -1997G/T (rs1107946) and risk of fracture

First author	Fracture					Control					*P* value
	GG	GT	TT	G	T	GG	GT	TT	G	T	
Husted 2009 [11]	199 (M/F 41/158)	84 (M/F 21/63)	8 (M/F 1/7)	482 (M/F 103/379)	100 (M/F 23/77)	216 (M/F 41/175)	61 (M/F 15/46)	6 (M/F 1/5)	493 (M/F 97/396)	73 (M/F 17/56)	-
Jin 2009 [23]	68	26	4	162	34	2353 (2247 perimenopausal and 106 postmenopausal)	734 (699 perimenopausal and 35 postmenopausal)	67 (65 perimenopausal and 2 postmenopausal)	5440 (5193 perimenopausal and 247 postmenopausal)	868 (829 perimenopausal and 39 postmenopausal)	
Urreizti 2012 [19]	156	43	4	355	51	301	89	7	691	103	0.772

Table 4. Studies included in the meta-analysis of *COL1A1* -1663indelT (rs2412298) and BMD) (g/cm^2)

First author	*II*		*ID*		*DD*		*I*		*D*		*P* value
	N	BMD	N	BMD	N	BMD	N	BMD	N	BMD	
Garcia-Giralt 2002 [13]	155	LS 0.897±0.131	84	LS 0.882±0.145	17	LS 0.932±0.110	-	-	-	-	ANOVA 0.239
Stewart 2006 [14]	1967	Baseline LS 1.059±0.177, FN 0.885±0.133; 6.5-year follow-up LS 1.040±0.443, FN 0.847±0.310	869	Baseline LS 1.054±0.176, FN 0.880±0.117; 6.5-year follow-up LS 1.037±0.324, FN 0.842±0.206	151	Baseline LS 1.019±0.233, FN 0.860±0.110; 6.5-year follow-up LS 1.008±0.208, FN 0.824±0.159					ANOVA Baseline LS 0.006, FN 0.024; Follow-up LS 0.117, FN 0.074
Gonzalez-Bofill 2011 [15]	1143	LS 1.025±0.138; FN 0.796±0.116; TH 0.916±0.118	510	LS 1.025±0.142; FN 0.794±0.113; TH 0.914±0.117	56	LS 1.048±0.119; FN 0.800±0.111; TH 0.929±0.128	-	-	-	-	ANOVA LS 0.49; FN 0.93; TH 0.67

Table 5. Studies included in the meta-analysis of *COL1A1* -1663indelT (rs2412298) and risk of fracture

First author	Fracture					Control					*P* value
	ins/ins (II)	*ins/del (ID)*	*del/del (DD)*	*Ins*	*Del*	*II*	*ID*	*DD*	*Ins*	*Del*	
Husted 2009 [11]	193 (M/F 42/151)	82 (M/F 17/65)	15 (M/F 4/11)	468 (M/F 101/367)	112 (M/F 25/87)	187 (M/F 33/154)	87 (M/F 23/64)	8 (M/F 1/7)	461 (M/F 89/372)	103 (M/F 25/78)	-
Jin 2009 [23]	69	23	6	161	35	2062 (1967 perimenopausal and 95 postmenopausal)	912 (869 perimenopausal and 43 postmenopausal)	156 (151 perimenopausal and 5 postmenopausal)	5036 (4803 perimenopausal and 213 postmenopausal)	1224 (1171 perimenopausal and 53 postmenopausal)	-
Urreizti 2012 [19]	127	65	11	319	87	240	138	19	618	176	0.921

Table 6. Meta-analysis of *COL1A1* -1997G/T polymorphism and BMD (g/cm^2)

Comparison	BMD (g/cm^2)	Number of Studies	Number of Subjects	Overall MD [95% CI]; *P*; I^2	Gender-based Subgroups MD [95% CI]; *P*; I^2	Ethnicity-based subgroups MD [95% CI]; *P*; I^2
GG vs GT	Lumbar Spine (LS) BMD	9 [10, 12-16, 20-22]	9725 vs 4131	-0.00 [-0.01, 0.00]; *P*=0.34; I^2=42	Women overall: -0.01 [-0.02, 0.00]; *P*=0.23; I^2=41; Post- or Peri-menopausal women: -0.01 [-0.02, 0.00]; *P*=0.21; I^2=49; Premenopausal women: -0.00 [-0.02, 0.02]; *P*=0.92; I^2=11 Men: 0.00 [-0.02, 0.03]; *P*=0.84; I^2=68	Asian population: -0.01 [-0.02, 0.01]; *P*=0.44; I^2=47; Caucasian population: -0.00 [-0.02, 0.02]; *P*=0.87; I^2=61; Spanish or Mexican: -0.01 [-0.04, 0.02]; *P*=0.61; I^2=63
GG vs TT	LS BMD	9 [10, 12-16, 20-22]	9725 vs 695	-0.00 [-0.02, 0.02]; *P*=0.96; I^2=53	Women overall: -0.00 [-0.02, 0.02]; *P*=0.85; I^2=60; Post- or Peri-menopausal women: -0.00 [-0.03, 0.02]; *P*=0.82; I^2=64; Premenopausal women: 0.00 [-0.05, 0.05]; *P*=0.94; I^2=55 Men: 0.01 [-0.02, 0.03]; *P*=0.57; I^2=0	Asian population: -0.01 [-0.03, 0.01]; *P*=0.35; I^2=30; Caucasian population: 0.03 [0.00, 0.06]; *P*=0.03; I^2=0; Spanish or Mexican: 0.02 [-0.01, 0.05]; *P*=0.25; I^2=0
GT vs TT	LS BMD	9 [10, 12-16, 20-22]	4131 vs 695	0.00 [-0.01, 0.02]; *P*=0.79; I^2=26	Women overall: 0.00 [-0.01, 0.02]; *P*=0.69; I^2=36;Post- or Peri-menopausal women: 0.00 [-0.02, 0.03]; *P*=0.72; I^2=47; Premenopausal women: 0.00 [-0.03, 0.04]; *P*=0.76; I^2=0 Men: -0.00 [-0.03, 0.02]; *P*=0.72; I^2=0	Asian population: -0.00 [-0.02, 0.01]; *P*=0.55; I^2=0; Caucasian population: 0.03 [-0.00, 0.06]; *P*=0.06; I^2=0; Spanish or Mexican: 0.03 [-0.03, 0.09]; *P*=0.30; I^2=58
GG vs GT	Femoral neck (FN) BMD	7 [10, 12, 14-16, 20, 21]	9072 vs 3941	-0.00 [-0.01, 0.01]; *P*=0.73; I^2=68	Women overall: -0.00 [-0.01, 0.01]; *P*=0.67; I^2=74; Post- or Peri-menopausal women: -0.01 [-0.02, 0.01]; *P*=0.41; I^2=79; Premenopausal women: 0.01 [-0.01, 0.03]; *P*=0.24; I^2=0 Men: 0.00 [-0.01, 0.01]; *P*=1.00; I^2=0	Asian population: -0.01 [-0.02, 0.01]; *P*=0.31; I^2=64; Caucasian population: 0.00 [-0.01, 0.02]; *P*=0.88; I^2=74
GG vs TT	FN BMD	7 [10, 12, 14-16, 20, 21]	9072 vs 677	-0.01 [-0.03, 0.02]; *P*=0.55; I^2=84	Women overall: -0.01 [-0.04, 0.02]; *P*=0.41; I^2=86;Post- or Peri-menopausal women: -0.02 [-0.06, 0.01]; *P*=0.20; I^2=88; Premenopausal women: 0.02 [-0.00, 0.05]; *P*=0.10; I^2=0 Men: 0.01 [-0.01, 0.03]; *P*=0.19; I^2=0	Asian population: -0.02 [-0.06, 0.02]; *P*=0.36; I^2=90; Caucasian population: 0.01 [-0.02, 0.05]; *P*=0.51; I^2=61
GT vs TT	FN BMD	7 [10, 12, 14-16, 20, 21]	3941 vs 677	-0.01 [-0.02, 0.01]; *P*=0.60; I^2=77	Women overall: -0.01 [-0.03, 0.01]; *P*=0.43; I^2=80; Post- or Peri-menopausal women: -0.02 [-0.05, 0.01]; *P*=0.29; I^2=84; Premenopausal women: 0.01 [-0.02, 0.04]; *P*=0.52; I^2=42Men: 0.01 [-0.01, 0.03]; *P*=0.18; I^2=0	Asian population: -0.01 [-0.04, 0.02]; *P*=0.53; I^2=87; Caucasian population: 0.01 [-0.01, 0.03]; *P*=0.40; I^2=0
GG vs GT	Total Hip (TH) BMD	3 [10, 15, 21]	1901 vs 926	0.01 [0.00, 0.02]; *P*=0.02; I^2=0	Women Overall: 0.01 [0.00, 0.02]; *P*=0.02; I^2=0; Post- or Peri-menopausal women: 0.01 [0.00, 0.02]; *P*=0.02; I^2=0	-
GG vs TT	TH BMD	3 [10, 15, 21]	1901 vs 170	0.01 [-0.01, 0.04]; *P*=0.17; I^2=26	Women Overall: 0.01 [-0.01, 0.04]; *P*=0.17; I^2=26; Post- or Peri-menopausal women: 0.01 [-0.02, 0.04]; *P*=0.48; I^2=46	-
GT vs TT	TH BMD	3 [10, 15, 21]	926 vs 170	0.01 [-0.01, 0.03]; *P*=0.48; I^2=0	Women Overall: 0.01 [-0.01, 0.03]; *P*=0.48; I^2=0; Post- or Peri-menopausal women: 0.00 [-0.02, 0.03]; *P*=0.78; I^2=0	-

Table 7. Meta-analysis of *COL1A1* -1997G/T polymorphism and risk of fracture

Comparison	Number of Studies	Number of Subjects	OR [95% CI]; *P*; I^2	Fracture type-based subgroups OR (95% CI); *P*; I^2
Biallelic Frequency Model (BFM) (T vs G)	3 [11, 19, 23]	4426	1.22 [0.97, 1.53]; *P*=0.09; I^2=20	Hip fracture: 1.12 [0.83, 1.52]; *P*=0.47; I^2=27
Biallelic Dominant Model (BDM) (T+ vs GG)	3 [11, 19, 23]	4426	1.23 [0.94, 1.61]; *P*=0.14; I^2=28	Hip fracture: 1.09 [0.80, 1.49]; *P*=0.58; I^2=9
Biallelic Recessive Model (BRM) (TT vs G+)	3 [11, 19, 23]	4426	1.42 [0.74, 2.73]; *P*=0.29; I^2=0	Hip fracture: 1.50 [0.67, 3.38]; *P*=0.33; I^2=0

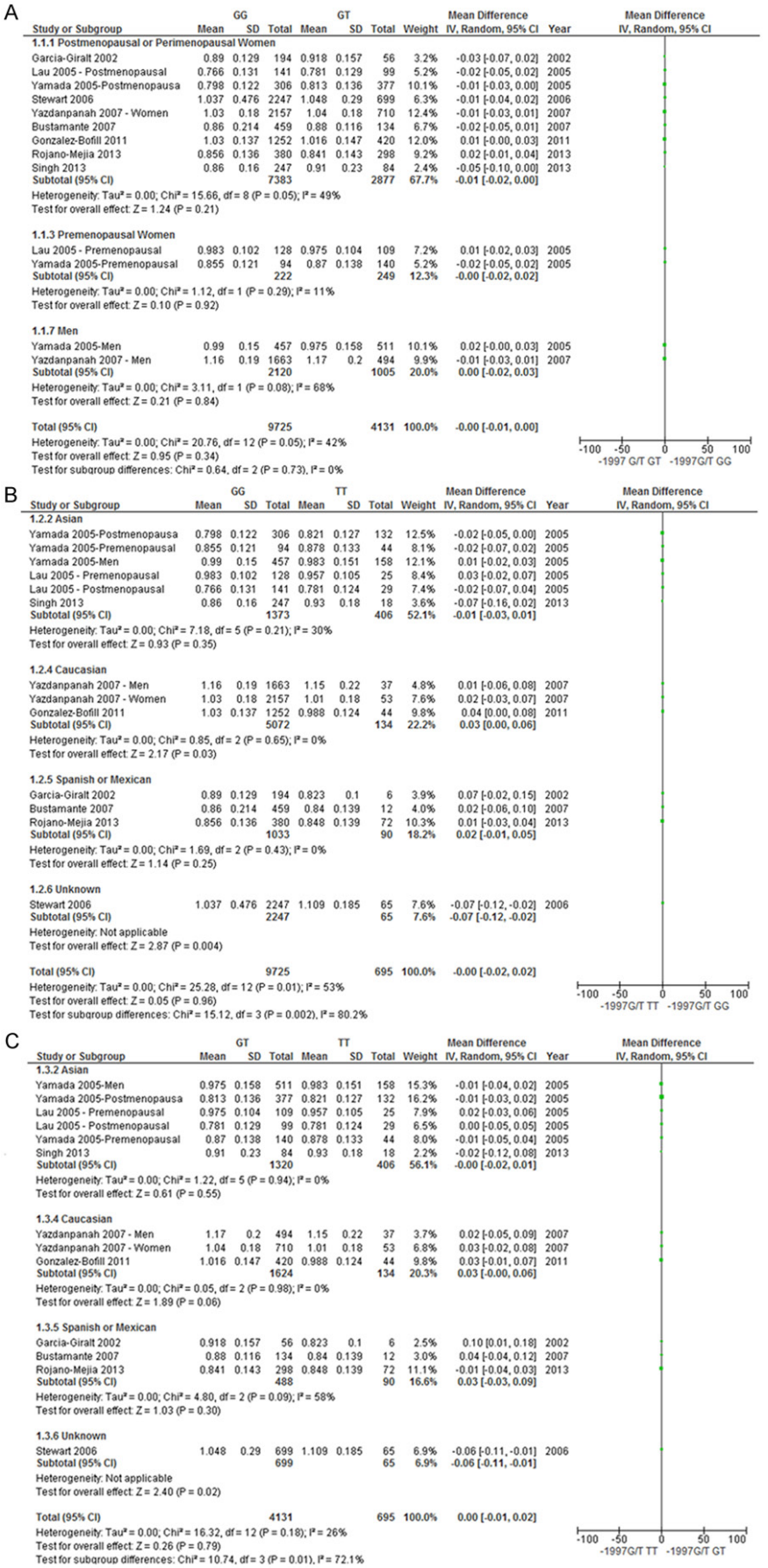

Figure 2. A. Forest plot of the *COL1A1 -1997G/T* polymorphism and Lumbar Spine (LS) BMD (g/cm^2) using random-effect model: GG homozygotes versus GT heterozygotes. The diamond stood for pooled effect. No significant different LS BMD for GG homozygotes versus GT heterozygotes. B. Forest plot of the *COL1A1 -1997G/T* polymorphism and LS BMD (g/cm^2) using random-effect model: GG homozygotes versus TT homozygotes. The diamond stood for pooled effect. No significant different LS BMD for GG homozygotes versus TT homozygotes except for Caucasian subgroup in which GG homozygotes had a significantly higher LS BMD than TT homozygotes. C. Forest plot of the *COL1A1 -1997G/T* polymorphism and LS BMD (g/cm^2) using random-effect model: GT heterozygotes versus TT homozygotes. The diamond stood for pooled effect. No significant different LS BMD for GT heterozygotes versus TT homozygotes although there was a trend of higher LS BMD associated with the GT genotype compared with the TT genotype in the Caucasian subgroup.

Results

Eligible studies and study characteristics

Figure 1 described our search process flow and results. From a total of 400 articles, 288 were excluded because they were irrelevant to our meta-analysis questions, were reviews or meeting abstracts, or contained no detailed

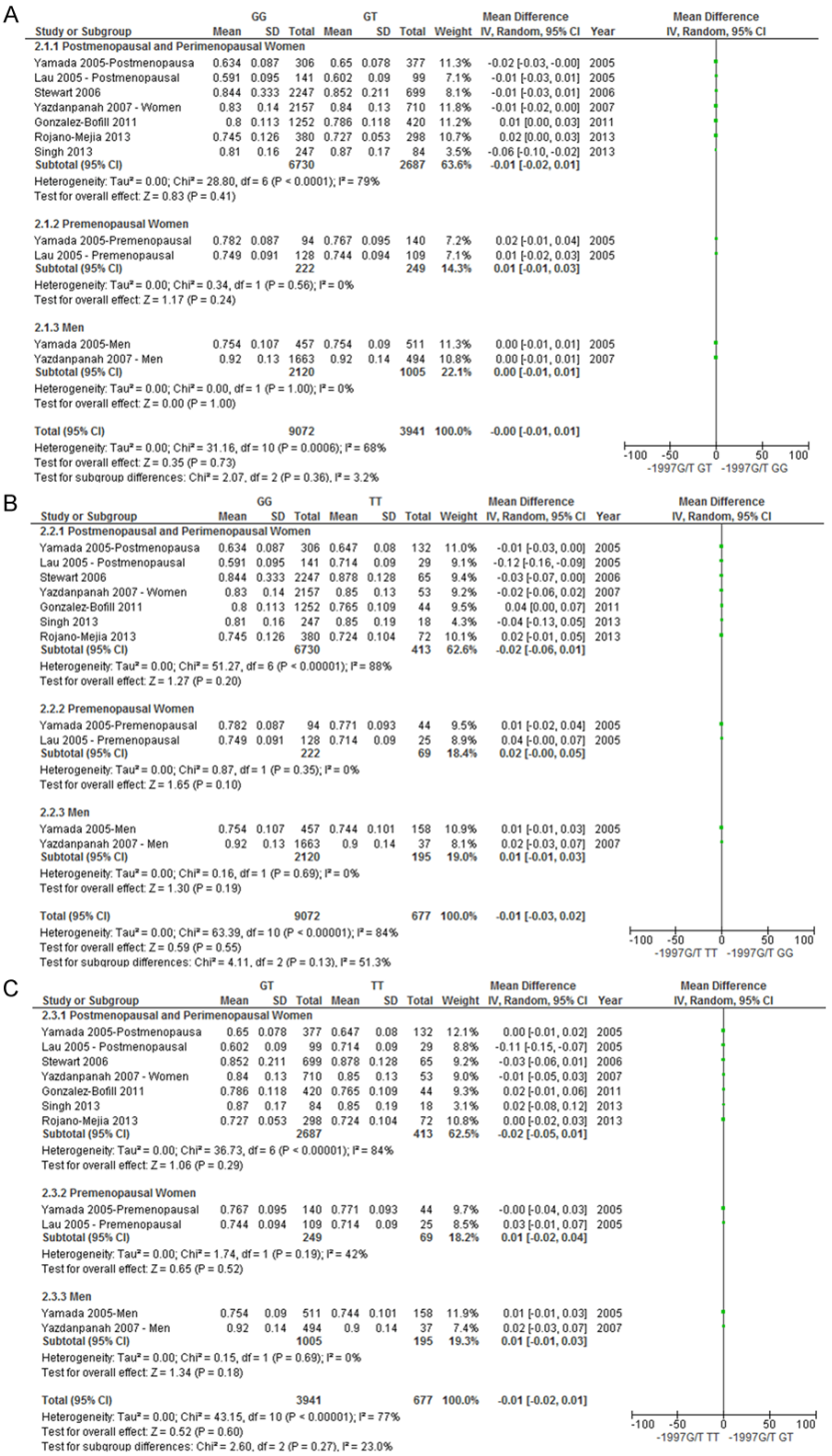

Figure 3. A. Forest plot of the *COL1A1 -1997G/T* polymorphism and Femoral Neck (FN) BMD (g/cm^2) using random-effect model: GG homozygotes versus GT heterozygotes. The diamond stood for pooled effect. No significant different FN BMD for GG homozygotes versus GT heterozygotes. B. Forest plot of the *COL1A1 -1997G/T* polymorphism and FN BMD (g/cm^2) using random-effect model: GG homozygotes versus TT homozygotes. The diamond stood for pooled effect. No significant different FN BMD for GG homozygotes versus TT homozygotes. C. Forest plot of the *COL1A1 -1997G/T* polymorphism and FN BMD (g/cm^2) using random-effect model: GT heterozygotes versus TT homozygotes. The diamond stood for pooled effect. No significant different LS BMD for GT heterozygotes versus TT homozygotes.

usable numbers needed for this meta-analysis. A total of 12 studies were included in our meta-analysis [10-16, 19-23] and relevant data from each of the 12 included studies were extracted and described in **Tables 1-5**.

The -1997G/T polymorphism and BMD and risk of fracture

As shown in **Tables 2** and **6**, 9 studies were included in the meta-analysis of the -1997G/T polymorphism and LS BMD with 14,551 subjects [10, 12-16, 20-22]; 7 studies were included in the meta-analysis of the -1997G/T polymorphism and FN BMD with 13690 subjects [10, 12, 14-16, 20, 21]; and 3 studies were included in the meta-analysis of the -1997G/T polymorphism and TH BMD with 2,997 subjects [10, 15, 21]. Further, as shown in **Tables 3** and **7**, 3 studies were included in the meta-analysis of the -1997G/T polymorphism and risk of fracture with 4426 subjects [11, 19, 23].

Our meta-analysis showed that overall, the -1997G/T polymorphism had no significant association with LS BMD: MD=-0.00 [-0.01, 0.00]; *P*=0.34; I^2=42 for GG vs GT (**Figure 2A**; **Table 6**); MD=-0.00 [-0.02, 0.02]; *P*=0.96; I^2=53 for GG vs TT (**Figure 2B**; **Table 6**); and MD=0.00 [-0.01, 0.02]; *P*=0.79; I^2=26 for

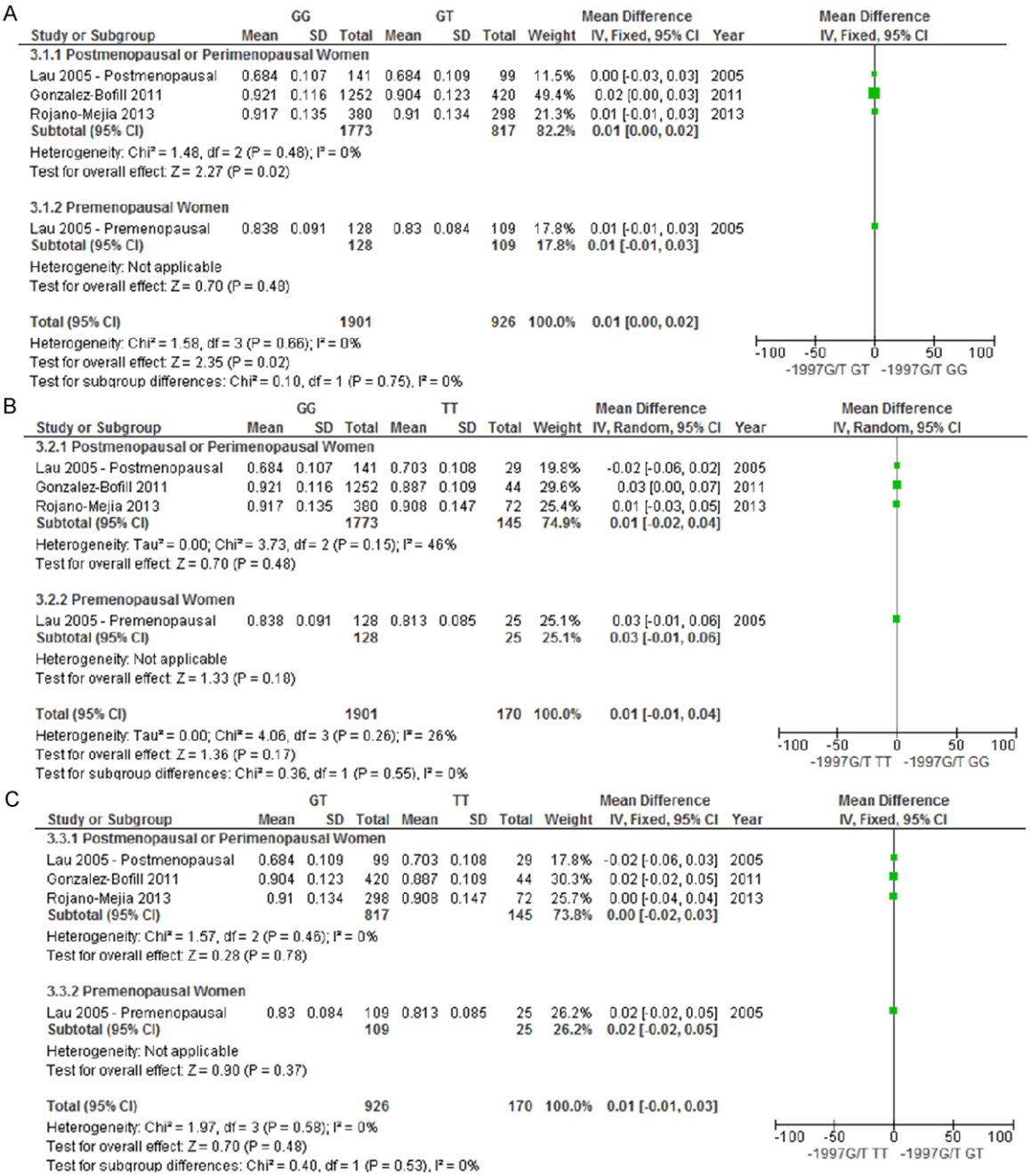

Figure 4. A. Forest plot of the *COL1A1 -1997G/T* polymorphism and Total Hip (TH) BMD (g/cm^2) using fixed-effect model: GG homozygotes versus GT heterozygotes. The diamond stood for pooled effect. GG homozygotes had significantly higher BMD than GT heterozygotes. B. Forest plot of the *COL1A1 -1997G/T* polymorphism and TH BMD (g/cm^2) using random-effect model: GG homozygotes versus TT homozygotes. The diamond stood for pooled effect. No significant different TH BMD for GG homozygotes versus TT homozygotes. C. Forest plot of the *COL1A1 -1997G/T* polymorphism and TH BMD (g/cm^2) using fixed-effect model: GT heterozygotes versus TT homozygotes. The diamond stood for pooled effect. No significant different TH BMD for GT heterozygotes versus TT homozygotes.

GT vs TT (**Figure 2C**; **Table 6**). Gender-based, ethnicity-based and menopausal status-based sub-population analysis did not show significant association except for Caucasian sub-population wherein subjects with the GG genotype had a significantly higher LS BMD than those with the TT genotype: MD=0.03 [0.00, 0.06]; *P*=0.03; I^2=0 (**Figure 2B**; **Table 6**), and wherein

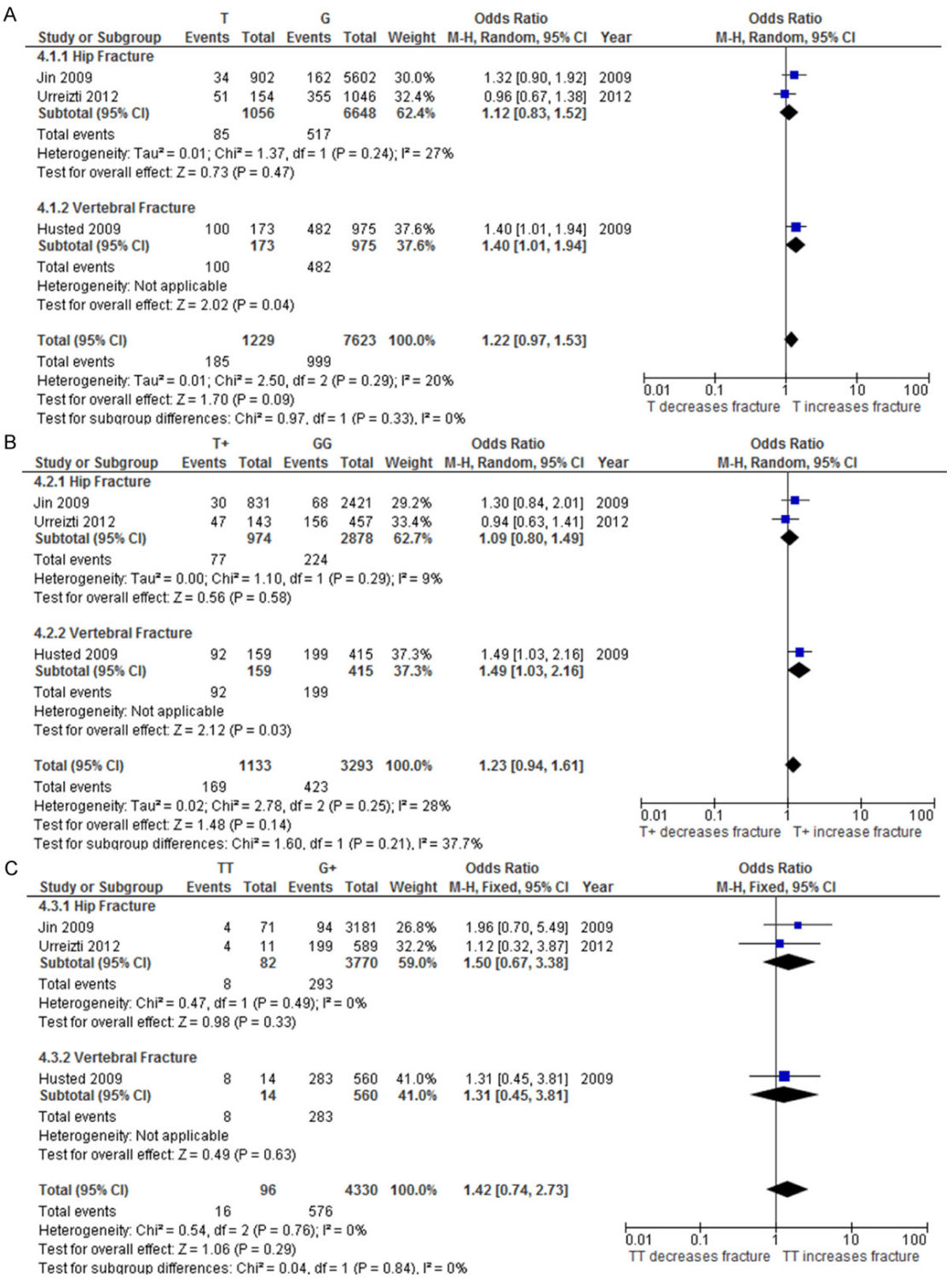

Figure 5. A. Forest plot of the *COL1A1 -1997G/T* polymorphism and the risk of fracture using biallelic frequency model (*T vs* G) (BFM) with a random-effect model. The diamond stood for pooled effect. No significant association between the *-1997G/T* polymorphism and risk of fracture in the BFM model. B. Forest plot of the *COL1A1 -1997G/T* polymorphism and the risk of fracture using biallelic dominant model (*T+ vs GG*) (BDM) with a random-effect model. The diamond stood for pooled effect. No significant association between the *-1997G/T* polymorphism and risk of fracture in the BDM model. C. Forest plot of the *COL1A1 -1997G/T* polymorphism and the risk of fracture using biallelic recessive model (*TT vs G+*) (BRM) with a fixed-effect model. The diamond stood for pooled effect. No significant association between the *-1997G/T* polymorphism and risk of fracture in the BRM model.

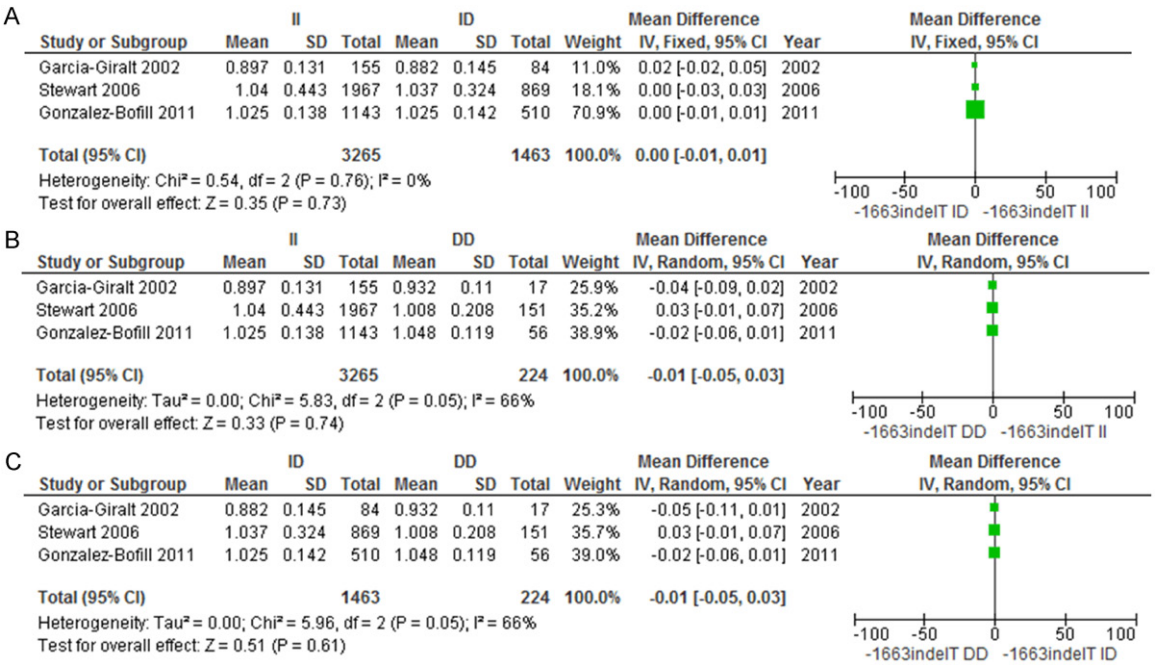

Figure 6. A. Forest plot of the *COL1A1 -1663indelT* polymorphism and Lumbar Spine (LS) BMD (g/cm^2) in postmenopausal or perimenopausal women using fixed-effect model: II homozygotes versus ID heterozygotes. The diamond stood for pooled effect. No significant different LS BMD for II homozygotes versus ID heterozygotes. B. Forest plot of the *COL1A1 -1663indelT* polymorphism and LS BMD (g/cm^2) in postmenopausal or perimenopausal women using random-effect model: II homozygotes versus DD homozygotes. The diamond stood for pooled effect. No significant different LS BMD for II homozygotes versus TDD homozygotes. C. Forest plot of the *COL1A1 -1663indelT* polymorphism and LS BMD (g/cm^2) in postmenopausal or perimenopausal women using random-effect model: ID heterozygotes versus DD homozygotes. The diamond stood for pooled effect. No significant different LS BMD for ID heterozygotes versus DD homozygotes.

Table 8. Meta-analysis of *COL1A1* -1663indelT (rs2412298) polymorphism and Lumbar Spine (LS) BMD (g/cm^2) in postmenopausal or perimenopausal women

Comparison	Number of Studies	Number of Subjects	Overall MD [95% CI]; *P*; I^2
II vs ID	3 [13-15]	3265 vs 1463	0.00 [-0.01, 0.01]; *P*=0.73; I^2=0
II vs DD	3 [13-15]	3265 vs 224	-0.01 [-0.05, 0.03]; *P*=0.74; I^2=66
ID vs DD	3 [13-15]	1463 vs 224	-0.01 [-0.05, 0.03]; *P*=0.61; I^2=66

there was a trend toward significant association between the GG genotype and higher LS BMD when compared to the GT genotype, although it did not reach the level of statistical significance: MD=0.03 [-0.00, 0.06]; *P*=0.06; I^2=0 (**Figure 2C**; **Table 6**). This indicated the possibility of a significant association between the G allele of the -1997G/T and higher LS BMD in Caucasian population. However, since there were only two studies included in the Caucasian sub-population [12, 15], whether said significant association does truly existed was still far from certain.

Our meta-analysis further showed that that overall, the -1997G/T polymorphism had no significant association with FN BMD: MD=-0.00 [-0.01, 0.01]; *P*=0.73; I^2=68 for GG vs GT (**Figure 3A**; **Table 6**); MD=-0.01 [-0.03, 0.02]; *P*=0.55; I^2=84 for GG vs TT (**Figure 3B**; **Table 6**); and MD=-0.01 [-0.02, 0.01]; *P*=0.60; I^2=77 for GT vs TT (**Figure 3C**; **Table 6**). Gender-based, ethnicity-based and menopausal status-based sub-population analysis did not show any significant association either.

As to the TH BMD, all of the 3 studies included in our analysis used only female subjects and our meta-analysis showed that women with the GG genotype had significantly higher TH BMD than those with the GT phenotype: MD=0.01 [0.00, 0.02]; *P*=0.02; I^2=0 (**Figure 4A**; **Table 6**),

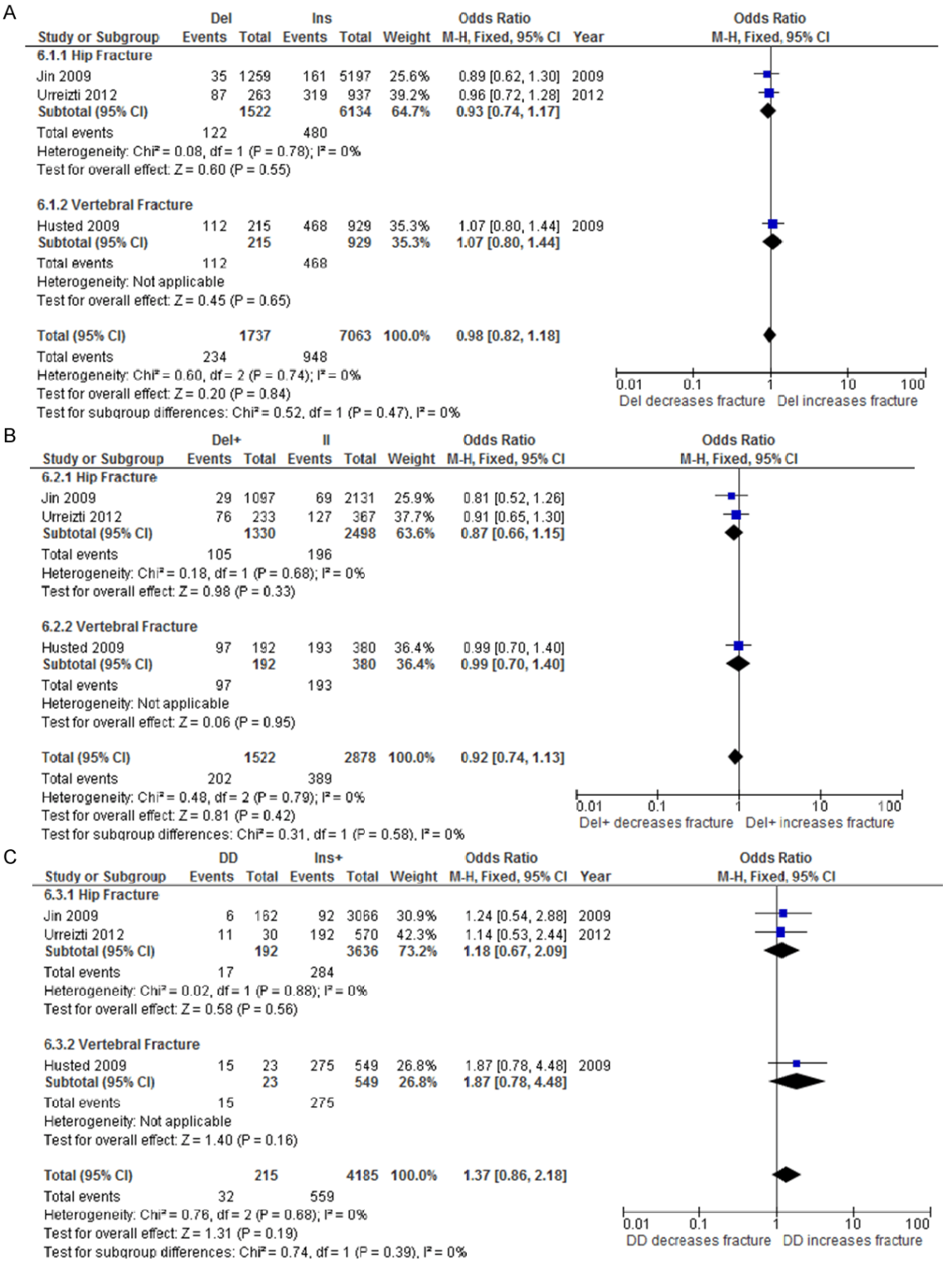

Figure 7. A. Forest plot of the *COL1A1 -1663indelT* polymorphism and the risk of fracture using BFM (*Del vs Ins*) with a fixed-effect model. The diamond stood for pooled effect. No significant association between the *-1663indelT* polymorphism and risk of fracture in the BFM model. B. Forest plot of the *COL1A1 -1663indelT* polymorphism and the risk of fracture using BDM (*Del+ vs II*) with a fixed-effect model. The diamond stood for pooled effect. No significant association between the *-1663indelT* polymorphism and risk of fracture in the BDM model. C. Forest plot of the *COL1A1 -1663indelT* polymorphism and the risk of fracture using BRM (*Ins+ vs DD*) with a fixed-effect model. The diamond stood for pooled effect. No significant association between the *-1663indelT* polymorphism and risk of fracture in the BRM model.

Table 9. Meta-analysis of *COL1A1* -1663indelT (rs2412298) polymorphism and risk of fracture

Comparison	Number of Studies	Number of Subjects	OR [95% CI]; *P*; I^2	Fracture type-based subgroups OR (95% CI); *P*; I^2
BFM (Del vs Ins)	3 [11, 19, 23]	4400	0.98 [0.82, 1.18]; *P*=0.84; I^2=0	Hip fracture: 0.93 [0.74, 1.17]; *P*=0.55; I^2=0
BDM (Del+ vs II)	3 [11, 19, 23]	4400	0.92 [0.74, 1.13]; *P*=0.42; I^2=0	Hip fracture: 0.87 [0.66, 1.15]; *P*=0.33; I^2=0
BRM (DD vs Ins+)	3 [11, 19, 23]	4400	1.37 [0.86, 2.18]; *P*=0.19; I^2=0	Hip fracture: 1.18 [0.67, 2.09]; *P*=0.56; I^2=0

menopausal status based sub-population analysis further showed that for postmenopausal or perimenopausal women, such significant association also held true: MD=0.01 [0.00, 0.02]; *P*=0.02; I^2=0 (**Figure 4A**; **Table 6**). However, our meta-analysis only included 3 studies and showed that women with the GG genotype did not have significantly different TH BMD compared to those with the TT genotype: MD=0.01 [-0.01, 0.04]; *P*=0.17; I^2=26 (**Figure 4B**; **Table 6**), nor did women with the GT genotype have a significantly different TH BMD compared to those with the TT genotype: MD=0.01 [-0.01, 0.03]; *P*=0.48; I^2=0 (**Figure 4C**; **Table 6**). Therefore, whether the significantly higher TH BMD associated with the GG genotype compared to the GT genotype was true still awaits further confirmation.

Additionally, our meta-analysis showed that the -1997G/T polymorphism had no significant association with risk of fracture using the three biallelic models. BFM: OR=1.22 [0.97, 1.53]; *P*=0.09; I^2=20 (**Figure 5A**; **Table 7**); BDM: OR =1.23 [0.94, 1.61]; *P*=0.14; I^2=28 (**Figure 5B**; **Table 7**); and BRM: 1.42 [0.74, 2.73]; *P*=0.29; I^2=0 (**Figure 5C**; **Table 7**). Fracture type-based sub-population analysis failed to reveal any significant association either (**Table 7**).

Between-studies heterogeneities varied with I^2 ranging from 0 to 88 (**Tables 6**, **7**); Further, symmetrical funnel plots for all of the meta-analyses performed indicated the presence of none or very little publication bias (data not shown).

The -1663indelT polymorphism and BMD and risk of fracture

Due to limited number of relevant studies available, we only did meta-analysis on association between the -1663indelT polymorphism and LS BMD in postmenopausal or perimenopausal women. As shown in **Table 4**, 3 studies were included in the meta-analysis of the -1663indelT polymorphism and LS BMD with 4,952 subjects [13-15]. Additionally, as shown in **Table 5**, 3 studies were included in the meta-analysis of the -1663indelT polymorphism and risk of fracture with 4,400 subjects [11, 19, 23].

Our meta-analysis did not reveal a significant association between the -1663indelT polymorphism and LS BMD in postmenopausal or perimenopausal women: MD=0.00 [-0.01, 0.01]; *P*=0.73; I^2=0 for II vs ID (**Figure 6A**; **Table 8**); MD=-0.01 [-0.05, 0.03]; *P*=0.74; I^2=66 for II vs DD (**Figure 6B**; **Table 8**); and MD=-0.01 [-0.05, 0.03]; *P*=0.61; I^2=66 for ID vs DD (**Figure 6C**; **Table 8**).

Further, our meta-analysis showed no significant association between the -1663indelT polymorphism and the risk of fracture using the three biallelic model. BFM: OR=0.98 [0.82, 1.18]; *P*=0.84; I^2=0 (**Figure 7A**; **Table 9**); BDM: 0.92 [0.74, 1.13]; *P*=0.42; I^2=0 (**Figure 7B**; **Table 9**); and BRM (**Figure 7C**; **Table 9**). Fracture-type based sub-population analysis did not reveal any significant association either (**Table 9**)

Between-studies heterogeneities varied with I^2 varying from 0 to 66 (**Tables 8**, **9**). Further, symmetrical funnel plots for all of the meta-analyses performed indicated the presence of none of very little publication bias (data not shown).

Discussion

In this meta-analysis, we analyzed association between the two *COL1A1* promoter polymorphisms, the -1997G/T and the -1663indelT polymorphisms and osteoporosis/BMD and risk of fracture. Our analysis revealed no significant association between the -1997G/T polymorphism and LS and FN BMD except for the Caucasian subpopulation wherein subjects with the GG genotype had a significantly higher LS BMD than those with the TT genotype and wherein there was a trend toward significant association between the GG genotype and higher LS BMD when compared to the GT genotype, although it did not reach the level of statistical significance, indicating the possibility of a significant association between the G allele

of the -1997G/T and higher LS BMD in Caucasian population. Our analysis did reveal that women, especially postmenopausal or perimenopausal women with the GG genotype had significantly higher TH BMD than those with the GT, although said higher TH BMD did not hold true for women with the GG genotype when compared to those with the TT genotype or for women with the GT genotype when compared to those with the TT phenotype. Additionally, our meta-analysis did not reveal significant association between the -1997G/T polymorphism and risk of fracture, between the -1663indelT polymorphism and LS BMD in postmenopausal or perimenopausal women, or between the -1663indelT polymorphism and the risk of fracture.

Our analysis confirmed findings of the meta-analysis done by Jin et al. [8] about lack of significant association between the -1997G/T or the -1663indelT polymorphism and risk of fracture and between the -1997G/T polymorphism and FN BMD, however, our result regarding whether the -1997G/T or the -1663indelT polymorphism was associated with LS BMD differed from Jin et al. which found that the GG genotype of the 1997G/T polymorphism was associated with significantly higher LS BMD than the GT genotype in female (P=0.02), whereas in the whole population, there was only a borderline significant association between this polymorphism and BMD using a fixed-effect model [8]. Jin et al. further found significant association between -1663indelT polymorphism and LS BMD using a fixed effect model (P=0.03) [8]. We think such difference of results were mainly due to three reasons. First, our meta-analysis included studies published later than Jin et al. [10, 15, 16, 19]. Secondly Jin et al. only included studies on subjects with fracture in its meta-analysis, it did not include studies such as Garcia-Giralt et al. [13], Lau et al. [21] and Jin et al. [23] which focused on the association between the -1997G/T or the -1663indelT polymorphism and BMD in subjects without regard to whether they had fracture or not, whereas our meta-analysis included all relevant studies regardless whether the subjects had fracture or not. Thirdly, Jin et al incorporate unpublished data into their analysis through corresponding with authors, while our analysis was based on published data only and thus our analysis on association between the -1997G/T polymorphism and BMD did not include studies such as Husted et al. [11] which did not publish detailed usable data regarding number of subjects with each of GG, GT and TT genotypes, and our analysis on association between the -1663indelT polymorphism and LS BMD did not include studies such as Husted et al. [11] and Bustamante et al. [22] which did not provide detailed number of subject with each of the II, ID and DD genotypes. We believe such different choice of studies could explain the difference in our result regarding the association between the -1997GT or the -1663indelT polymorphism and LS BMD.

Since our analysis on association between the -1997G/T polymorphism and LS BMD included 9 studies as opposed to 5 studies included in the meta-analysis done by Jin et al. [8], we believe our result were indeed more robust as it incorporated more recent data and provided a more comprehensive picture. We found no significant association between the -1997G/T polymorphism and LS BMD except for the Caucasian subpopulation wherein subjects with the GG genotype had a significantly higher LS BMD than those with the TT genotype and wherein there was a trend toward significant association between the GG genotype and higher LS BMD when compared to the GT genotype, although it did not reach the level of statistical significance, indicating the possibility of a significant association between the G allele of the -1997G/T and higher LS BMD in Caucasian population. However, since there were only two studies included in the Caucasian sub-population [12, 15], whether said significant association hold true need further confirmation by more studies.

We further performed analysis on association between the -1997G/T polymorphism and TH BMD, such analysis was not performed by Jin et al. [8]. We found that women, especially postmenopausal or perimenopausal women with the GG genotype had significantly higher TH BMD than those with the GT, however, since our analysis only included 3 studies [10, 15, 21] and said higher TH BMD did not hold true for women with the GG genotype when compared to those with the TT genotype or for women with the GT genotype when compared to those with the TT phenotype, it is still doubtful whether said higher TH BMD associated with the GG genotype was true, more studies are needed to further confirm or dispute our finding.

Overall, our meta-analysis suggested the possibility of the -1997G/T and the -1663indelT polymorphisms playing very little, if any role in osteoporosis and fracture, however, alternative possibilities exist. One strong alternative possibility is that the -1997G/T and the -1663indelT polymorphisms did not individually play a role in osteoporosis and fracture, rather, they function as parts of a haplotype. Studies have shown that interaction among the +1245G/T, the -1997G/T and the -1663indelT polymorphisms regulated *COL1A1* transcription and gene expression [7, 29]. Since these three polymorphisms were all in strong linkage disequilibrium (LD) [15], several groups have studied effect of the haplotype on osteoporosis/BMD and fracture. A rare haplotype consisting of the minor alleles of all of the 3 polymorphisms (-1997T/-1663delT/+1245T) was found to be strongly associated with hip fracture and being overrepresented in a cohort of 98 Caucasian hip fracture cases [23]. However, a later study, Urreizti et al. found no significant association between this rare haplotype and hip fracture in a Spanish population although it did found a small but significant association between another haplotype, -1997G/-1663insT/+1245T and hip fracture [19]. Ethnicity difference might explain such difference in result [19]. Further, Gonzalez-Bofill et al. found that the haplotype -1997T/-1663insT/+1245G was associated with reduced BMD and increase bone turn over when compared to the haplotype -1997G/-1663insT/+1245G [15]. On the other hand, there have also be studies that showed that the haplotype -1997T/-1663insT/+1245G did not influence BMD [11, 12, 22] or was associated with increased BMD [14]. Based on these findings, Gonzalez-Bofill deduced that the haplotype -1997G/-1663insT/+1245G was likely to be associated with healthy bone, while the haplotype -1997G/-1663del/+1245T and the haplotype -1997T/-1663insT/+1245G might negatively affect BMD and bone turnover, and ethnicity might be a contributing factor in determine which of the 2 haplotypes played a more important role [15]. Functional study of these 3 polymorphisms seemed to support a role of haplotypes consisting of these 3 polymorphisms in regulating bone health. It has been found that the -1663delT allele increased *COL1A* transcription possibly by binding to the transcription factors Nmp4 and Osterix, while the -1997T allele binds osteoblast-produced protein and purified Sp1 stronger than the -1997G allele, and the +1245T allele had a higher affinity for Sp1 than the +1245G allele thus leading to faster *COL1A1* transcription [15]. It has also been found that the *COL1A1* transcription-enhancing activity associated with the -1663delT allele was only seen in combination with the -1997T allele, and that the haplotypes -1997G/-1663del/+1245T and -1997T/-1663insT/+1245G led to enhanced *COL1A1* transcription compared to the haplotype -1997G/-1663insT/+1245G [15]. Based on this result, one future direction of study would be more haplotype studies in order to further determine their role in regulating bone health and in osteoporosis and fracture.

Another possible factor leading to at least part of our negative findings is the limited number of studies and relative small sample sizes for analysis on association between the -1997G/T or the -1663indelT polymorphism and fracture and for analysis on association between the -1663indelT polymorphism and BMD, such lack of statistical power might very well substantially contributed to our negative finding and is one important limitation of our analysis.

Our analysis has some limitation. First, as mentioned above, lack of statistical power due to limited number of studies for some parts of our analysis might affect our result; and secondly, since we only included published data, we excluded a couple of studies which did not published detailed usable data necessary for our analysis.

On the other hand, our analysis also had its strength. First, guideline for designing and reporting systemic review of genetic association studies was followed during our meta-analysis [30-32]. Secondly, we used all three biallelic models (BFM, BDM and BRM) to analysis the association between the -1997G/T and the -1663indelT polymorphisms and fracture and results from these three models were consistent, and as such, we believe that at least results generated from this part of our analysis were indeed robust.

Conclusions

Our results suggested that it was possible that the *COL1A1* -1997G/T and the -1663indelT polymorphisms might individually played very little role in osteoporosis and fracture, although more studies are needed especially for the analysis of association between these two poly-

morphisms and fracture. Haplotype studies may become one important future direction of study to further elucidate whether and how various *COL1A1* polymorphisms affect bone health, osteoporosis and fracture.

Disclosure of conflict of interest

None.

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