RESEARCH ARTICLE

Association of *Lactase* 13910 C/T polymorphism with bone mineral density and fracture risk: a meta-analysis

YOUGEN WU[1,2], YINGHUA LI[1], YUNQING CUI[1], YUNJIAO ZHOU[1], QINGQING QIAN[2*], AND YANG HONG[1,2,3*]

[1] *Central Laboratory, The Fifth People's Hospital of Shanghai, Fudan University, Shanghai 200240, China;*

[2] *Medical Institution Conducting Clinical Trials for Human Used Drug, The Fifth People's Hospital of Shanghai, Fudan University, Shanghai 200240, China;*[3] *Department of Osteology, The Fifth People's Hospital of Shanghai, Fudan University, Shanghai 200240, China.*

Running title: LCT C13910T gene polymorphism with BMD and fracture risk.

* To whom correspondence should be addressed.

Medical Institution Conducting Clinical Trials for Human Used Drug, The Fifth People's Hospital of Shanghai, Fudan University, 128 Ruili Road, Minhang District, Shanghai 200240, China. Tel.: +86 21 24289472; fax: +86 21 24289472; E-mail: hongyangcm@163.com (Yang Hong)

E-mail: moneyqingqing@126.com (Qingqing Qian)

Abstract

A number of studies have investigated the association of lactase (*LCT*, C/T-13910) gene polymorphism with bone mineral density (BMD) and fracture risk, but previous results have been inconclusive. In this study, a meta-analysis was performed to quantify the association of *LCT* (C/T-13910) polymorphism with BMD and fracture risk. Eligible publications were searched in the PubMed, Web of Science, Embase databases, Google Scholar, Yahoo, and Baidu. Pooled weighted mean difference (WMD) or odds ratio (OR) with their 95% confidence interval (CI) were calculated

using a fixed- or random-effects model. A total of 9 articles with 8,871 subjects were investigated in the present meta-analysis. Overall, the TT/TC genotypes of *LCT* 13910 C/T polymorphism showed significantly higher BMD than those with the CC genotype at femur neck (FN) (WMD = 0.011g/cm^2, 95% CI = 0.004-0.018, P = 0.003). Besides, *LCT* 13910 C/T polymorphism may decrease the risk of any site fractures (for TT vs. TC+CC, OR = 0.813, 95 % CI = 0.704-0.938, p = 0.005; for T allele vs. C allele, OR = 0.885, 95 % CI = 0.792-0.989, p = 0.032). However, there was no significant association of *LCT* 13910 C/T polymorphism with BMD at lumbar spine and risk of vertebral fractures under all genetic contrast models (all P values were more than 0.05). The meta-analysis suggests that there are significant effects of *LCT* 13910 C/T polymorphism on bone mineral density and fracture risk. Large-scale studies with different ethnic populations will be needed to further investigate the possible race-specific effect of *LCT*-13910 C/T polymorphism on BMD and fracture risk.

Keywords. lactase; polymorphism; bone mineral density; fracture; meta-analysis.

Introduction

Osteoporosis is a common bone disorder characterized by decrease in bone mineral density (BMD) and skeletal microarchitecture deterioration, which leads to increased bone fragility and fracture risk. Although osteoporosis and fracture are influenced by many environmental factors such as exercise and nutrients, including calcium and vitamin D (Uusi-Rasi *et al.* 1998; Valimaki *et al.* 2004), genetic factors also play an important role in regulating BMD and pathogenesis of fracture (Stewart and Ralston 2000). Twin studies have indicated that heritability can account for 50–85% of the variance in BMD (Pocock *et al.* 1987).

In recent years, several candidate genes, including estrogen receptor (ESR) (Deng *et al.* 2015), vitamin D receptor (VDR) (Shen *et al.* 2014), calcitonin receptor (CTR) (Xiong *et al.* 2015), collagen type1α1 (COL1A1) (Zintzaras *et al.* 2011), osteoprotegerin (OPG) (Guo *et al.* 2014), low-density lipoprotein receptor-related protein 5 gene (LRP5) (Xu *et al.* 2014), beta-2 adrenergic receptor (B2AR) (Veldhuis-Vlug *et al.* 2015), and interleukin-6 (IL-6) (Wang *et al.* 2013) have been

demonstrated to be associated with BMD and osteoporosis. The genome-wide association studies (GWASs) also identified novel loci associated with BMD or fracture risk, including GPR177, WNT16, MEF2C, and Rap1A (Karasik and Cohen-Zinder 2012; Richards *et al.* 2012). Although polymorphisms of those genes alone have limited capability towards the prediction of risk in individuals, they provide insight into the biological pathways and functional interaction network influencing BMD variation and the risk of fracture (Hsu and Kiel 2012).

The lactase (*LCT*) gene codes for the lactase enzyme which is responsible for lactose hydrolysis into glucose and galactose in the small-intestine mucosa. A single nucleotide polymorphism (SNP), a C to T change residing 13,910 base pairs upstream of the *LCT* gene at chromosome 2q21–22, which shows complete association with lactase activity. The C/C-13910 genotype in a homozygous form is associated with lactose non-persistence and genotypes C/T-13910 and T/T-13910 with lactase persistence. Lactase non-persistence, may also have an impact on calcium intake, and subsequently on bone metabolism, bone mineral density and bone fracture incidence (Enattah *et al.* 2004).

In 2004 Obermayer-Pietsch *et al*. first reported that *LCT* (C/T-13910) polymorphism was associated with calcium intake, bone density and fractures in Austrian postmenopausal women (Obermayer-Pietsch *et al.* 2004). Since then, several studies regarding the association between *LCT* (C/T-13910) polymorphism and bone density and fracture have been published. However, the results have been inconsistent (Enattah *et al.* 2005a; Enattah *et al.* 2005b; Gugatschka *et al.* 2007; Obermayer-Pietsch *et al.* 2007; Bacsi *et al.* 2009; Kull *et al.* 2009; Smith *et al.* 2009; Agueda *et al.* 2010; Koek *et al.* 2010; Marozik *et al.* 2013). Nowadays, there is no meta-analysis focused on the relationship between *LCT* 13910 C/T gene polymorphism and bone density and fracture risk across different studies. Therefore, it is important to perform a meta-analysis to evaluate the relationships, with the intention to understand the real situation.

Materials and Methods

Literature and search strategy

PubMed, Web of Science, Embase, Google Scholar, Yahoo, and Baidu were searched for eligible

articles. The search strategy used combinations of the following key terms: “lactose” or “lactase” and “polymorphism” or “variant” or “mutation” and “bone mineral density” or “BMD” or “fracture” (The literature search was updated on June 30, 2016). The reference lists of reviews and retrieved studies were identified by a manual search. The publication language was restricted to English.

Inclusion criteria and data extraction

Eligible studies had to meet the following criteria: (1) evaluated the association of *LCT*-13910C/T polymorphism with BMD or fracture risk, (2) provided sufficient data for calculation of weighted mean difference (WMD) or odds ratio (OR) with 95% confidence interval (CI), and (3) review articles were excluded. For BMD phenotype, the following data was extracted: (1) first author’s name, (2) year of publication, (3) country of origin, (4) ethnicity, (5) age, (6) gender, (7) type of BMD phenotype, (8) sample size across three genotypes, and (9) mean and standard deviation of BMD across three genotypes. For fracture phenotype, the following information was extracted from each study: (1) name of the first author, (2) year of publication, (3) country of origin, (4) ethnicity, (5) age, (6) gender, (7) type of fracture, (8) sample size in cases and controls, and (9) genotype distributions of cases and controls. Data was extracted from all eligible publications independently by two authors (Yougen Wu and Yinghua Li) and disagreement was resolved by discussion until consensus was reached.

Statistical analysis

The association between *LCT*-13910 C/T polymorphism and BMD was estimated by a pooled WMD and 95% CI under co-dominant model, dominant model, and recessive model, respectively. The association between *LCT*-13910 C/T polymorphism and fracture risk was estimated by calculating a pooled OR and 95% CI under three genetic models above, respectively. The significance of the pooled WMD or OR was determined by a Z test ($p < 0.05$ was considered statistically significant). Cochran’s Chi square-based Q statistic test was performed to evaluate possible heterogeneity among studies. If heterogeneity existed ($P < 0.1$) across studies, the random effects model was used (Dersimonian and Laird 2015); otherwise, the fixed effects model was adopted (Mantel and Haenszel 1959). I^2 statistic was calculated to quantify the proportion of the total heterogeneity across studies.

I^2 values of 25, 50, and 75% were used as evidence of low, moderate, and high heterogeneity, respectively. Publication bias was assessed by Egger's test (Egger *et al.* 1997) and funnel-plot analysis. $P < 0.05$ was considered statistically significant. Subgroup analyses based on type of fracture (any fracture vs. vertebral fracture), and sex (male vs. female) were performed. Sensitivity analysis was conducted by deleting each study at a time to assess the stability of the results. Data analyses were performed using STATA version 11.0 (Stata Corporation, College Station, TX, USA).

Results

Characteristics of the studies

The literature search identified a total of 22 potentially relevant articles. Of these, 11 articles, which did not provide sufficient data for the calculation of WMD, OR and 95%CI, were excluded. If more than one study was included in one paper, they were considered as separate study in the meta-analysis. All of these studies reported on Caucasions. For BMD phenotypes, 9 studies from 7 articles analyzed BMD of femoral neck (Enattah *et al.* 2004; Obermayer-Pietsch *et al.* 2004; Obermayer-Pietsch *et al.* 2007; Kull *et al.* 2009; Agueda *et al.* 2010; Koek *et al.* 2010; Marozik *et al.* 2013), and 8 studies from 7 articles analyzed BMD of lumbar spine (Enattah *et al.* 2004; Obermayer-Pietsch *et al.* 2004; Obermayer-Pietsch *et al.* 2007; Kull *et al.* 2009; Agueda *et al.* 2010; Koek *et al.* 2010; Marozik *et al.* 2013). For fracture, 7 studies from 7 articles analyzed fracture at any site (Obermayer-Pietsch *et al.* 2004; Enattah *et al.* 2005a; Enattah *et al.* 2005b; Obermayer-Pietsch *et al.* 2007; Bacsi *et al.* 2009; Smith *et al.* 2009; Agueda *et al.* 2010), and 4 studies from 4 articles analyzed vertebral fracture (Enattah *et al.* 2004; Obermayer-Pietsch *et al.* 2007; Bacsi *et al.* 2009; Agueda *et al.* 2010). A flow chart summarizing the process of studies exclusion/inclusion is depicted in Figure 1. The detailed characteristics of all the studies and the main results in this meta-analysis are shown in Table 1, 2, and 3.

BMD phenotypes

For BMD of femoral neck, 9 studies with a total of 8871 subjects were identified for the data analysis. There was a significant association between *LCT*-13910 C/T polymorphism and BMD of

femoral neck under a dominant model (TT + TC vs. CC: WMD = 0.011 g/cm^2, 95%CI = 0.004–0.018 g/cm^2, p = 0.003, Fig. 2). Similar significant associations were observed when women and men genotypes were analyzed separately (Table 4).

For BMD of lumbar spine, 8 studies with a total of 8740 subjects were identified for the data analysis. There was no significant difference in BMD values at the lumbar spine under four genetic contrast models (Table 4). $I^2 > 75.0$ % was observed in overall analyses. Sensitivity analysis was conducted to assess the influence of each study on the overall pooled WMD. The exclusion of Enattah *et al*., 2004 study (Enattah *et al.* 2004) made the biggest drop for heterogeneity values and still no significant difference was observed in BMD values at the lumbar spine under four genetic contrast models (Table 4). The study by Enattah *et al*., 2004 was considered the most influenced on the pooled WMD for the association of *LCT* 13910C/T polymorphism with BMD at lumbar spine (Fig. 3). When stratified by gender, significant association was found only in men, but not in women (Table 4).

Fractures

7 studies with a total of 5,919 subjects were identified for the analysis on *LCT*-13910 C/T polymorphism and fracture. The overall result showed that there was significant association between this polymorphism and fracture risk (TT vs. TC+CC: OR = 0.813; 95% CI = 0.704–0.938, p = 0.005, Fig. 4 (a); T allele vs. C allele, OR = 0.885, 95 % CI = 0.792–0.989, p = 0.032, Fig. 4 (b)). Thus, individuals with C allele have obviously increased risk of any site fractures compared those with T allele. However, there was no statistically significant association between this polymorphism and vertebral fractures risk under all genetic models (Table 5).

Potential publication bias

Funnel plots and Egger's test were performed to access the publication bias of literatures. As showed in Fig. 5 and Fig. 6, the shapes of the funnel plots did not reveal any evidence of obvious asymmetry. Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry. No publication bias could be detected for the association of *LCT*-13910 C/T polymorphism with BMD phenotypes (BMD of femoral neck: TT vs. CC, p = 0.577; TC vs. CC, p = 0.181; TT+TC vs. CC, p =

0.076; TT vs. TC+CC, p = 0.349); BMD of lumbar spine: TT vs. CC, p = 0.380; TC vs. CC, p = 0.232; TT+TC vs. CC, p = 0.198; TT vs. TC+CC, p =0.962) and fracture risk (TT vs. CC, p = 0.500; TC vs. CC, p = 0.389; TT+TC vs. CC, p = 0.642; TT vs. TC+CC, p = 0.990).

Discussion

In 2004 Obermayer-Pietsch *et al*. (Obermayer-Pietsch *et al.* 2004) first reported that C/C-13910 genotype was associated with subjective milk intolerance, reduced milk calcium intake and reduced BMD at the hip and the lumbar spine and predisposed to bone fractures in postmenopausal women. However, the results from other articles did not find significant correlation between *LCT*-13910 C/T polymorphism and BMD, and fracture (Enattah *et al.* 2004; Enattah *et al.* 2005a; Kull *et al.* 2009; Smith *et al.* 2009; Agueda *et al.* 2010; Koek *et al.* 2010). Specially, several studies suggested the LCT-13910 C/T polymorphism was associated with subjective milk intolerance, reduced milk calcium intake and, therefore, increased bone loss (Obermayer-Pietsch *et al.* 2004; Enattah *et al.* 2005b; Obermayer-Pietsch *et al.* 2007; Koek *et al.* 2010). Thus, calcium intake may act as modifier that affect the association of LCT-13910 C/T polymorphism with BMD or fracture risk. Our data supported a significant association of *LCT*-13910 C/T polymorphism with BMD at femoral neck and risk of any site fractures among Caucasian populations, which is consistent with previous studies (Obermayer-Pietsch *et al*. 2004; Enattah *et al.* 2005b). However, we did not detect any association of this polymorphism with BMD at lumbar spine, and vertebral fractures risk among Caucasian populations. In addition, the sensitivity analysis results showed that Enattah *et al*., 2004 study (Enattah *et al.* 2004) was the source of heterogeneity. The conclusion remained unchanged even after the forementioned study was excluded. The current meta-analysis has particular strengths. First, when the heterogeneity obviously existed, we explored and analyzed the sources of heterogeneity. Second, the present meta-analysis was based primarily on effect estimates and 95% CIs adjusted for age, body height, and body weight. Third, there was no significant publication bias.

The current meta-analysis has some limitations. First, the associations of *LCT*-13910 C/T polymorphism with BMD and fracture risk may be affected by ethnicity; however, we didn't perform further subgroup analysis by ethnicity owing to the lack of different ethnic populations studies. Thus,

further association studies with large sample size of different ethnic populations will be needed to assess the possible race-specific effect of *LCT*-13910 C/T polymorphism on BMD and fracture risk. Second,, environmental factors, such as calcium intake, physical exercise, alcohol consumption, and smoking, have been shown to influence BMD, osteoporosis, and fracture (Enattah *et al.* 2004). The effects of gene–gene and gene–environment interaction were not addressed in this meta-analysis due to lack of sufficient and uniform information in original studies.

In summary, despite the limitations, our meta-analysis found that *LCT*-13910 C/T polymorphism was significantly associated with BMD at femoral neck, and with any site fractures risk, but this polymorphism was not associated with BMD at lumbar spine, or with risk of vertebral fractures.

Acknowledgments

This study was funded by the Scientific Research Project of Shanghai Municipal Health and Family Planning Commission (Grant No. 201540203).

Conflict of Interest:

All authors have no conflicts of interest.

References

Agueda, L., R. Urreizti, M. Bustamante, S. Jurado, N. Garcia-Giralt, A. Diez-Perez *et al.* 2010 Analysis of three functional polymorphisms in relation to osteoporosis phenotypes: replication in a Spanish cohort. *Calcif Tissue Int.* **87,** 14-24.

Bacsi, K., J. P. Kosa, A. Lazary, B. Balla, H. Horvath, A. Kis *et al.* 2009 LCT 13910 C/T polymorphism, serum calcium, and bone mineral density in postmenopausal women. *Osteoporos Int.* **20,** 639-645.

Deng, W., J. C. Han, L. Chen and W. L. Qi. 2015 Estrogen receptor alpha gene PvuII polymorphism and risk of fracture in postmenopausal women: a meta-analysis. *Genet Mol Res.* **14,** 1293-1300.

DerSimonian, R., and N. Laird. 2015 Meta-analysis in clinical trials revisited. *Contemp Clin Trials.* **45,** 139-145.

Egger, M., G. Davey Smith, M. Schneider and C. Minder. 1997 Bias in meta-analysis detected by a simple, graphical test. *BMJ.* **315,** 629-634.

Enattah, N., T. Pekkarinen, M. J. Valimaki, E. Loyttyniemi and I. Jarvela. 2005a Genetically defined adult-type hypolactasia and self-reported lactose intolerance as risk factors of osteoporosis in Finnish postmenopausal women. *Eur J Clin Nutr.* **59,** 1105-1111.

Enattah, N., V. V. Valimaki, M. J. Valimaki, E. Loyttyniemi, T. Sahi and I. Jarvela. 2004 Molecularly defined lactose malabsorption, peak bone mass and bone turnover rate in young finnish men. *Calcif Tissue Int.* **75,** 488-493.

Enattah, N. S., R. Sulkava, P. Halonen, K. Kontula and I. Jarvela. 2005b Genetic variant of lactase-persistent C/T-13910 is associated with bone fractures in very old age. *J Am Geriatr Soc.* **53,** 79-82.

Gugatschka, M., A. Hoeller, A. Fahrleitner-Pammer, H. Dobnig, P. Pietschmann, S. Kudlacek *et al.* 2007 Calcium supply, bone mineral density and genetically defined lactose maldigestion in a cohort of elderly men. *J Endocrinol Invest.* **30,** 46-51.

Guo, L., K. Tang, Z. Quan, Z. Zhao and D. Jiang. 2014 Association between seven common OPG genetic polymorphisms and osteoporosis risk: a meta-analysis. *DNA Cell Biol.* **33,** 29-39.

Hsu, Y. H., and D. P. Kiel. 2012 Clinical review: Genome-wide association studies of skeletal phenotypes: what we have learned and where we are headed. *J Clin Endocrinol Metab.* **97,** E1958-1977.

Karasik, D., and M. Cohen-Zinder. 2012 Osteoporosis genetics: year 2011 in review. *Bonekey Rep.* **1,** 114.

Koek, W. N., J. B. van Meurs, B. C. van der Eerden, F. Rivadeneira, M. C. Zillikens, A. Hofman *et al.* 2010 The T-13910C polymorphism in the lactase phlorizin hydrolase gene is associated with differences in serum calcium levels and calcium intake. *J Bone Miner Res.* **25,** 1980-1987.

Kull, M., R. Kallikorm and M. Lember. 2009 Impact of molecularly defined hypolactasia, self-perceived milk intolerance and milk consumption on bone mineral density in a population sample in Northern Europe. *Scand J Gastroenterol.* **44,** 415-421.

Mantel, N., and W. Haenszel. 1959 Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst.* **22,** 719-748.

Marozik, P., I. Mosse, V. Alekna, E. Rudenko, M. Tamulaitiene, H. Ramanau *et al.* 2013 Association Between Polymorphisms of VDR, COL1A1, and LCT genes and bone mineral density in Belarusian women with severe postmenopausal osteoporosis. *Medicina (Kaunas).* **49,** 177-184.

Obermayer-Pietsch, B. M., C. M. Bonelli, D. E. Walter, R. J. Kuhn, A. Fahrleitner-Pammer, A. Berghold *et al.* 2004 Genetic predisposition for adult lactose intolerance and relation to diet, bone density, and bone fractures. *J Bone Miner Res.* **19,** 42-47.

Obermayer-Pietsch, B. M., M. Gugatschka, S. Reitter, W. Plank, A. Strele, D. Walter *et al.* 2007 Adult-type hypolactasia and calcium availability: decreased calcium intake or impaired calcium absorption? *Osteoporos Int.* **18,** 445-451.

Pocock, N. A., J. A. Eisman, J. L. Hopper, M. G. Yeates, P. N. Sambrook and S. Eberl. 1987 Genetic determinants of bone mass in adults. A twin study. *J Clin Invest.* **80,** 706-710.

Richards, J. B., H. F. Zheng and T. D. Spector. 2012 Genetics of osteoporosis from genome-wide association studies: advances and challenges. *Nat Rev Genet.* **13,** 576-588.

Shen, H., J. Xie and H. Lu. 2014 Vitamin D receptor gene and risk of fracture in postmenopausal women: a meta-analysis. *Climacteric.* **17,** 319-324.

Smith, G. D., D. A. Lawlor, N. J. Timpson, J. Baban, M. Kiessling, I. N. Day *et al.* 2009 Lactase persistence-related genetic variant: population substructure and health outcomes. *Eur J Hum Genet.* **17,** 357-367.

Stewart, T. L., and S. H. Ralston. 2000 Role of genetic factors in the pathogenesis of osteoporosis. *J Endocrinol.* **166,** 235-245.

Uusi-Rasi, K., H. Sievanen, I. Vuori, M. Pasanen, A. Heinonen and P. Oja. 1998 Associations of physical activity and calcium intake with bone mass and size in healthy women at different ages. *J Bone Miner Res.* **13,** 133-142.

Valimaki, V. V., H. Alfthan, E. Lehmuskallio, E. Loyttyniemi, T. Sahi, U. H. Stenman *et al.* 2004 Vitamin D status as a determinant of peak bone mass in young Finnish men. *J Clin Endocrinol Metab.* **89,** 76-80.

Veldhuis-Vlug, A. G., L. Oei, P. C. Souverein, M. W. Tanck, F. Rivadeneira, M. C. Zillikens *et al.* 2015 Association of polymorphisms in the beta-2 adrenergic receptor gene with fracture risk and bone mineral density. *Osteoporos Int.* **26,** 2019-2027.

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

Xiong, Q., L. Xin, L. Zhang, Z. Mao and P. Tang. 2015 Association between calcitonin receptor I gene polymorphism and bone mineral density: A meta-analysis. *Exp Ther Med.* **9,** 65-76.

Xu, G. Y., Y. Qiu and H. J. Mao. 2014 Common polymorphism in the LRP5 gene may increase the risk of bone fracture and osteoporosis. *Biomed Res Int.* **2014,** 290531.

Zintzaras, E., C. Doxani, T. Koufakis, A. Kastanis, P. Rodopoulou and T. Karachalios. 2011 Synopsis and meta-analysis of genetic association studies in osteoporosis for the focal adhesion family genes: the CUMAGAS-OSTEOporosis information system. *BMC Med.* **9,** 9.

Received 20 August 2016, in revised form 6 February 2017; accepted 20 March 2017
Unedited version published online: 24 March 2017

Table 1: Characteristics of studies of the association between the LCT 13910 C/T polymorphism and femoral neck BMD

First author	Country	Ethnicity	Age	Number	Gender (F/M)	TT		TC		CC	
						n	BMD(g/cm^2)	n	BMD(g/cm^2)	n	BMD(g/cm^2)
Enattah（2004）	Finland	Caucasion	18.3-20.6	234	M	70	1.153 (0.022)	124	1.153 (0.019)	40	1.139 (0.027)
Obermayer-Pietsch (2004)	Austria	Caucasion	62±9	258	F	72	0.72 ± 0.12	125	0.68 ± 0.12	61	0.67 ± 0.11
Obermayer-Pietsch (2007)	Austria	Caucasion	65±10	73	F	41	0.72±0.12	-	-	32	0.67±0.11
Kull (2009)	Estonia	Caucasion	49.7 ± 12.5	356	F and M	101	1.058±0.14	168	1.054±0.15	87	1.084±0.16
Agueda (2010)	Spain	Caucasion	57.6 ± 8.2	477	F	81	0.68 (0.01)	212	0.67 (0.01)	184	0.67 (0.01)
Koek-1 (2010)	The Netherlands	Caucasion	69.0 ± 8.8	6367	F and M	3064	0.87 ± 0.1	2638	0.87 ± 0.1	665	0.86 ± 0.1
Koek-2 (2010)	The Netherlands	Caucasion	75.7 ± 6.6	844	F and M	435	0.71 ± 0.1	324	0.69 ± 0.1	85	0.70 ± 0.1
Marozik-1 (2013)	Belarus	Caucasion	56.7 (7.4)	131	F	30	0.8 (0.035)	65	0.865 (0.025)	36	0.825 (0.029)
Marozik-2 (2013)	Belarus	Caucasion	56.7 (7.4)	131	F	30	0.8 (0.045)	65	0.845 (0.029)	36	0.814 (0.031)

Table 2: Characteristics of studies of the association between the LCT 13910C/T polymorphism and lumbar spine BMD

First author	Country	Ethnicity	Age	Number	Gender (F/M)	TT		TC		CC	
						n	BMD(g/cm^2)	n	BMD(g/cm^2)	n	BMD(g/cm^2)
Enattah（2004）	Finland	Caucasion	18.3-20.6	234	M	70	1.207 (0.019)	124	1.221 (0.016)	40	1.176 (0.023)
Obermayer-Pietsch (2004)	Austria	Caucasion	62±9	258	F	72	0.91 ± 0.16	125	0.84 ± 0.14	61	0.84 ± 0.19
Obermayer-Pietsch (2007)	Austria	Caucasion	65±10	73	F	41	0.91±0.16	-	-	32	0.84±0.19
Kull (2009)	Estonia	Caucasion	49.7 ± 12.5	356	F and M	101	1.161±0.21	168	1.157±0.18	87	1.174±0.2
Agueda (2010)	Spain	Caucasion	55.5 ± 8.7	477	F	144	0.85 (0.01)	375	0.85 (0.01)	340	0.85 (0.01)
Koek-1 (2010)	The Netherlands	Caucasion	69.0 ± 8.8	6367	F and M	3064	1.09 ± 0.2	2638	1.09 ± 0.2	665	1.08 ± 0.2
Koek-2 (2010)	The Netherlands	Caucasion	75.7 ± 6.6	844	F and M	435	0.98 ± 0.2	324	0.97 ± 0.2	85	0.93 ± 0.2
Marozik (2013)	Belarus	Caucasion	56.7 (7.4)	131	F	30	0.905 (0.067)	65	0.990 (0.034)	36	0.958 (0.037)

Table 3 Characteristics of studies of the association between the LCT 13910 C/T polymorphism and fracture

Study	Country	Ethnicity	Age	Number	Gender (F/M)	Fracture type

Obermayer-Pietsch (2004)	Austria	Caucasion	62 ± 9	258	F	Any Fracture
Enattah-a (2005)	Finland	Caucasion	69 (62–78)	453	F	Any fracture; Vertebral fracture
Enattah-b (2005)	Finland	Caucasion	85 - 98	483	F and M	Any Fracture
Obermayer-Pietsch (2007)	Austria	Caucasion	65 ± 10	73	F	Any fracture; Vertebral fracture
Smith (2009)	UK	Caucasion	60 - 79	3344	F	Any Fracture
Bácsi (2009)	Hungary	Caucasion	62 ± 10	595	F	Any fracture; Vertebral fracture
Agueda (2010)	Spain	Caucasion	57.6 ± 8.2	713	F	Any fracture; Vertebral fracture

Table 4 WMDs and 95% CIs of the association between LCT 13910C/T polymorphism and bone mineral density

Contrasts	No. of studies (sample size)	TT vs. CC			TC vs. CC[a]			TT+TC vs. CC			TT vs. TC+CC		
		WMD[95% CI]	P value	I^2(P)	WMD[95% CI]	P value	I^2(P)	WMD[95% CI]	P value	I^2(P)	WMD[95% CI]	P value	I^2(P)
Femoral neck													
Overall	9 (8871)	0.005 [-0.005, 0.014]	p = 0.31	76.6% (p = 0.000)	0.012 [-0.001, 0.024]	p = 0.063	91.5% (p = 0.000)	0.011 [0.004,0.018]	**p = 0.003**	68.6% (p = 0.001)	-0.001 [-0.012, 0.010]	p = 0.806	92.6% (p = 0.000)
Overall[b]	8 (8637)	0.003 [-0.008, 0.015]	p = 0.588	79.0% (p = 0.000)	0.011 [-0.004, 0.026]	p = 0.149	92.1% (p = 0.000)	0.010 [0.002, 0.018]	**p = 0.013**	65.9% (p = 0.005)	-0.002 [-0.015, 0.012]	p = 0.812	93.4% (p = 0.000)
Sex[c]													
Women	5 (1070)	0.006 [-0.016, 0.028]	p = 0.581	87% (p = 0.000)	0.021 [-0.004, 0.046]	p = 0.107	95.7%(p = 0.000)	0.015 [0.003, 0.027]	**p = 0.017**	73.9% (p = 0.004)	-0.001 [-0.035, 0.033]	p = 0.971	96% (p = 0.000)
Men	1 (234)	0.014 [0.004, 0.024]	**p = 0.005**	NA	0.014 [0.005, 0.023]	**p = 0.002**	NA	0.014 [0.005, 0.023]	**p = 0.002**	NA	0.003 [-0.003, 0.010]	p = 0.277	NA
Lumbar spine													
Overall	8 (8740)	0.012 [-0.008, 0.031]	p = 0.237	91.1% (p = 0.000)	0.018 [-0.005, 0.041]	p = 0.126	95.9% (p = 0.000)	0.018 [-0.002, 0.037]	p = 0.077	93.8% (p = 0.000)	-0.002 [-0.013, 0.010]	p = 0.778	85.6% (p = 0.000)
Overall[b]	7 (8506)	0.007 [-0.015, 0.029]	p = 0.521	79.3% (p = 0.000)	0.012 [-0.005, 0.029]	p = 0.159	77.8% (p = 0.000)	0.000 [-0.001, 0.002]	p = 0.807	39.4% (p = 0.129)	0.001 [-0.017, 0.020]	p = 0.895	87.4% (p = 0.000)
Sex[c]													
Women	4 (939)	0.009 [-0.034, 0.053]	p = 0.679	86.9% (p = 0.000)	0.013 [-0.014, 0.040]	p = 0.359	89% (p = 0.000)	0.000 [-0.001, 0.001]	p = 0.921	26% (p = 0.256)	0.009 [-0.044, 0.062]	p = 0.739	93.4% (p = 0.000)
Men	1 (234)	0.031 [0.023, 0.039]	**p = 0.000**	NA	0.045 [0.037, 0.053]	**p = 0.000**	NA	0.040 [0.032, 0.048]	**p = 0.000**	NA	-0.003 [-0.009, 0.003]	p = 0.324	NA

WMD, weighted mean difference; CI, confidence interval.

[a] The study by Obermayer-Pietsch et al., 2007 was not included in the meta-analysis since it didn't present the data on TC genotype.

[b] The results of when the study of Enattah *et al*., 2004 was excluded.

[c] The studies by Kull et al., 2009 and Koek *et al*., 2010 did not present the sex-specific data.

Table 5 Odds ratios and heterogeneity results for the association of LCT 13910C/T polymorphism with fractures risk

asts	No. of	TT vs. CC	TC vs. CC[a]	TT+TC vs. CC	TT vs. TC+CC	T allele vs. C allele

	studies (sample size)	OR[95% CI]	P value	I^2(P)	OR[95% CI]	P value	I^2(P)	OR[95% CI]	P value	I^2(P)	OR[95% CI]	P value	I^2(P)	OR[95% CI]	P value	I^2
acture																
rall	7(5919)	0.684[0.432, 1.084]	p = 0.106	74.5% (p = 0.001)	0.769[0.522, 1.133]	p = 0.184	74.9% (p = 0.001)	0.738[0.500, 1.090]	p = 0.127	76.3% (p = 0.000)	0.813[0.704, 0.938]	**p = 0.005**	19.3% (p = 0.282)	0.885 [0.792, 0.989]	**p = 0.032**	10.7% (
Fracture																
rall	4(1834)	1.086[0.676, 1.745]	p = 0.732	0% (p = 0.662)	0.951[0.673, 1.346]	p = 0.778	0% (p = 0.570)	0.979[0.709, 1.354]	p = 0.900	0.0% (p = 0.662)	1.104[0.724, 1.682]	p = 0.646	0% (p = 0.849)	1.007[0.775, 1.309]	p = 0.958	0.0% (p

y by B. M. Obermayer-Pietsch (2007) et al. was not included in the meta-analysis since it didn't present the data on TC genotype.

Figure 1. Flow chart of meta-analysis for exclusion/inclusion of studies

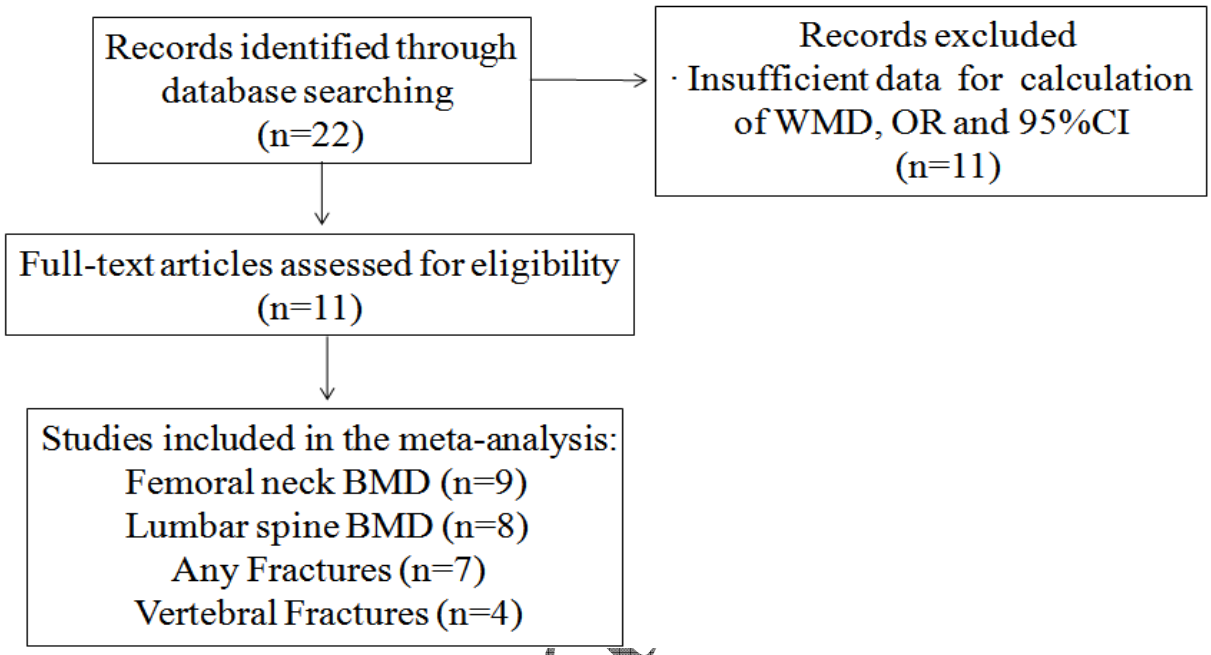


Figure 2. Meta-analysis of the association between LCT 13910 C/T polymorphism and BMD of femoral neck under dominant model (TT + TC vs. CC)

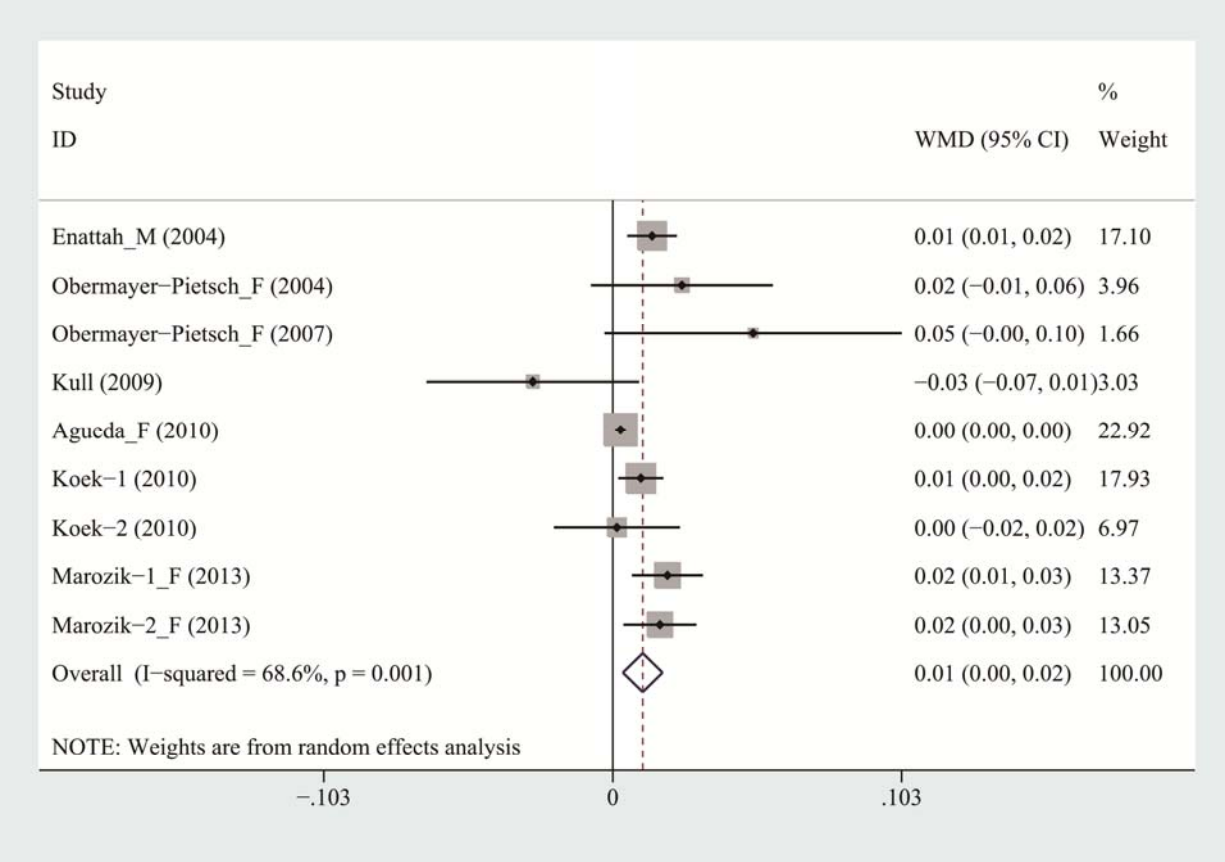


Figure 3. Sensitivity analysis of association between LCT 13910 C/T polymorphism and BMD of Lumbar spine

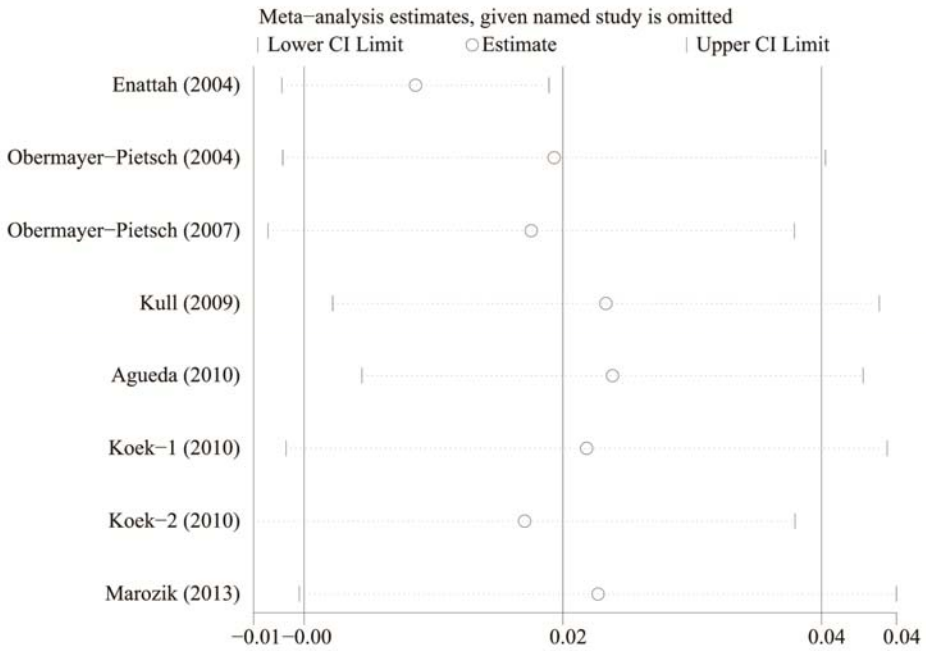
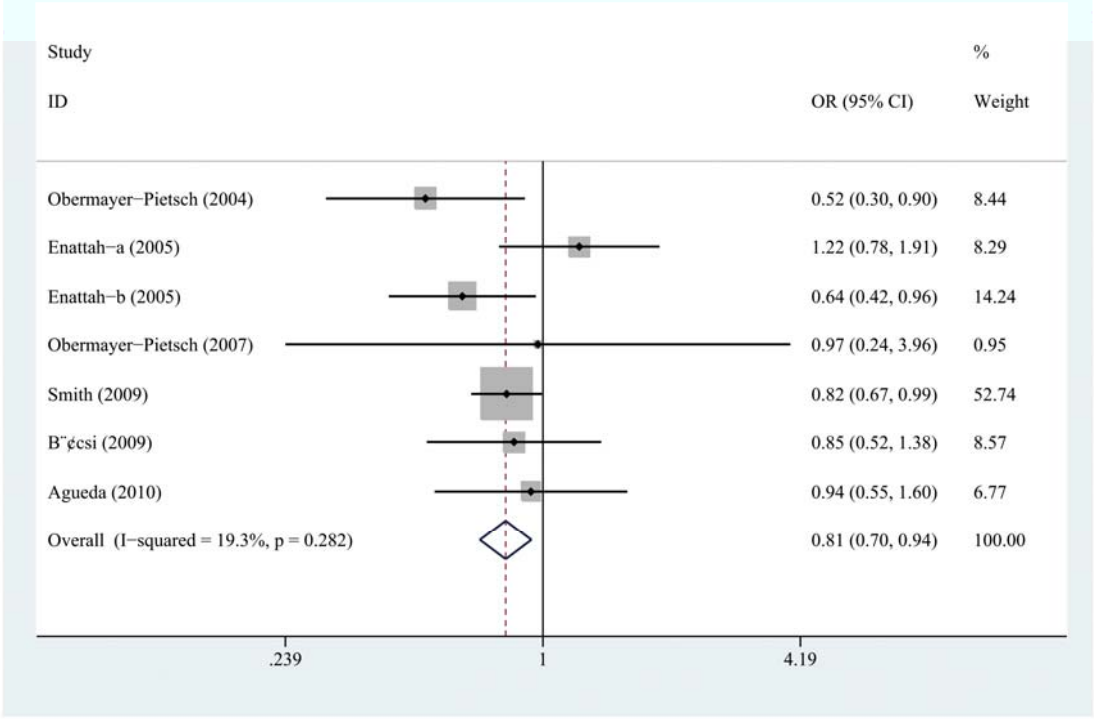


Figure 4. Meta-analysis of the association between LCT 13910 C/T polymorphism and fracture risk under (a) recessive model (TT vs. TC+CC), and (b) allele model (T allele vs. C allele)

(a)


Study
ID
OR (95% CI)
%
Weight
Obermayer−Pietsch (2004)
0.52 (0.30, 0.90)
8.44
Enattah−a (2005)
1.22 (0.78, 1.91)
8.29
Enattah−b (2005)
0.64 (0.42, 0.96)
14.24
Obermayer−Pietsch (2007)
0.97 (0.24, 3.96)
0.95
Smith (2009)
0.82 (0.67, 0.99)
52.74
B¨¢csi (2009)
0.85 (0.52, 1.38)
8.57
Agueda (2010)
0.94 (0.55, 1.60)
6.77
Overall (I−squared = 19.3%, p = 0.282)
0.81 (0.70, 0.94)
100.00
.239
1
4.19

(b)

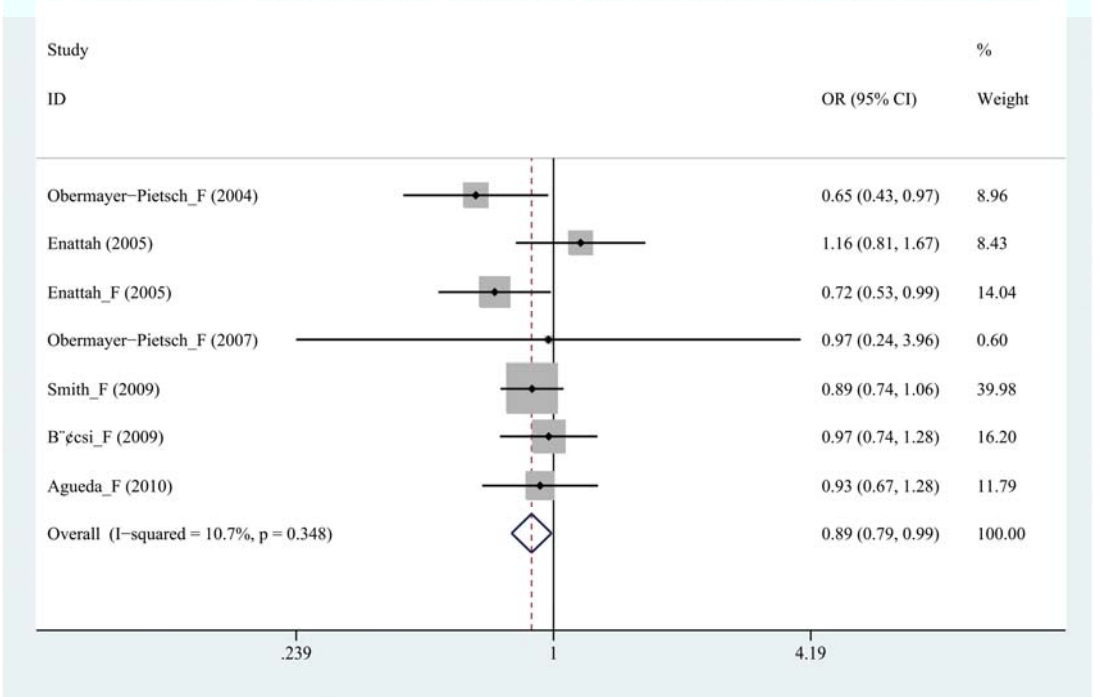

Study
ID
OR (95% CI)
%
Weight
Obermayer−Pietsch_F (2004)
0.65 (0.43, 0.97)
8.96
Enattah (2005)
1.16 (0.81, 1.67)
8.43
Enattah_F (2005)
0.72 (0.53, 0.99)
14.04
Obermayer−Pietsch_F (2007)
0.97 (0.24, 3.96)
0.60
Smith_F (2009)
0.89 (0.74, 1.06)
39.98
B¨¢csi_F (2009)
0.97 (0.74, 1.28)
16.20
Agueda_F (2010)
0.93 (0.67, 1.28)
11.79
Overall (I−squared = 10.7%, p = 0.348)
0.89 (0.79, 0.99)
100.00
.239
1
4.19

Figure 5. Funnel plot for FN BMD between LCT C13910T TT/TC and CC

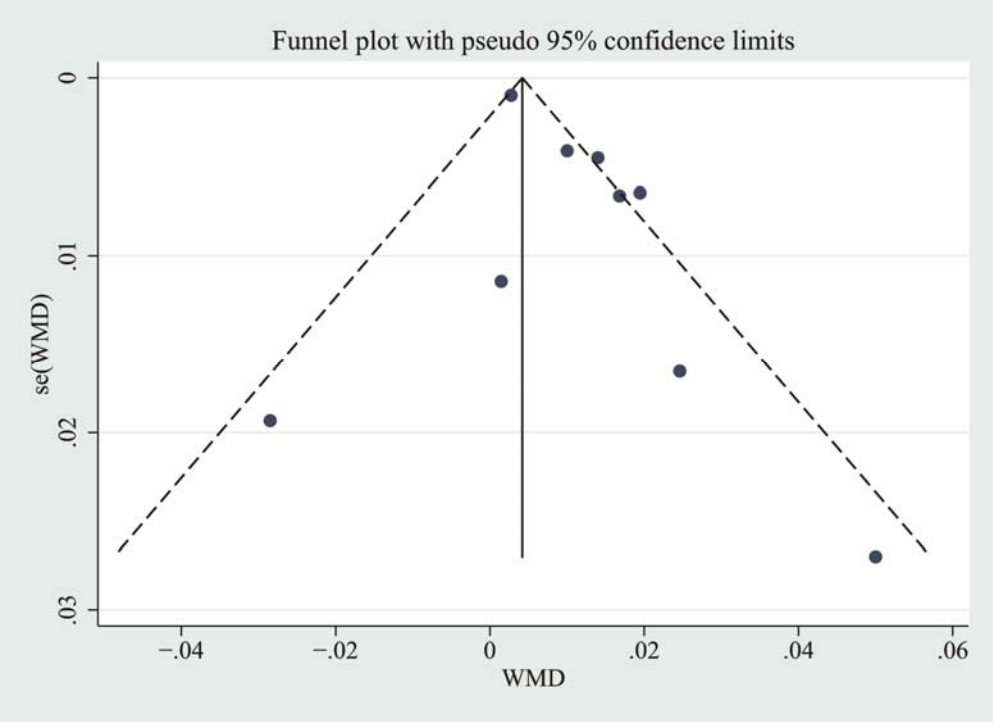


Figure 6. Funnel plot for Any Fracture between LCT C13910T TT and TC/CC