SYSTEMATIC REVIEW AND META-ANALYSIS OF THE ASSOCIATION BETWEEN VITAMIN D LEVELS AND THE SINGLE NUCLEOTIDE POLYMORPHISMS, *rs7041*, *rs4588*, AND *rs2282679* OF THE GROUP-SPECIFIC COMPONENT GENE (GC)

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ABSTRACT

Background: Previous literature suggested that certain single nucleotide polymorphisms (SNP) of group-specific component gene (GC) (i.e. rs7041, rs4588, and rs2282679) were associated with serum vitamin D level.

Objectives: To assess effects of GC gene including rs7041, rs4588, and rs2282679 on serum vitamin D level.

Methods: Studies were located from Medline via PubMed and Scopus databases from initiation to 17th January 2015. All observational studies, published in English were selected if they studied the effect of interested SNPs on serum vitamin D level. Mean differences (MD) of serum vitamin D level between genotypes of each SNP were estimated using multivariate meta-analysis.

Results: A total of 44 studies met inclusion criteria, 25, 20, and 27 studies had data for rs4588, rs7041, and rs2282679, respectively. For rs4588; genotype effect of overall pooled MDs of 25 hydroxy vitamin D (25(OH)D) levels, MD1 (AA versus CC) and MD2 (AC versus CC) for Caucasian adults were, -4.119 (-5.408, -2.831) and -1.871 (95%CI; -2.644, -1.097), and homogenous with the I2 of 25% and 0%, respectively. For rs7041, pooled MD of 25(OH)D level between genotypes TT versus GG (MD1) and TG versus GG (MD2) were, -2.707 (95%CI: -4.91, -0.504) and -1.407 (95%CI: -2.203, -0.612) in Caucasians, with I2 71.4% and 0%, respectively. For rs2282679, pooled MD of 25(OH)D level between genotypes TT versus GG (MD1) and TG versus GG (MD2) for Caucasians were, -3.598 (95%CI: -5.086, -2.111) and -1.841 (95%CI: -2.743, -0.94), with I2 of 76% and 80.4%, respectively.

Conclusions: The SNPs rs4588 and rs2282679 showed negative association with level of serum 25(OH)D.

KEY WORDS: VITAMIN D/rs7041/ rs4588/rs2282679/GC

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LIST OF ABBREVIATIONS

1,25(OH)2D	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
BMI	Body mass index
CI	Confidence interval
GC	Group-specific component
HWE	Hardy-Weinberg equilibrium
MD	Mean difference
n	Number of subjects
OR	Odds ratio
RANK	Receptor Activator of Nuclear Factor Kappa
RANKL	Receptor Activator of Nuclear Factor Kappa Ligand
SD	Standard deviation
SE	Standard error
SNP	Single nucleotide polymorphism

CHAPTER I
INTRODUCTION

1.1 Vitamin D synthesis

Vitamin D is a fat soluble vitamin, which can be acquired from various foods (e.g., fish, liver oils, certain plants and fungi) and synthesized in the skin when exposed to the ultra violet blue radiation from sunlight. The synthesis pathway is described in Figure 1.1[1].

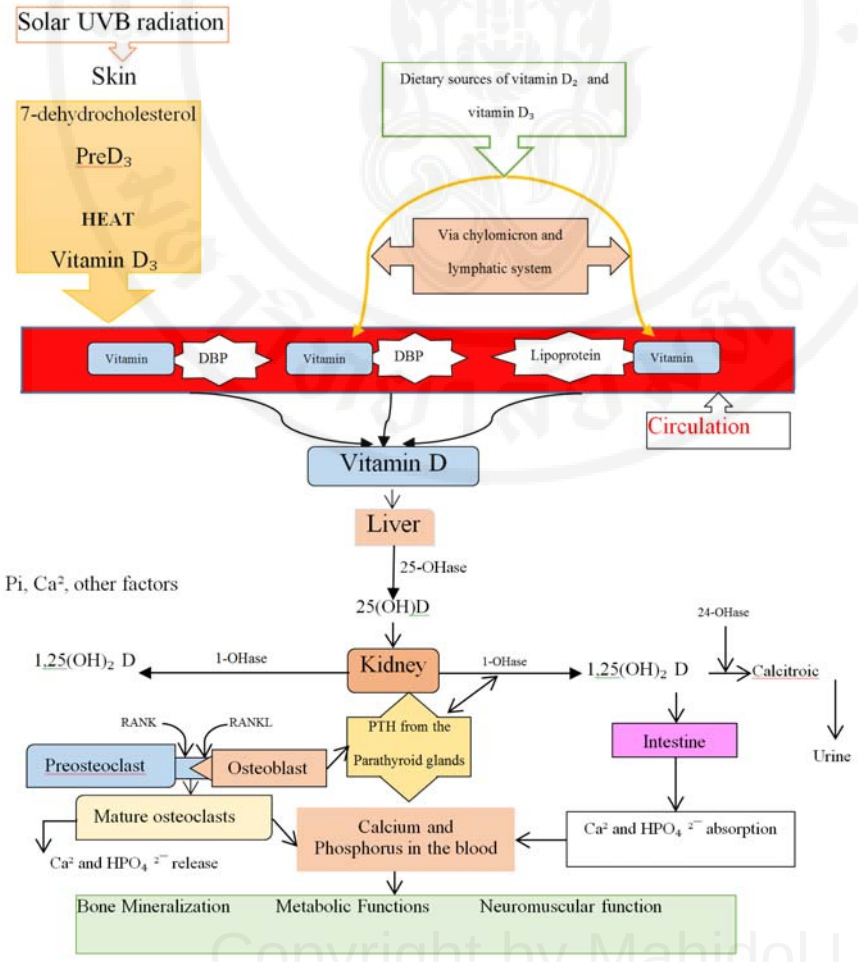

Figure 1.1 Vitamin D synthesis

Two types of vitamin D (i.e., vitamin D_2 (cholecalciferol) and vitamin D_3 (ergocalciferol)) are transported in the blood stream, where D_2 is bounded to chylomicrons and lipoproteins whereas vitamin D_3, from the skin, is bounded to a group-specific component (GC), which is also known as vitamin D-binding protein[1]. Vitamin D_3 and vitamin D_2 are converted to 25-hydroxyvitamin D (25(OH)D) in the liver by the enzyme 25-hydroxylase (CYP2R1). 25(OH)D is transformed in the kidneys to the active form of vitamin D known as 1,25-dihydroxyvitamin D (1,25(OH)2D), which binds to the vitamin D receptor to exert its biological effect. It also plays a role in the absorption of calcium and phosphorus from the intestines, as well as stimulating the expression of Receptor Activator of Nuclear Factor Kappa Ligand (RANKL) on the osteoblasts. This causes it to interact with the Receptor Activator of Nuclear Factor Kappa (RANK) on proteoclasts and subsequently induces the mature osteoclastic activity, resulting in the release of calcium and phosphorus[1]. Also 1,25(OH)2D acts as its own inhibitor and causes 24-hydroxylase (CYP24A1) to convert it to its biologically inactive form as calcitroic acid which is water-soluble that is excreted in the bile. 25(OH)D is the main form of vitamin D found in the blood, which makes it a good biomarker of a body's vitamin D level.

Besides sun exposure and diet, serum levels of 25(OH)D can also be affected by other factors such as supplementary vitamin D intake, race, age, sex, obesity, skin color, and tea intake [2]. Genetic factors may also contribute to the serum levels of vitamin D [29, 63, 68].

1.2 Functions of Vitamin D

Vitamin D plays a role in several functions of the body, such as regulating calcium and phosphate homeostasis and playing a maintenance role in the smooth functioning of the musculoskeletal system. Vitamin D has also been implicated in the sustenance of a healthy immune system, cardiovascular function, respiratory function, and in the development of the brain as well as having anti-cancer effects [3].

Vitamin D deficiency can increase risk of developing osteoporosis [4], multiple sclerosis [5], rickets, metabolic syndrome, hypertension [6], autoimmune diseases [7], cardiovascular diseases [8], type 1 and type 2 diabetes mellitus [9 10 11] as well as several

types of cancer [12] [13] [14]. Vitamin D deficiency is also commonly found in patients with rheumatoid arthritis [4], and liver fibrosis. High vitamin D levels may be favorably associated with chronic inflammatory diseases such as multiple sclerosis, and psoriasis [15].

1.3 Genes involved with the vitamin D synthesis pathway

Single nucleotide polymorphisms (SNP, pronounced as “snips), are the most common type of genetic variations among people. Each SNP represents a difference in a single DNA building block, called a nucleotide. For example, a SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA [16]. A SNP is somewhat like a point mutation, but much more frequent in its occurrence [17].

SNPs occur in at least 1% of the population for it to be called a SNP. SNPs can be classified into 2 main categories, i.e., linked and causative/disease SNPs, see Figure 2. Linked SNPs (also called indicative SNPs) are not on the disease genes, but they are found nearby [17]. They do not affect the gene expression of proteins, but they do correspond to a particular drug response or the risk of a disease. Causative/disease SNPs can be coding or non-coding SNPs. Coding SNPs are found within the coding region that affect the amino-acid sequence of the protein whereas non-coding SNPs are found in the regulatory sequences of the gene and can change the timing, location or level of the gene expression [17].

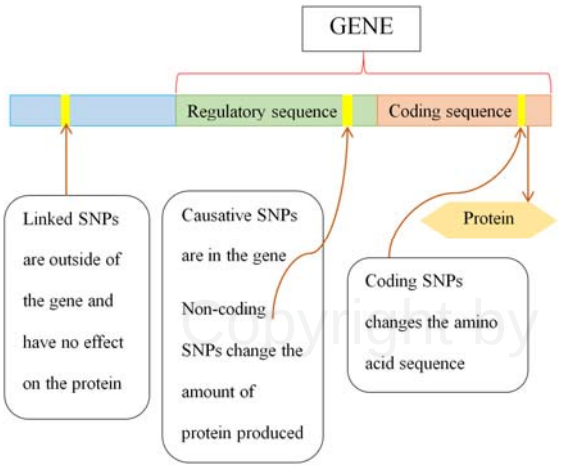

Figure 1.2 Types of SNPs diagram

Some of the following genes are involved in the vitamin D synthesis pathway. First, the vitamin D-binding protein is encoded by the GC that transports vitamin D3 in the blood [18]. Vitamin D3 and vitamin D2 are also converted to 25(OH)D in the liver by the enzyme 25-

hydroxylase which is encoded by the gene CYP2R1 [19].

There have been several studies which assessed the association between genes and serum 25(OH)D levels. These SNPs included GC genes (e.g., rs7041 [20-22], rs4588 [23-27], and rs2282679 [28, 29]), and other genes (e.g., rs1155563 [30], rs222016, rs222020, and rs222029 [31], rs2298849 [18], rs842999, rs12512631, rs16846876 and rs17467825) [32] which found to significantly associate with serum levels of 25(OH)D. Among these SNPs, the GC gene at rs7041, rs4588, and rs2282679 were the most common SNPs that had been studied. Genetic risk score analysis also suggested that carriers with no risk alleles of rs4588 and rs842999 had significantly higher serum 25(OH)D concentrations compared to carriers of all risk alleles [32], or in another word, having the risk genotype of rs7041 was associated with lower levels of 25(OH)D [20, 29]. In addition, subjects who carried risk allele of GC at rs2282679 were increased risk of developing vitamin D deficiency [33, 34].

1.4 Rationale for conducting a systematic review

Associations between GC genes and levels of serum 25(OH)D were controversial, i.e., some studies [29] found significant associations whereas some other studies [22, 35] did not. This might be because some of those individual studies had small sample sizes [20, 22, 24, 33, 34, 36-38], and thus could not detect the gene effects whereas some studies with larger sample sizes could. Increasing the sample size by conducting a systematic review to identify these relevant studies as many as possible, and then pool them together using meta-analysis should be able to increase power to detect gene effects. Therefore, a systematic review and meta-analysis was performed with following research questions.

1.5 Research question

Is there an association between the vitamin D level and the SNPs, including, rs7041, rs4588, and rs2282679 of the GC gene?

1.6 Research objectives

1.6.1 To estimate minor allele effects on serum 25(OH)D levels by estimating the pooled mean differences (MDs) between

- T vs G for rs7041

- A vs C for rs4588

- G vs T for rs2282679

1.6.2 To estimate genotype effects on serum 25(OH)D levels by estimating the pooled MDs between

- TT vs GG and TG vs GG for rs7041

- AA vs CC and AC vs CC for rs4588

- GG vs TT and GT vs TT for rs2282679

1.6.3 To estimate allele effects on vitamin D deficiency or /insufficiency by estimating the pooled odds ratios (OR) of

- T vs G for rs7041

- A vs C for rs4588

- G vs T for rs2282679

1.6.4 To estimate the genotype effects on vitamin D deficiency/ insufficiency by estimating the pooled ORs of

- TT vs GG and TG vs GG for rs7041

- AA vs CC and AC vs CC for rs4588

- GG vs TT and GT vs TT for rs2282679

CHAPTER II
MATERIALS AND METHODS

2.1 Search strategy

Relevant studies were located from Medline via PubMed and Scopus databases from initiation to January 2015. Reference lists of included studies were explored for additional relevant studies. The search terms were constructed according to P, I, and O domains of PICO, as described in Appendix A. Search strategies were then constructed by combining search terms with 'OR' within the same domain, and 'AND' for between the domains, as described below for PubMed and Scopus:

PubMed:

The 3 domains were constructed using the search terms:

– ((("allele") OR "genotype") OR "polymorphism") OR "polymorphisms") OR "SNPs") OR "single nucleotide polymorphisms"))

– ((("rs4588") OR "rs7041") OR "rs2282679") OR "Thr420Lys") OR "Glu416Asp") OR "group specific component") OR "GC gene") OR "GC loci") OR "GC locus"))

– ((("1,25(OH)2D") OR "1,25-dihydroxyvitamin D") OR "25(OH)D") OR "25 hydroxyvitamin D") OR "vitamin D"))

The 3 terms were then combined with 'AND' as follows:

– ((("allele") OR "genotype") OR "polymorphism") OR "polymorphisms") OR "SNPs") OR "single nucleotide polymorphisms")) AND ((("rs4588") OR "rs7041") OR "rs2282679") OR "Thr420Lys") OR "Glu416Asp") OR "group specific component") OR "GC gene") OR "GC loci") OR "GC locus")) AND ((("1,25(OH)2D") OR "1,25-dihydroxyvitamin D") OR "25(OH)D") OR "25 hydroxyvitamin D") OR "vitamin D"))

Scopus:

The 3 domains were constructed using the search terms:

– ("allele" OR "genotype" OR "polymorphism" OR "polymorphisms " OR "SNPs" OR "single nucleotide polymorphisms")

– ("rs4588" OR "rs7041" OR "rs2282679" OR "Thr420Lys" OR "G lu416Asp" OR "group specific component" OR "GC gene" OR "GC loci" OR "GC locus")

– ("1, 25(OH)2D" OR "1, 25-dihydroxyvitamin D" OR "25(OH)D" OR "25 hydroxyvitamin D" OR "vitamin D")

The 3 terms were then combined with 'AND' as follows:

– ("allele" OR "genotype" OR "polymorphism" OR "polymorphisms " OR "SNPs" OR "single nucleotide polymorphisms") AND ("rs4588" OR "rs7041" OR "rs2282679" OR "Thr420Ly s" OR "Glu416Asp" OR "group specific component" OR "GC gene" OR "GC loci" OR "GC locus") AND ("1, 25(OH)2D" OR "1, 25-dihydroxyvitamin D" OR "25(OH)D" OR "25 hydroxyvitamin D" OR "vitamin D"

2.2 Selection of studies

Inclusion criteria

All observational studies published in English were included, if they met all of the following criteria.

– Studied in human.

– Studied the effect of genotypes or alleles of any of the following SNPs: rs2282679, rs7041, or rs4588.

– Measured any of the following outcomes: serum 25(OH)D, or serum 1,25(OH)2D levels for continuous outcomes; or vitamin D insufficiency or deficiency for dichotomous outcomes.

– Have sufficient data for pooling, i.e. mean and standard deviation (SD) or the beta coefficients and its standard error for continuous outcome (e.g., serum 25(OH)D levels) between allele/genotype groups; reported the contingency table of

interested allele/genotypes and vitamin D insufficiency or deficiency; or odds ratio (OR) and the 95% CI the for dichotomous outcomes).

Studies that did not meet with our inclusion criteria were coded, with an ineligibility criterion coding, see Appendix B.

2.3 Study selection

Initial studies were identified from Medline via PubMed and Scopus and then managed using the EndNote software X7 by remove duplicates. The studies were independently selected based on the title and abstracts by two reviewers (IA and TA), studies that did not meet the eligibility criteria were eliminated with providing the reasons. The full text articles were retrieved and read to make decisions to include in the review. Results of study selection between the two reviewers were then validated. Any disagreements were solved by discussion and consensus. A kappa test was applied to assess agreements between the two reviewers.

2.4 Data extraction

Summary data were extracted independently by two reviewers (IA and TA) using a standardized data extraction form, see Appendix C. The data extraction forms consisted of general characteristics of studies and studied subjects, The general characteristics of the studies included the author, journal, year of publication, type of study design, types of SNP, and whether the HWE was checked or not. The characteristics of the studied subjects were those that may be associated with the vitamin D level. These included, age, gender, obesity, physical activity, kidney disease, liver disease, sun exposure, alcohol intake, and vitamin D supplement.

Data for pooling were continuous and dichotomous outcomes. For continuous outcome, total number of studied subjects, mean, and SD of the serum vitamin D levels by allele/genotype groups were extracted. For those studies did not report mean and SD but instead reported the beta coefficients and the SE, these data

were extracted and combined with estimated MDs from those studies reported mean and SD.

For dichotomous outcome, the frequencies of the alleles and the genotypes between outcome groups were collected. ORs and along with 95% CI were used for those studies did not report frequency data. Any disagreement was resolved by consensus. Data cleaning and checking were performed separately for each study.

2.5 Risk of bias assessment

The risk of bias was assessed by modifying the form constructed by Thakkinstian et al[39]. The forms were used separately by type of study design (i.e., the case-control, cohort, and cross-sectional study), see Appendix D. The forms consisted of 6 domains as follows:

For case control studies

2.5.1 Selection bias

2.5.1.1 Representativeness of cases and controls assessed whether the cases and controls were consecutive/randomly selected from their population with a clearly defined random frame.

2.5.2 Information bias

2.5.2.1 Ascertainment of vitamin D level assessed whether the method of vitamin D measurement was clearly given, and also whether definition of vitamin D deficiency or insufficiency was clearly defined.

2.5.2.2 Ascertainment of genotyping examination assessed whether the genotyping was done with blinding, whether the error rate was reported and whether any quality control measures were taken.

2.5.3 Confounding bias.

2.5.3.1 Population stratification assessed whether any population stratification was done.

2.5.3.2 Confounding variables assessed whether any

confounding variables were adjusted in analysis.

2.5.4 Multiple testing.

2.5.4.1 Assessed whether any adjustment was done for multiple testing.

2.5.5 Selective reporting.

2.5.5.1 Assessed whether the study reported the results of all polymorphisms mentioned in objectives, regardless significant or not.

2.5.6 HWE.

2.5.6.1 Assessed whether Hardy-Weinberg equilibrium (HWE) was checked.

For cross sectional and cohort studies

2.5.7 Selection bias.

2.5.7.1 Representativeness assessed whether the study subjects were consecutive/randomly selected from the target population with a clearly defined random frame.

2.5.8 Information bias.

2.5.8.1 Ascertainment of vitamin D level assessed whether the method of vitamin D measurement was clearly described.

2.5.8.2 Ascertainment of genotyping examination assessed whether the genotyping was done with blinding, whether the genotyping error rate was reported and whether any quality control measures were taken.

2.5.9 Confounding bias.

2.5.9.1 Population stratification assessed whether any population stratification was done.

2.5.9.2 Confounding variables assessed whether any confounding variables were adjusted when means were compared between allele/genotype groups.

2.5.10 Multiple testing.

2.5.10.1Assessed whether any adjustment was done for multiple testing.

2.5.11 Selective reporting.

2.5.11.1Assessed whether the study reported the results of all polymorphisms mentioned in objectives, regardless significant or not.

2.5.12 HWE.

2.5.12.1 Assessed whether HWE was checked.

Each item was asked whether there was low risk of bias and four possible answers were:

- Yes, meant there was low risk of bias.
- No, meant there was high risk of bias
- Unclear, if the information was not clearly given and thus could not make the decision to be yes or no.
- Inapplicability meant that the item could not be assessed.

2.6 Studied polymorphism

The three SNPs studied were rs7041, rs4588, and rs2282679.

The SNP rs2282679 has the alleles T (major) and G (minor) and the genotypes TT, GT, and GG.

The SNP rs7041 has the alleles G (major) and T (minor) and the genotypes GG, TG, and TT.

The SNP rs4588 has the alleles C (major) and A (minor) and the genotypes CC, AC, and AA.

2.7 Outcome of interest

The outcome of interest was the serum vitamin D reported according to the individual studies as:

2.7.1 25(OH)D levels

The 25(OH)D levels were reported in the units of ng/ml. For those studies reported as nmol/l, they were converted to ng/ml by dividing with the conversion factor of 2.496[40].

2.7.2 Vitamin D deficiency or vitamin D insufficiency

Vitamin D was classified as deficiency or insufficiency according to original studies. Briefly, the 25(OH)D < 20 ng/ml and 21 – 29 ng/ml were respectively considered as vitamin D deficiency and insufficiency, as for the American Endocrine Society clinical practice guideline[41].

2.8 Statistical analysis

2.8.1 Kappa test for agreement in study selection.

Percent agreement of study selection between 2 reviewers was assessed using Kappa statistic. The kappa was calculated using the equation below[42].

$$K = \frac{(O - E)}{(1 - E)}$$

Where is K the Kappa, O is the observed agreement, and E is the expected agreement.

Observed agreement is the total number of agreements (%) in both included and excluded studies by both reviewers (IA and TA), i.e., (a +d) as shown in Table 2.1. Expected agreement was calculated using equation given below [42].

$$E = \left(\left(\frac{n_1}{n}\right)\left(\frac{m_1}{n}\right)\right) + \left(\left(\frac{n_0}{n}\right)\left(\frac{m_0}{n}\right)\right)$$

Where E is the expected agreement, n_1 is the total number of included studies by reviewer 1, m_1 is the total number of included studies by reviewer 2, n_0 is the total number of excluded studies by reviewer 1, m_0 is the total number of excluded studies by reviewer 2, n is the total number of selected studies

Level of agreement can be categorized as poor, slightly poor, fair, moderate, substantial, and perfect chance of agreement if the Kappa statistic ranged from 0.0 to <0.2 , 0.2 to < 0.4, 0.4 to < 0.6, 0.6 to <0.8, 0.8 to <1, and 1.0, respectively.

Table 2.1 Example of observed and expected values of both reviewers for calculating Kappa

Observed		Reviewer 2 (TA)		
		Included	Excluded	Total
Reviewer 1 (IA)	Included	a	b	m_1
	Excluded	c	d	m_0
Total		n_1	n_0	n
Expected		Reviewer 2 (TA)		
		Included	Excluded	Total
Reviewer 1 (IA)	Included	e_{11}	e_{12}	m_1
	Excluded	e_{21}	e_{22}	m_0
Total		n_1	n_0	n

2.8.2 HWE

The HWE was assessed only in the control groups if the study design was case-control whereas the whole data was used for cohort/cross-sectional studies. Suppose C and A was the major and minor alleles respectively. The HWE was assessed to see if the distribution of the genotypes CC, AC, and AA in the control group were complied with frequency of p^2, 2pq, and q^2 respectively. We used the Chi square or Fisher's exact test where appropriate (i.e., if the sample size was too small in more than 20% of the cells) to see whether they deviated from the HWE. A sensitivity analysis was done for those studies which showed Hardy-Weinberg disequilibrium (HWD).

2.8.3 Pooling minor allele prevalence

The minor allele frequency of the studied SNP was pooled using data from control groups if the design was case-control study; otherwise the whole data was used. Where the data for type of subject (i.e., children and adults) and ethnicity were available, we pooled the prevalence of minor allele, stratified accordingly by ethnicity and type of subjects.

Overall prevalence of minor allele A was pooled using the inverse variance method. This was done according to the equation for pooling as given[43].

$$\bar{p} = \frac{\sum w_i p_i}{\sum w_i}$$

Where $\bar{p}$ was the pooled prevalence of the minor allele, p_i was the prevalence of the minor allele in each study, and w_i was the inverse variance $\left(\frac{1}{\text{var}(p_i)}\right)$, which was the weight of each study.

The 95% CI was calculated using the using the equation as given [43].

$$95\%CI = \bar{p} \pm \frac{1.96}{\sqrt{\sum wi}}$$

Preparing the data for pooling prevalence.

As for minor and major A and C alleles, a total number of A and C alleles, total number of alleles (A +C) in the control population were calculated from genotype data as follows:

Total A allele = $2 \times$ n(AA) + n (AC)

Total C allele = $2 \times$ n(CC) + n(AC)

Total number of alleles = Total A allele + Total C allele.

The 'pmeta' command from STATA was used to estimate the pooled prevalence of the minor allele A along with its 95% CI, and also heterogeneity parameters. If the heterogeneity was present, a random-effect model was applied for pooling.

2.8.4 Assess the gene effects

We applied both per-allele and per genotype approaches for assessing gene effects as follows.

2.8.4.1 Per-allele approach.

Suppose that A and C were minor and major alleles, respectively.

2.8.4.1.1 For continuous outcome

For 25(OH)D outcome, the MD of 25(OH)D between C and A alleles along with its variance and 95% CIs were estimated for each study. For those studies provided only coefficients and the SE of allele effect, these data were later appended with calculated MDs. Unstandardized MD was applied to pool MDs across studies, using the equations below:

$$\hat{D} = \frac{\sum_{i=1}^{k} w_i d_i}{\sum_{i=1}^{k} w_i}$$

$$d_i = \left(\bar{x}_{1i} - \bar{x}_{2i}\right),\ \text{var}(d_i) = \frac{sd_{1i}^2}{n_{1i}} + \frac{sd_{2i}^2}{n_{2i}},\ wi = \frac{1}{\text{var}(d_i)}$$

$$95\%CI = d_i \pm 1.96\sqrt{\text{var}(d_i)}$$

Where $\hat{D}$ is the pooled unstandardized mean difference, and d_i is the unstandardized mean differences. $\bar{x}_{1i}$ and $\bar{x}_{2i}$ are the means of 25(OH)D levels in subjects with C allele and A allele. w_i is the weight, and $\text{var}(d_i)$ is the variance of the unstandardized mean differences. n_{1i} and n_{2i} are the number of A and C alleles. sd_{1i}^2 and sd_{2i}^2 are the SDs of the MDs for subjects with A and C alleles.

The fixed-effect model was applied if there was no heterogeneity; otherwise a random-effect model was applied.

2.8.4.1.2 Dichotomous outcome

For studies which reported the frequency data of A and C alleles between controls and vitamin D deficiency/insufficiency, log OR along with its variance and the 95%CI were estimated for each study. Some studies did not report the frequency of genotype data but instead provided the ORs and the 95%CIs. In that case, this data were later combined with ORs of those studies that reported frequency of genotype data. The pooled OR was then estimated using inverse variance method if the heterogeneity was not present, otherwise a random-effect model using the Der-Simonian and Laird method was applied.

For the fixed effects model, the equation was given below:

$$\ln \hat{OR}_{pooled} = \frac{\sum_j^k w_i \ln OR_j}{\sum_j^k w_i}$$

$$OR_j = \frac{a_j d_j}{b_j c_j}$$

$$\text{var}\ln(OR_j) = \ln\left(\frac{1}{a_j} + \frac{1}{b_j} + \frac{1}{c_j} + \frac{1}{d_j}\right)$$

$$w_j = \frac{1}{\text{var}_j}$$

$$95\%CI \ln(\hat{OR})_j = \ln(\hat{OR})_j \pm 1.96\sqrt{\text{var}\ln(\hat{OR})_j}$$

$\ln \hat{OR}_{pooled}$ is the pooled log (OR) of allele A versus C, $\ln OR_j$ is the natural logarithm of OR of each study, $\text{var}\ln(OR_j)$ is the variance of the log (OR), and w_j is the weight. The frequencies of C alleles for cases and controls are a and b respectively. The frequencies of A alleles for cases and controls are c and d respectively.

2.8.4.2 The genotype approach.

2.8.4.2.1 For continuous outcomes

The MD_1 (AA versus CC) and MD_2 (AC versus CC) and their respective SEs were estimated for each study. A multivariate meta-analysis with a random-effect model was applied for pooling genotype effects of MD_1 and MD_2. A maximum likelihood function was used for estimation of the pooled MDs assuming zero variance-covariance between MD_1 and MD_2. In addition, pooled MD_3 (AA versus AC) was then estimated using a linear combination of $MD_1 - MD_2$ from the multi-variate meta-analysis model.

2.8.4.2.2 For dichotomous outcomes

For dichotomous outcome, the data were reshaped from wide format to long format with 3 × 2 observations per study. The summary long format data were then expanded to be individual patient data using 'expand' command. The log-scales for OR_1 (AA versus CC), OR_2 (AC versus CC), variance of $\ln(OR_1)$, $\ln(OR_2)$, and covariance ($\ln OR_1$ and $\ln OR_2$) were then estimated using the 'mvmeta make' command with logit model. Summary ORs and their variances were then appended to these estimations for those studies where frequency data were not available. The multi-variate meta-analysis was then used to pool OR_1 and OR_2 across studies simultaneously. In addition, the pooled OR_3 (AA versus AC) was then estimated using a linear combination of $\ln OR_1 - \ln OR_2$ from the multi-variate meta-analysis model.

2.8.5 Assess the heterogeneity.

To check for heterogeneity of gene effects, we used the Cochran Q test and quantified the degree of heterogeneity (I^2). The p value < 0.1 from Cochran Q test or the $I^2 \geq 25$ was considered as presence of heterogeneity. If heterogeneity was present, we pooled the data using the random-effect model; otherwise the fixed-effect model was used. The null hypothesis for heterogeneity states that there is no heterogeneity among the effect size (e.g., ORs/MDs) across studies. Heterogeneity was assessed by the following equations:

2.8.5.1 Assessing the heterogeneity for pooling minor allele prevalence.

$$Q = \sum w_i (p_i - \bar{p})^2$$

Where Q is the heterogeneity, $\bar{p}$ is the pooled prevalence of the allele, p_i is the prevalence of the allele in each study, and w_i is the inverse variance ($1/\text{var}(p_i)$).

If heterogeneity was present, then the between-study variation (τ^2) was estimated with the equation given below[43]:

$$\tau^2 = \frac{Q-(k-1)}{\sum w_i - \frac{\sum {w_i}^2}{w_i}} \quad \text{if } Q > \text{k-1 or 0 otherwise.}$$

The weight could be calculated taking into account for τ^2 as

$$w_i^* = \frac{1}{var(p_i) + \tau^2}$$

The pooled prevalence could then be calculated with the random-effect equation as

$$\overline{p^*} = \frac{\sum w_i{}^* p_i}{\sum w_i{}^*}$$

And the 95% CI could be estimated using the equation

$$96\%CI = \overline{p^*} \pm \frac{1.96}{\sqrt{\sum w_i{}^*}}$$

2.8.5.2 Heterogeneity for OR.

$$Q = \sum w_i(\widehat{\theta_1} - \widehat{\theta_p})^2$$

$$\widehat{\theta_p} = ln\widehat{OR}_p = \frac{\sum_{i=1}^{k} w_i ln\widehat{OR}_i}{\sum_{i=1}^{k} w_i}$$

$$\widehat{\theta_i} = ln\widehat{OR}_i$$

In the above equations, Q is heterogeneity, w_i and $var\left(ln\widehat{OR}_i\right)$ are the variance and weight as defined previously[44-46].

2.8.5.3 Heterogeneity for MD

$$Q = \sum_{i}^{k} w_i(d_i - \widehat{D})^2$$

$$\widehat{D} = \frac{\sum_{i}^{k} w_i d_i}{\sum_{i}^{k} w_i}$$

$$w_i = \frac{1}{var(d_i)}$$

Q is heterogeneity Chi-square test, w_i is weight which is the

inverse variance, $var(d_i)$ is the variance of MD as defined previously, d_i is the unstandardized MD , and $\widehat{D}$ is the pooled MD. The subscript i stands for the study i.

A meta-regression analysis was done to explore for the source of heterogeneity by adding the covariables (e.g., age, sex, obesity, physical activity, kidney disease, liver disease, ethnicity, sun exposure, season, country, alcohol intake, and vitamin D supplements) into the model one by one, if the studies reported the covariables needed. If adding the variable could decrease the between-study variation , or the I^2, this would indicate that added variable may be a source of heterogeneity. A subgroup analysis by this variable was then done next[44-47].

2.8.6 Publication bias

For both dichotomous and continuous data, publication bias was assessed by plotting a funnel plot, which plots the log OR (for dichotomous outcome) and the MD (for continuous outcomes) on the x-axis and the SE of the log OR or the SE of the MD on the y-axis. If there was asymmetry, this suggested that there was the possibility of small study effects, or it could be due to heterogeneity or publication bias. An Egger test was done to test for asymmetry. We also did a contour enhanced funnel plot to differentiate between publication bias and heterogeneity.

STATA version 13[48] was used to perform all statistical analyses. P value of < 0.05 was considered as statistically significant, except in the Cochran Q test where the p value of <0.1 was use.

CHAPTER III
RESULTS

3.1 Study selection

As described in Figure 3.1, 559 studies were identified from the two main databases, of which 43 studies[10, 12, 20-29, 33-38, 49-73] were finally met inclusion criteria.

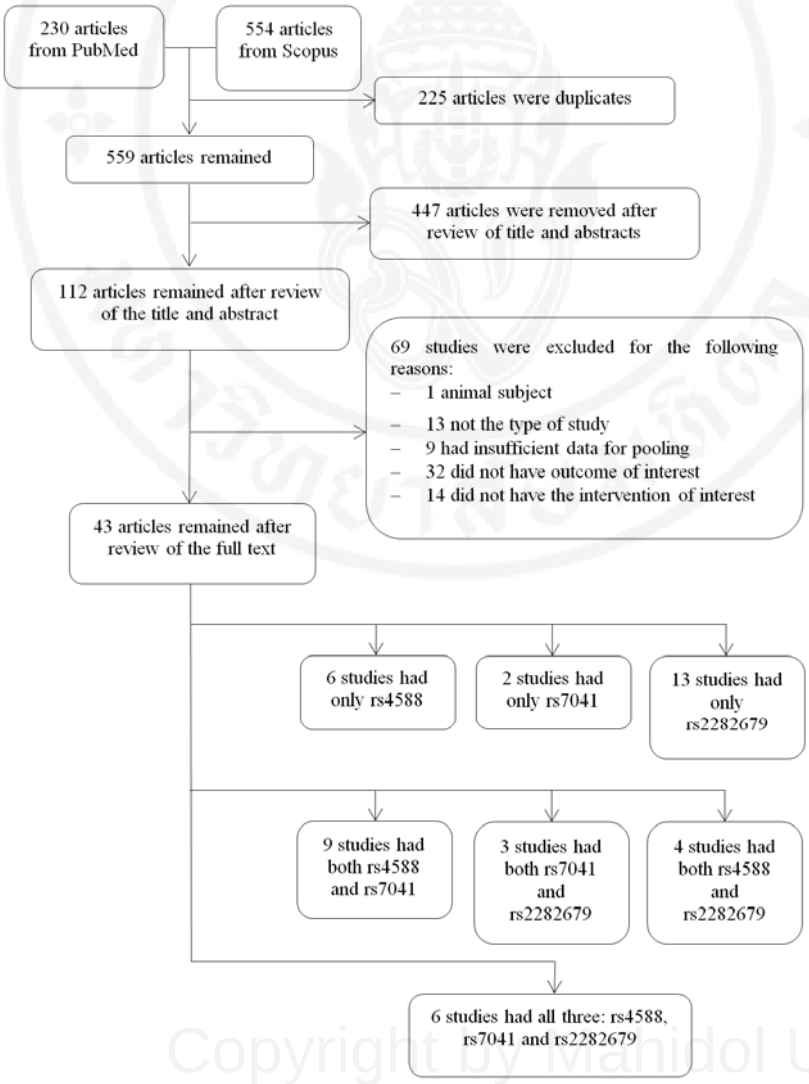

Figure 3.1 Flow chart of the study selection

Among the 559 studies, 447 studies were excluded after review of the title and abstract. From the remaining 112 studies, 69 studies were excluded after review of the full text. Of which, 13 studies[14, 74-85] were not observational studies, 1 study[86] was not done in human, 14 studies[7, 87-99] did not have the gene of interest, 32 studies[11, 16, 100-129] did not have the outcome of interest, and 9 studies[130-138] did not have sufficient data for pooling. Among 43 studies selected, 6[12, 23, 36, 50, 53, 58], 2[54, 59], and 13[10, 28, 49, 51, 52, 55, 62, 65-67, 69, 71, 72] studies had data for rs4588, rs7041, and rs2282679, respectively. Nine studies[20, 22, 24, 27, 37, 38, 60, 61, 64] had data for both rs4588 and rs7041, 3 studies[29, 63, 68] had data for both rs7041 and rs2282679, 4 studies[25, 26, 57, 70] had data for both rs4588 and rs2282679, and 6 studies[21, 33-35, 56, 73] had data for all three SNPs. Agreement for selection of studies between the two reviewers was described in Table 3.1, which suggested 97.2% agreement with the Kappa statistic of 0.779. Characteristics of these included studies were described as follows:

Table 3.1 Selected studies by reviewers

		Reviewer 1 (TA)		
		Included	Excluded	Total
Reviewer 2 (IA)	Included	29	6	35
	Excluded	9	488	497
Total		38	494	532

3.2 Characteristics of included selection

Characteristic of included studies were described in Table 3.2 Among 43 studies, most studies were done on Caucasian (n =22) and Asian populations (n=12), and only a few studies conducted in Africans (n=4), Hispanics (n=3), and some (n = 9) had mixed ethnicities; some studies reported for more than one ethnicity. Most common study designs were cross-sectional (n = 25), followed by case-controls (n = 10), cohort studies (n = 7), and 1 study [66] had reported both cross sectional and cohort study designs. Mean age and body mass index ranged from 5.9 to 83.8 and 17.4

to 48.6, respectively. Male percentage ranged from 0 to 100. Most studies (n = 19) used radioimmunoassay method for measurement of vitamin D.

3.3 Risk of bias

We assessed the risk of bias as described in Tables 3.3-3.4. Among 43 studies, the risk of bias was highest in selective reporting (11/43, 25.58%), followed by confounding bias (8/43, 18.60%) and HWE (7/43, 16.28%). The risk of bias was lowest in adjustment for multiple testing (1/43, 2.33%), followed by representativeness (2/43, 4.65%), and ascertainment of 25(OH)D level of controls in case control studies and for the non-exposure group in other study designs (2/43, 4.65%).

3.4 rs4588

Twenty five[12, 20-27, 33-38, 50, 53, 56-58, 60, 61, 64, 70, 73] studies were included in the pooling for the SNP rs4588, out of which, 4[33, 34, 37, 38] studies had data for pooling OR for vitamin D deficiency/insufficiency, 18[12, 20, 22-27, 35, 36, 50, 53, 56, 58, 60, 61, 64, 73] studies had data for pooling MD for vitamin D level, and 3 studies[21, 57, 70] had both data. From the 7 with vitamin D deficiency/insufficiency, 5[21, 33, 34, 37, 38] studies reported frequency data for genotypes, 2[57, 70] studies reported ORs and 95% CIs for additive model.

For 21 studies with vitamin D level, 10 studies[12, 20, 22-25, 27, 36, 53, 61] reported means by genotypes , 1 study[26] reported MD, 2 studies[58, 64] reported MD by combined genotypes (AC and AA), 6 studies[21, 35, 56, 57, 70, 73] reported MD for additive model, 2 studies[50, 60] reported MD for allele data.

Table 3.2 Characteristics of the included studies

First Author, Year, (Reference No.)	Study design	Age group	Ethnicity	Mean Age	BMI	% Male	Method of measurement of vitamin D
Sinotte, 2009 [27]	Cross-sectional study	Adult	Caucasian	46.8	25.2	0	Radioimmunoassay
Ahn, 2010 [49]	Case-control study	Adult	Caucasian			60.9	Chemilluminescence assay
Bu, 2010 [35]	Cross-sectional study	Mixed[a]	Caucasian	53	27.0		Radioimmunoassay
Janssens, 2010 [20]	Case-control study	> 50 years	Caucasian		26.9	79.0	Radioimmunoassay
Wang, 2010[67]	Cross-sectional study	> 50 years	Caucasian	48.7	26.9	36	Radioimmunoassay, Chemilluminescence assay, ELISA, Mass spectrometry
Gozdzik, 2011[53]	Cross-sectional study	Adult	South Asian	22.3	23.7	35.6	Liquid chromatography
	Cross-sectional study	Adult	East Asian	20.5	22.4	29.1	Liquid chromatography
	Cross-sectional study	Adult	Caucasian	20.9	23.5	34.7	Liquid chromatography
Signorello, 2011 [62]	Cohort study	Adult	African	51.9	28.3	50.7	Radioimmunoassay
Azad, 2012 [12]	Cross-sectional study	Adult	Mixed[b]			79	Not given
Berry, 2012 [50]	Cross-sectional study	Adult	Caucasian				ELISA
Hibler, 2012 [54]	Cross-sectional study	Adult	Caucasian	66.0	27.9	66.1	Radioimmunoassay
Kitanaka, 2012 [33]	Case-control study	Children	Asian			86.7	Not given
Lu, 2012 [56]	Cross-sectional study	> 50 years	Asian	58.6	24.4	44.3	Radioimmunoassay
Muindi, 2012 [57]	Case-control study	Adult	Mixed			54.2	Liquid chromatography
Petta, 2012 [59]	Cross-sectional study	Mixed	Mixed	52.8		49.2	Liquid chromatography
Suzuki, 2012 [64]	Cross-sectional study	Adult	Asian	72.1	22.1	47.5	Radioimmunoassay
Theodoratou, 2012 [28]	Case-control study	Adult	Caucasian	60.7	26.8	56.5	Liquid chromatography

Table 3.2 Characteristics of the included studies (continued)

First Author, Year, (Reference No.)	Study design	Age group	Ethnicity	Mean Age	BMI	% Male	Method of measurement of vitamin D
Trummer, 2012 [66]	Cross-sectional study	Adult	Caucasian	55.3	48.6	64	Radioimmunoassay
	Cohort study	Adult	Caucasian	83.8	25.7	15	Radioimmunoassay
Buijsse, 2013 [10]	Cohort study	Adult	Mixed	50.6	26.0	43.1	Mass chromatography
Carpenter, 2013 [23]	Cross-sectional study	Children	Mixed				Radioimmunoassay
Cheung, 2013 [51]	Cross-sectional study	Mixed	Asian	48.4	22.8		Radioimmunoassay
Foucan, 2013 [52]	Cross-sectional study	Adult	African	46	27	42	Chemilluminescence assay
Ismail, 2013 [55]	Case-control study	Children	African	7.8		54.9	ELISA
Nimitphong, 2013 [58]	Case-control study	Adult	Mixed	36.2	22.8	18.0	Liquid chromatography
Perna, 2013 [26]	Cohort study	> 50 years	Caucasian	62			Chemilluminescence assay
Powe, 2013 [60]	Cross-sectional study	Adult	Caucasian	48.3	29.8	44	Radioimmunoassay
	Cross-sectional study	Adult	African	48.3	29.4	44.3	Radioimmunoassay
Robien, 2013 [61]	Cohort study	Adult	Asian	55.7	22.8	43.7	Chemilluminescence assay
Santos, 2013 [37]	Cross-sectional study	Children	Hispanic	13.2		0	Radioimmunoassay
Singh, 2013 [22]	Cross-sectional study	Adult	Mixed		26.3	27.3	Liquid chromatography
Trummer, 2013 [65]	Cohort study	> 50 years	Caucasian	62.6	22.6	69.7	Radioimmunoassay
Zhang, 2013 [70]	Cross-sectional study	Adult	Asian		23.3	20.5	Electrochemiluminescense
Gilbert, 2014 [73]	Case-control study	> 50 years	Caucasian	62.6		100	Mass spectrometry
Laursen, 2014 [29]	Cross-sectional study	Mixed	Mixed	42.4		29	Chemilluminescence assay
Leong, 2014 [72]	Case-control study	Adult	Mixed	65.7	27.5	34.1	Chemilluminescence assay
Li, 2014 [21]	Cross-sectional study	Adult	Asian	48.6	23.6	62.5	Radioimmunoassay

Table 3.2 Characteristics of the included studies (continued)

First Author, Year, (Reference No.)	Study design	Age group	Ethnicity	Mean Age	BMI	% Male	Method of measurement of vitamin D
Navas-Nazario, 2014 [24]	Cohort study	Children	Hispanic	5.9		47	Radioimmunoassay
Nissen, 2014 [25]	Cross-sectional study	Adult	Caucasian		25.5	50.5	Liquid chromatography
	Cross-sectional study	Children	Caucasian		17.4	52.0	Liquid chromatography
Pekkinen, 2014 [36]	Cross-sectional study	Children	Caucasian	12.9	19.7	30.7	Liquid chromatography
Strawbridge, 2014 [63]	Cross-sectional study	> 50 years	Caucasian		26.8	48.1	Chemilluminescence assay
Suaini, 2014 [34]	Cross-sectional study	Children	Caucasian				Liquid chromatography
	Cross-sectional study	Children	Asian				Liquid chromatography
Thongthai, 2014 [71]	Cross-sectional study	Adult	Asian	47.8	23.6	61.4	Liquid chromatography
Touvier, 2014 [38]	Cross-sectional study	Adult	Caucasian			46	Chemilluminescence assay
Wang, 2014 [68]	Case-control study	Adult	Hispanic	52.8	29.7	0	Liquid chromatography
	Case-control study	Adult	Caucasian	56.8	26.8	0	Liquid chromatography
Yoshida, 2014 [69]	Cohort study	Mixed	Asian	64.3	21.2	12.3	Radioimmunoassay

[a] The age range of these studies was not specified, [b] The ethnicity of these study populations was not specified

BMI; body mass index

Table 3.3 Risk of bias assessment

	Selection bias			Information bias			Confounding bias			
First Author, Year, (Reference No.)	Rep[a]	Rep of cont[b]	Diff part[c]	Ascertainment of vitamin D (cases/exposure group)	Ascertainment of vitamin D (controls/non-exposure group)	Genotyp[d]	Population stratification	Multp Test[e]	Selective reporting	HWE
Case-control studies										
Ahn, 2010 [49]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Janssens, 2010 [20]	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Kitanaka, 2012 [33]	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes
Muindi, 2012 [57]	No	No	No	Yes	Yes	Yes	No	Yes	No	No
Theodoratou, 2012 [28]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Ismail, 2013 [55]	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes	No
Nimitphong, 2013 [58]	Yes	Yes	No	Yes	Yes	No	No	No	Yes	No
Gilbert, 2014 [73]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Leong, 2014 [72]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Wang, 2014 [68]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cross-sectional and cohort studies										
Sinotte, 2009 [27]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Bu, 2010 [35]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Wang, 2010[67]	Yes			Yes	Yes	Yes	Yes	Yes	No	Yes
Gozdzik, 2011[53]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 3.3 Risk of bias assessment (continued)

	Selection bias			Information bias			Confounding bias			
First Author, Year, (Reference No.)	Rep[a]	Rep of cont[b]	Diff part[c]	Ascertainment of vitamin D (cases/exposure group)	Ascertainment of vitamin D (controls/non-exposure group)	Genotyp[d]	Population stratification	Multp Test[e]	Selective reporting	HWE
Signorello, 2011 [62]	Yes			Yes	Yes	Yes	Yes	Yes	No	Yes
Azad, 2012 [12]	No			No	No	Yes	No	Yes	No	Yes
Berry, 2012 [50]	Yes			Yes	Yes	Yes	Yes	Yes	No	Yes
Hibler, 2012 [54]	Yes			Yes	Yes	Yes	No	Yes	No	Yes
Lu, 2012 [56]	Yes			Yes	Yes	Yes	Yes	Yes	No	Yes
Petta, 2012 [59]	Yes			Yes	Yes	Yes	No	Yes	Yes	No
Suzuki, 2012 [64]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Trummer, 2012 [66]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Buijsse, 2013 [10]	Yes			Yes	Yes	Yes	No	Yes	Yes	Yes
Carpenter, 2013 [23]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	No
Cheung, 2013 [51]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	No
Foucan, 2013 [52]	Yes			Yes	Yes	No	Yes	Yes	No	Yes
Perna, 2013 [26]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Powe, 2013 [60]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Robien, 2013 [61]	Yes			Yes	Yes	Yes	Yes	Yes	No	Yes
Santos, 2013 [37]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes

Table 3.3 Risk of bias assessment (continued)

	Selection bias			Information bias			Confounding bias			
First Author, Year, (Reference No.)	Rep[a]	Rep of cont[b]	Diff part[c]	Ascertainment of vitamin D (cases/exposure group)	Ascertainment of vitamin D (controls/non-exposure group)	Genotyp[d]	Population stratification	Multp Test[e]	Selective reporting	HWE
Singh, 2013 [22]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Trummer, 2013 [65]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Zhang, 2013 [70]	Yes			Yes	Yes	Yes	Yes	Yes	No	Yes
Laursen, 2014 [29]	Yes			Yes	Yes	Yes	No	Yes	Yes	No
Li, 2014 [21]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Navas-Nazario, 2014[24]	Yes			Yes	Yes	Yes	No	Yes	Yes	Yes
Nissen, 2014 [25]	Yes			Yes	Yes	Yes	No	Yes	Yes	Yes
Pekkinen, 2014 [36]	Yes			Yes	Yes	Yes	Yes	Yes	No	Yes
Strawbridge, 2014 [63]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Suaini, 2014 [34]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Thongthai, 2014 [71]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Touvier, 2014 [38]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes
Yoshida, 2014 [69]	Yes			Yes	Yes	Yes	Yes	Yes	Yes	Yes

[a] Representativeness of cases in case control studies and subjects in other study designs, [b] representativeness of controls in case control studies, [c] differential participation of cases and controls, [d] method of genotyping, [e] adjustment for multiple testing

HWE; Hardy-Weinberg equilibrium

Table 3.4 Summary of risk of bias assessment

Domain	Items assessed for risk of bias	low risk of bias		Moderate to high risk of bias	
		Frequency	%	Frequency	%
Selection bias	Representativeness	41	95.35	2	4.65
	Representativeness of controls in case control studies	7	70.00	3	30.00
	Differential participation for case and control	4	40.00	6	60.00
Information bias	Ascertainment of 25hydroxyvitamin D level	40	93.02	3	6.98
	Ascertainment of 25hydroxyvitamin D level of controls in case control studies and for the non-exposure group in other study designs	41	95.35	2	4.65
	Ascertainment of genotyping	39	88.64	4	9.30
Confounding bias	Population stratification	35	81.40	8	18.60
Multiple testing	Adjusted for multiple comparisons	42	97.67	1	2.33
Selective reporting	Selective reporting	32	74.42	11	25.58
HWE	Hardy-Weinberg equilibrium	36	83.72	7	16.28

3.4.1 Minor allele prevalence

Data used for pooling minor allele prevalence from 21 studies were described by ethnicity and type of subjects, see Table 3.5. Data for pooling minor allele prevalence was not available in 4 studies[34, 35, 50, 57]. HWE was checked for each study, for some studies where genotype data were not provided, we relied on assessing HWE from what was reported in the articles There were 4 studies [23, 24, 36, 37] in children Caucasians, 1 study [25] reported for adult and children Caucasians, 7 studies [12, 20, 22, 26, 27, 38, 73] in adult Caucasians, 2 studies [34, 53] reported in both Caucasians and Asians, 1 study [33] in children Asians, and 6 studies [21, 56, 58, 61, 64, 70] in adult Asians. The pooled minor A allele prevalence were estimated in children Caucasians, adult Caucasians, children Asians and adult Asians. The pooled prevalence across ethnicity and type of subjects were not much different with corresponding pooled prevalence of 0.232 (95%CI: 0.190, 0.274), 0.287 (95%CI: 0.278, 0.296), 0.281 (95%CI: 0.158, 0.404), and 0.293 (95%CI: 0.275, 0.312), respectively.

3.4.2 Vitamin D level

The overall MDs for genotype data for AA versus CC (MD_1) and AC versus CC (MD_2) were described by ethnicity and type of subjects, see Table 3.6. There were 11 studies conducted in Caucasians. Among 4 studies[23-25, 36] in children, MD_1 was high heterogeneity (I^2 = 76%) and no heterogeneity for MD_2 (I^2 = 0%). The pooled MD_1 and MD_2 were respectively -3.705 (95%CI: -6.241, -1.169) and -2.211 (95%CI: -2.930, -1.491), which could be interpreted that subjects with AA and AC genotypes had 25(OH)D about 3.7 and 2.2 ng/ml significantly lower than subjects with CC genotype, see Figure 3.2 a-b. Publication bias for pooling MDs was assessed where at least 3 studies were used for pooling using a funnel plot, which graphed SE on y-axis against MD on x-axis. For children Caucasians, the funnel plot suggested a little deviated from the funnel for MD_1 but not for MD_2, see Figure 3.3 a-b. This corresponded with the Egger's tests which yielded non-significant asymmetry of the funnels for both MD_1 and MD_2, with (Coefficient = -0.555, SE = 8.599, P = 0.954) and (Coefficient = -1.976, SE = 1.919, P = 0.411) respectively.

Among 7[12, 20, 22, 25-27, 53] studies in adults, the MDs were not much heterogeneous with the estimated I^2s of 25% and 0% for MD_1 and MD_2, respectively.

The corresponding pooled MDs were -3.619 (95%CI: -4.978, -2.261) and -1.601 (95%CI: -2.369, -0.833); which similar gene effects in children Caucasians i.e., adult Caucasians with AA and AC genotypes had 3.6 ng/ml and 1.6 ng/ml lower levels of vitamin D compared to those who have CC genotypes, see Figure 3.4 a-b. Publication bias was assessed for adult Caucasians, the funnel plots looked symmetry for MD_1 but not for MD_2, see Figure 3.5 a-b. This was corresponded with the Egger's tests which suggested symmetry for MD_1 (Coefficient = -0.836, SE = 0.788, P = 0.337) but asymmetry for MD_2 and (Coefficient = -1.144, SE = 0.328, P = 0.018). A contour-enhanced funnel plot was performed for MD_2 (see Figure 3.5 c) which indicated that some studies were missing, which could be either high quality with negative results or low quality with positive results. Thus, publication bias might be present for this pooling.

Only 2 studies[53, 61] in adult Asians were available for pooling MDs, which were homogenous for both MDs (I^2 = 0%) with MD_1 and MD_2 of -4.042 (95%CI: (-5.621, -2.462) and, -1.722 (95%CI: -2.754, -0.69), respectively. There were no studies in children Asians.

Six studies[21, 35, 56, 57, 70, 73] reported the genotype effects using the additive model, 3 studies in adult Caucasians, and 3 studies in adult Asians. The pooled MDs were -1.932 (95%CI: -2.719, -1.146) and -0.03 (95%CI: -0.037, 0.022) in Caucasians and Asians, respectively. The gene effects were mild heterogeneous in adult Caucasians (I^2 = 4%) but very heterogeneous in adult Asians (I^2 = 91.9%), see Figure 3.6 a-b. This could be interpreted that for every one copy of A allele increased in adult Caucasians, the 25(OH)D level significantly decreased 1.93 ng/ml; but this was not replicated in Asians. For pooling additive effects, a funnel plot suggested symmetry of the funnel for adult Caucasians but not for adult Asians, see Figure 3.7 a-b. The Egger's tests were non-significant for both MDs with (Coefficient = 1.105, SE = 2.206, P = 0.704) and (Coefficient = 0.023, SE = 3.672, P = 0.996), respectively.

3.4.3 Vitamin D deficiency/insufficiency

Among 5 studies [21, 33, 34, 37, 38] assessed A vs C allele effect on vitamin D deficiency/insufficiency, 2 [37, 38] and 2 studies [21, 33] were Caucasians and Asians, and 1 study [34] conducted in both ethnicities. Their data have been described in Table 3.7.

Pooling these ORs stratified by ethnicity found homogeniety (I^2 = 0%) for both Caucasians and Asians with the pooled OR of 1.522 (95%CI: 1.336, 1.733) and 1.255 (95%CI: 1.058, 1.487), respectively. This suggested that Caucasians and Asians, who had A allele were approximately at 52% and 26% significantly higher risk of developing vitamin D deficiency/insufficiency than those Caucasians and Asians who had C allele. Publication bias for pooling ORs was assessed. For pooling allele effect (A versus C) in Caucasians and Asians, funnel plots showed symmetry for both, respectively, see Figure 3.8 a-b. The Egger's tests were non-significant for both Caucasians and Asians, with (Coefficient = 1.300, SE = 0.414, P = 0.196) and (Coefficient = -0.807, SE = 0.455, P = 0.327) respectively.

Genotype data for AA versus CC (OR_1) and AC versus CC (OR_2) were described by ethnicity, see Table 3.8. Three studies each were conducted in Caucasians and Asians, respectively. In Caucasians, the genotype effects were homogenous for both ORs (I^2 = 0%) with the pooled OR_1 and pooled OR_2 2.207 (95%CI: 1.629, 2.99) and 1.529 (95%CI: 1.28, 1.826), respectively. This suggested that Caucasians with AA genotype and AC genotypes were approximately 2.21 and 1.53 times higher risk of developing vitamin D deficiency/insufficiency than those Caucasians with CC genotype. For publication bias, funnel plots also showed no evidence of asymmetry for both genotypic ORs in Caucasians, see Figure 3.9 a-b. This was corresponded with the Egger's tests, which suggested non-significant of asymmetry for both OR1 and OR2 with (Coefficient = 0.693, SE = 0.059, P = 0.054) and (Coefficient = -1.397, SE = 0.687, P = 0.291), respectively.

The genotype effects were also homogenous (I^2 = 0% for both ORs) in Asians, with the pooled OR_1 and pooled OR_2 of 1.578 (95%CI: 1.06, 2.348) and 1.251 (95%CI: 0.991, 1.58), respectively. This suggested that Asians with AA and AC genotypes were approximately at 58% and 25% significantly higher risk of developing vitamin D deficiency/insufficiency than those Asians with CC genotype. There was no evidence of publication bias for pooling genotypic effects in Asians, see Figure 3.10 a-b. The Egger's tests were non-significant for both OR_1 and OR_2, with (Coefficient = -0.862, SE = 0.377, P = 0.263) and (Coefficient = -0.325, SE = 0.272, P = 0.444) respectively.

Table 3.5 Pooling the prevalence of minor A allele of rs4588

Author, Year, (Reference No.)	Ethnicity	Type of subjects	Total number of alleles	Minor A allele frequency	Prevalence of the minor allele (95% CI)	HWE
Carpenter, 2013 [23]	Caucasian	Children	1550	274	0.177 (0.158, 0.196)	0.847
Santos, 2013, [37]	Caucasian	Children	396	106	0.268 (0.224, 0.311)	0.511
Navas-Nazario, 2014 [24]	Caucasian	Children	668	141	0.211 (0.18, 0.242)	0.772
Pekkinen, 2014 [36]	Caucasian	Children	466	87	0.187 (0.151, 0.222)	0.035[a]
Nissen, 2014 [25]	Caucasian	Children	688	184	0.267 (0.234, 0.301)	0.321
Suaini, 2014 [34]	Caucasian	Children	968	278	0.287 (0.259, 0.316)	0.065
Pooled			4340	964	0.232 (0.190, 0.274)	
Azad 27	Caucasian	Adult	912	281	0.308 (0.278, 0.338)	0.415
Janssens, 2010 [20]	Caucasian	Adult	300	75	0.25 (0.201, 0.299)	0.114
Perna, 2013 [26]	Caucasian	Adult	7482	2092	0.28 (0.269, 0.29)	0.6
Singh, 2013 [22]	Caucasian	Adult	356	100	0.281 (0.234, 0.328)	0.987
Sinotte, 2009 [27]	Caucasian	Adult	1466	430	0.293 (0.27, 0.317)	0.483
Touvier, 2014 [38]	Caucasian	Adult	3548	1071	0.302 (0.287, 0.317)	0.77
Gilbert, 2014 [73]	Caucasian	Adult	1796	508	0.283 (0.262, 0.304)	0.604
Gozdzik, 2011[53]	Caucasian	Adult	222	62	0.279 (0.22, 0.338)	0.757
Nissen, 2014 [25]	Caucasian	Adult	828	229	0.277 (0.246, 0.307)	0.567
Pooled			16910	4848	0.287 (0.278, 0.296)	

Table 3.5 Pooling the prevalence of minor A allele of rs4588 (continued)

Author, Year, (Reference No.)	Ethnicity	Type of subjects	Total number of alleles	Minor A allele frequency	Prevalence of the minor allele (95% CI)	HWE
Kitanaka, 2012 [33]	Asian	Children	132	29	0.22 (0.149, 0.29)	0.559
Suaini, 2014 [34]	Asian	Children	142	49	0.345 (0.267, 0.423)	0.741
Pooled			274	78	0.281 (0.158, 0.404)	
Li, 2014 [21]	Asian	Adult	2398	709	0.296 (0.277, 0.314)	0.94
Lu, 2012 [56]	Asian	Adult	6350	2008	0.316 (0.305, 0.328)	0.345
Nimitphong, 2013 [58]	Asian	Adult	78	23	0.295 (0.194, 0.396)	0.045[a]
Robien, 2013 [61]	Asian	Adult	958	251	0.262 (0.234, 0.29)	0.148
Suzuki, 2012 [64]	Asian	Adult	274	68	0.248 (0.197, 0.299)	0.475
Zhang, 2013 [70]	Asian	Adult	5794	1848	0.319 (0.307, 0.331)	0.36
Gozdzik, 2011[53]	Asian	Adult	398	107	0.269 (0.225, 0.312)	0.453
Pooled			16524	5092	0.293 (0.275, 0.312)	

[a] These 2 studies had Hardy Weinberg disequilibrium but was included in the analysis after a sensitivity analysis

CI; confidence interval, HWE; Hardy-Weinberg Equilibrium

Table 3.6 Describes genotype data and pooled mean difference of vitamin D levels for rs4588

First Author, Year, (Reference No.)	CC			AC			AA			MD_1	MD_2
	n	mean	SD	n	mean	SD	n	mean	SD		
Children Caucasians											
Carpenter, 2013 [23]	526	27.16	9.62	224	25.12	4.41	25	24.4	6.01	-2.764 (-5.259, -0.268)	-2.043 (-3.047, -1.039)
Navas-Nazario, 2014 [24]	207	28.41	12.38	113	24.88	6.05	14	26.08	5.25	-2.33 (-5.556, 0.896)	-3.53 (-5.552, -1.508)
Nissen, 2014 [25]	181	30.57	6.88	142	28.08	6.33	21	23.12	5.25	-7.45 (-9.909, -4.991)	-2.49 (-3.935, -1.045)
Pekkinen, 2014 [36]	159	18.31	5.84	61	16.79	4.72	13	16.26	3.5	-2.05 (-4.158, 0.058)	-1.52 (-3.012, -0.028)
Pooled MD										-3.705 (-6.241, -1.169)	-2.211 (-2.930, -1.491)
Adult Caucasians											
Azad, 2012 [12]	222	26.88	8.81	187	24.59	8.01	47	21.79	8.41	-5.09 (-7.759, -2.421)	-2.29 (-3.921, -0.659)
Gozdzik, 2011[53]	57	26.82	9.38	46	25.22	8.49	8	22.66	7.49	-4.16 (-9.893, 1.573)	-1.6 (-5.057, 1.857)
Janssens, 2010 [20]	88	25.80	8.80	49	23.8	8.6	13	20.6	4.6	-5.2 (-8.304, -2.096)	-2 (-5.03, 1.03)

Table 3.6 Describes genotype data and pooled mean difference of vitamin D levels for rs4588 (continued)

First Author, Year, (Reference No.)	CC			AC			AA			MD_1	MD_2
	n	mean	SD	n	mean	SD	n	mean	SD		
Nissen, 2014 [25]	219	29.69	9.68	161	27.92	9.08	34	25.48	9.78	-4.21 (-7.739, -0.681)	-1.77 (-3.67, 0.13)
Perna, 2013 [26]										-2.20 (-3.298, -1.102)	-0.92 (-1.469, -0.371)
Singh, 2013 [22]	92	26.56	11.26	72	23.84	10.3	14	29.41	21	2.85 (-8.363, 14.063)	-2.72 (-6.03, 0.59)
Sinotte, 2009 [27]	370	26.92	7.70	296	25.32	7.58	67	23.64	7.87	-3.28 (-5.321, -1.239)	-1.6 (-2.767, -0.433)
Pooled MD										-3.619 (-4.978, -2.261)	-1.601 (-2.369, -0.833)
Adult Asians											
Gozdzik, 2011[53]	106	15.62	6.55	79	13.96	5.11	14	12.37	4.04	-3.25 (-5.706, -0.794)	-1.66 (-3.341, 0.021)
Robien, 2013 [61]	267	27.52	7.01	173	25.76	6.72	39	22.92	6	-4.6 (-6.662, -2.538)	-1.76 (-3.068, -0.452)
Pooled MD										-4.042 (-5.621, -2.462)	-1.722 (-2.754, -0.69)

Table 3.6 Describes genotype data and pooled mean difference of vitamin D levels for rs4588 (continued)

First Author, Year, (Reference No.)	CC			AC			AA			MD_1	MD_2
	n	mean	SD	n	mean	SD	n	mean	SD		
Additive model											
Adult Caucasians											
Bu, 2010 [35]										-0.693 (-2.575, 1.188)	
Muindi, 2012 [57]										-2.43 (-4.429, -0.431)	
Gilbert, 2014 [73]										-2.14 (-3.1, -1.18)	
Pooled MD										-1.932 (-2.719, -1.146)	
Adult Asians											
Lu, 2012 [56]										-0.031 (-0.038, -0.023)	
Zhang, 2013 [70]										0.163 (0.06, 0.267)	

Table 3.6 Describes genotype data and pooled mean difference of vitamin D levels for rs4588 (continued)

First Author, Year, (Reference No.)	CC			AC			AA			MD_1	MD_2
	n	mean	SD	n	mean	SD	n	mean	SD		
Li, 2014 [21]										-1.24 (-1.946, -0.534)	
Pooled MD										-0.03 (-0.037, 0.022)	

MD_1; mean difference of AA versus CC, MD_2; mean difference of AC versus CC, n; number of subjects, SD; standard deviation

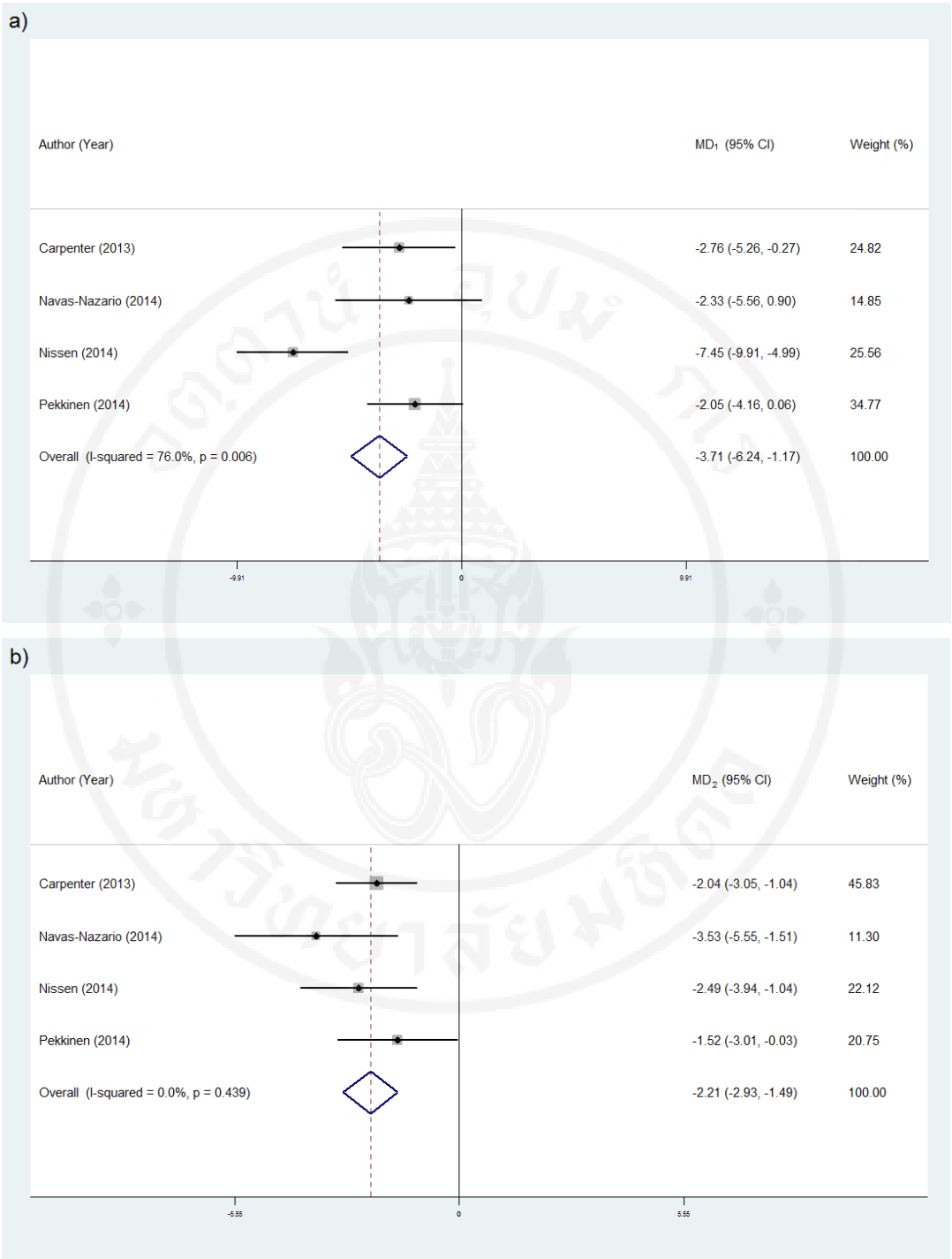

Figure 3.2 Forest plot of pooled MD in children Caucasians for rs4588 a) MD_1 for AA versus CC, b) MD_2 for AC versus CC

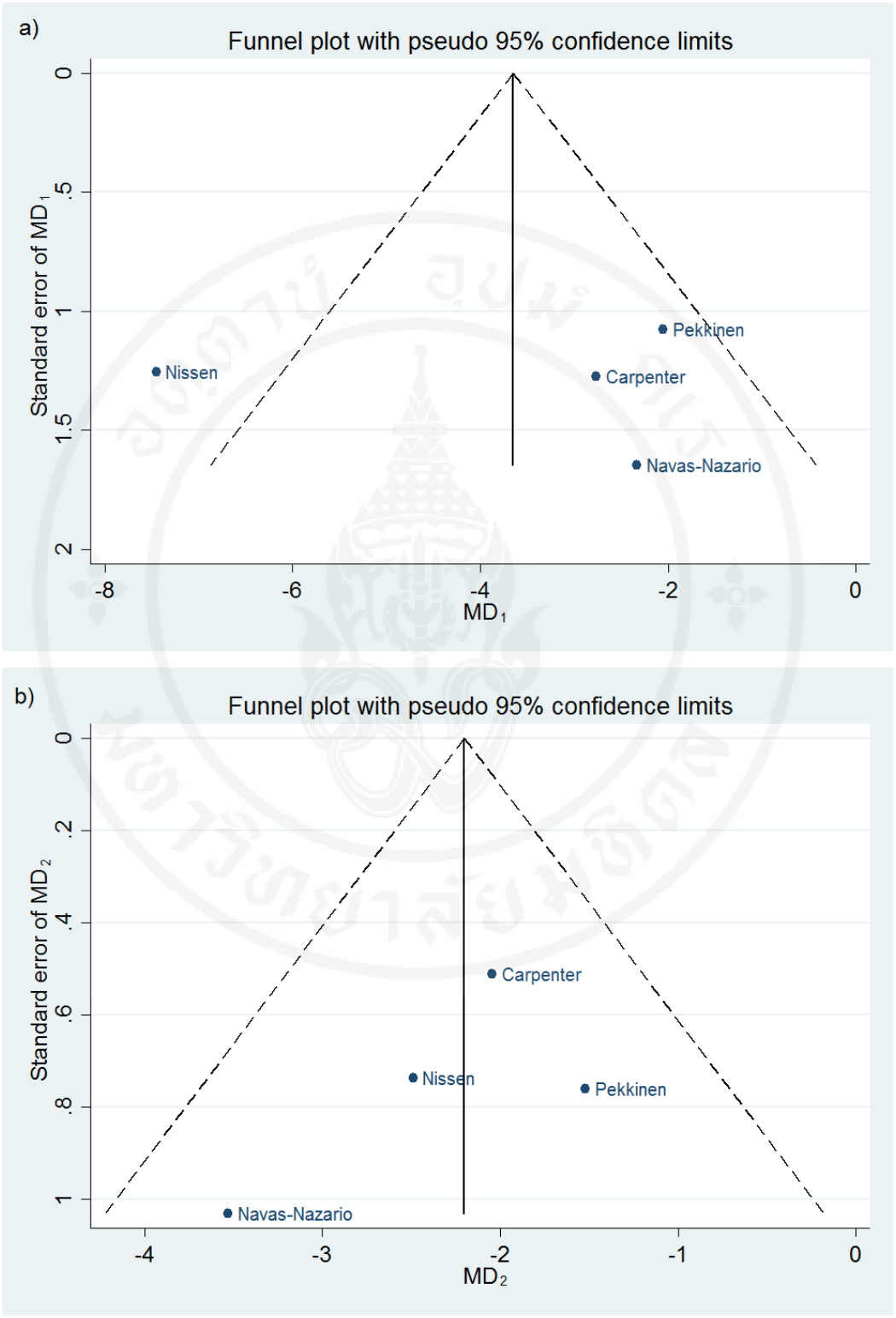

Figure 3.3 Funnel plots for pooling MD_1 and MD_2 for rs4588 in children Caucasian studies a) MD_1 (AA versus CC), b) MD_2 (AC versus CC)

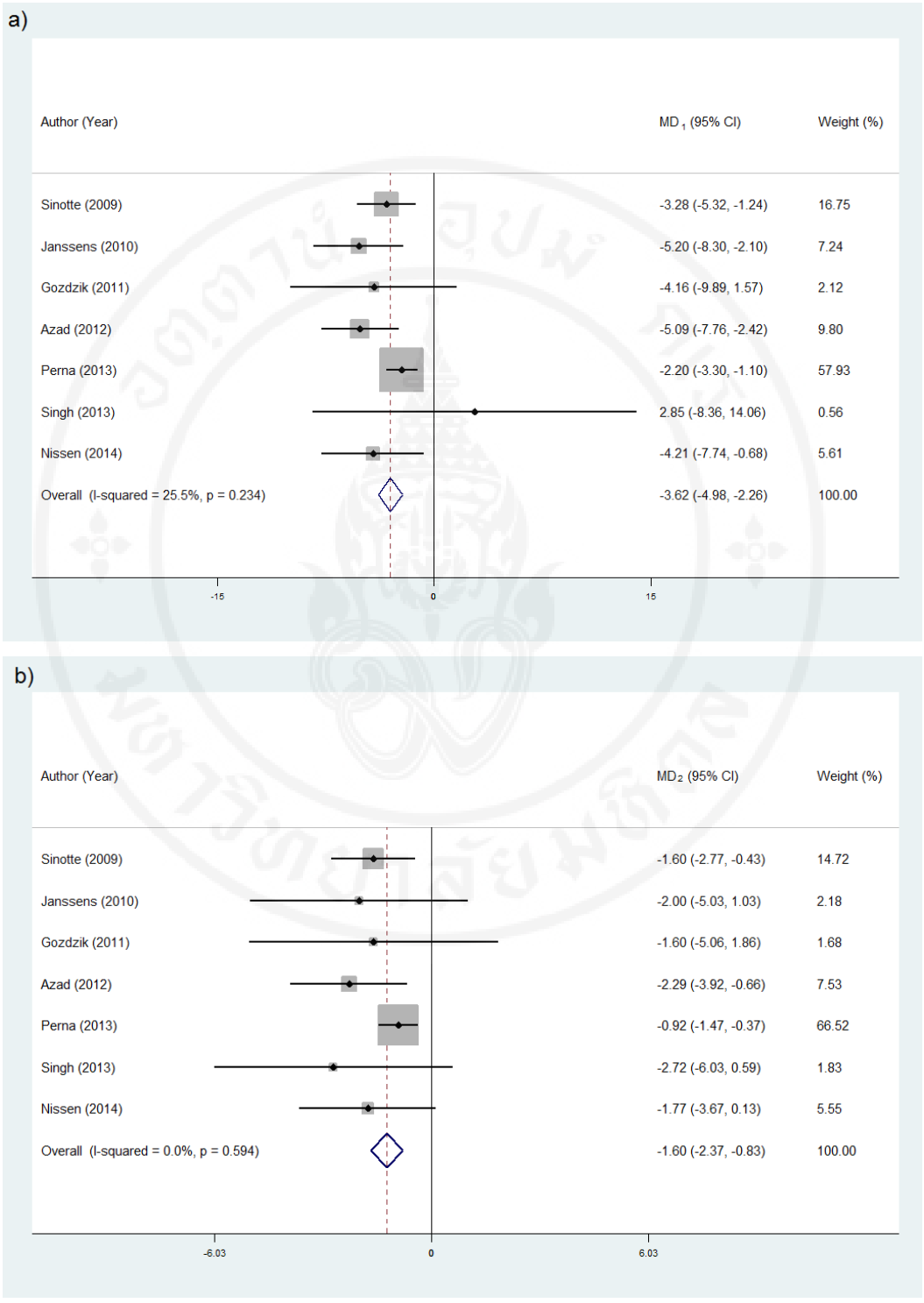

Figure 3.4. Forest plot of pooled MD in adult Caucasians for rs4588 a) MD_1 for AA versus CC, b) MD_2 for AC versus CC

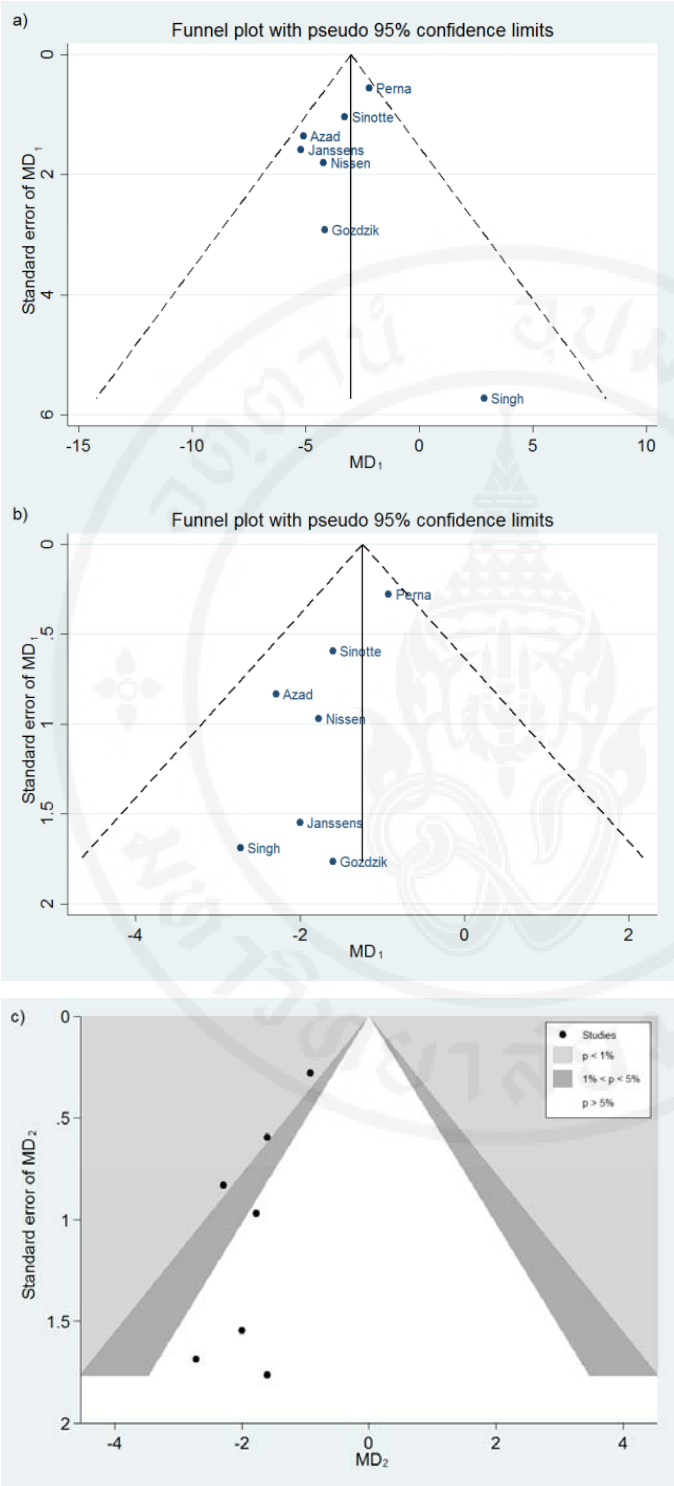

Figure 3.5 Funnel plots for pooling MD_1 and MD_2 for rs4588 in adult Caucasians studies a) MD_1 (AA versus CC), b) MD_2 (AC versus CC), c) Contour enhanced funnel plot for MD_2

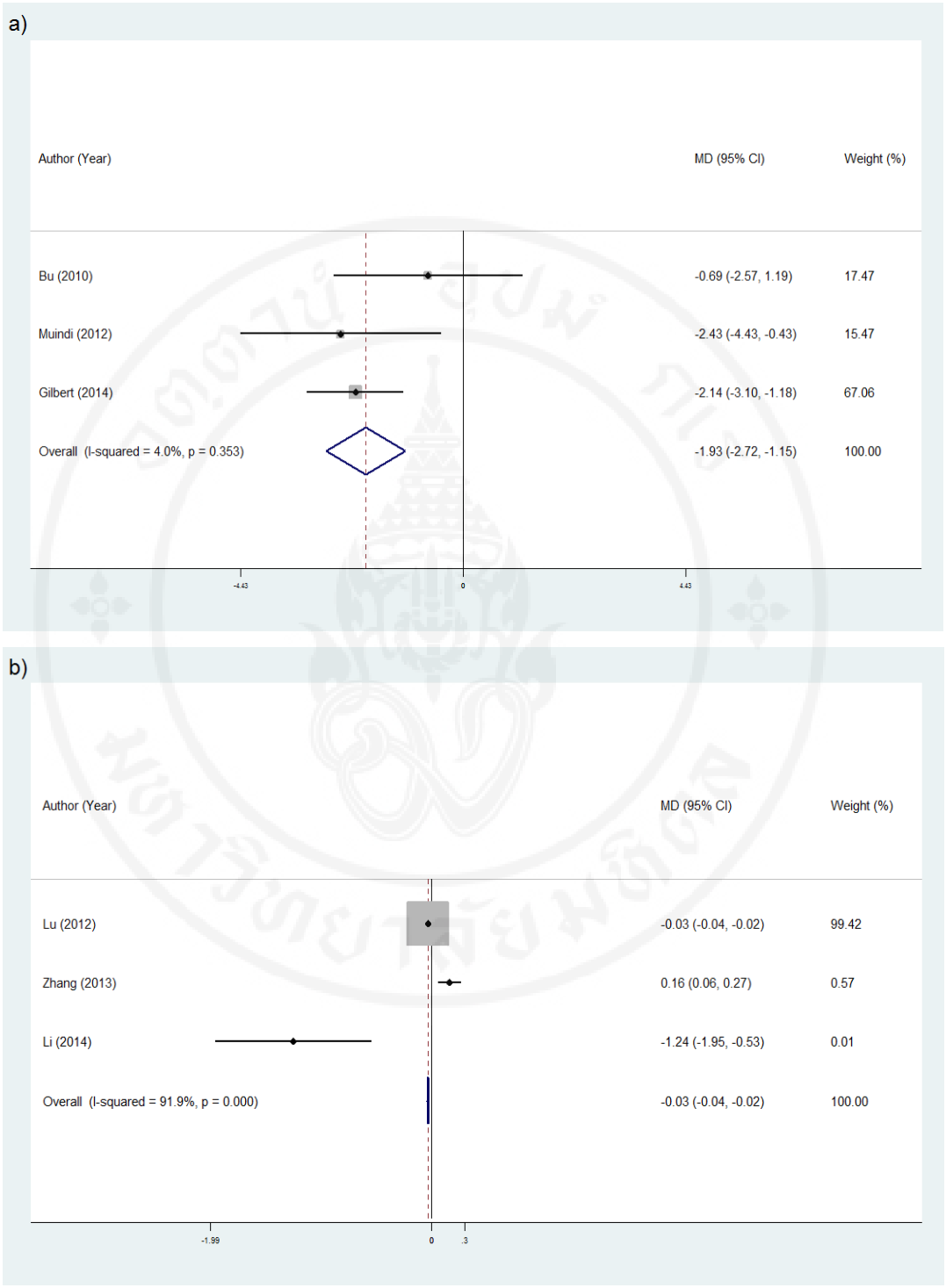

Figure 3.6 Forest plot of pooling MDs as additive effect for rs4588 a) adult Caucasians, b) adult Asians

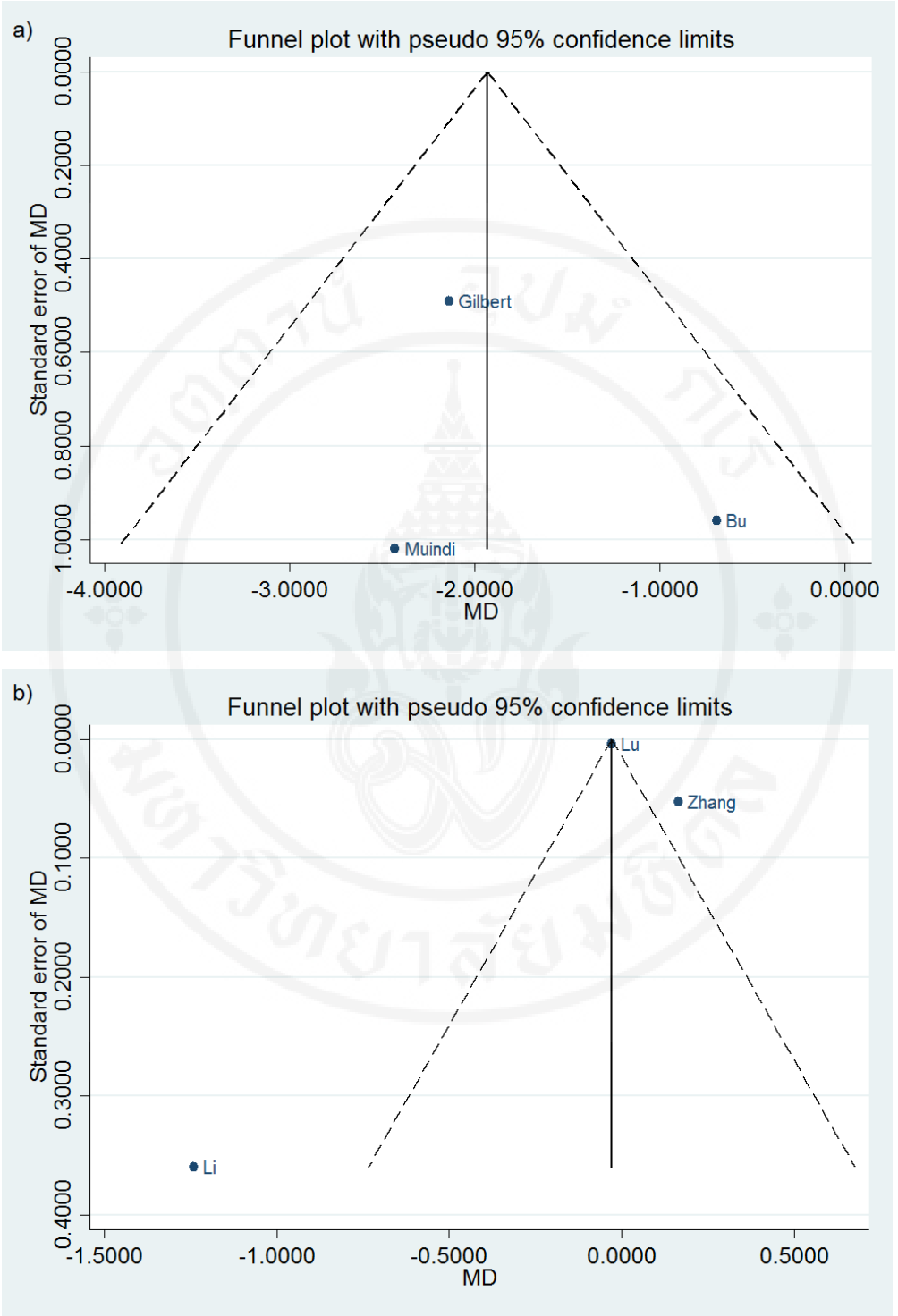

Figure 3.7 Funnel plot of pooling MDs as additive effect for rs4588 a) adult Caucasians, b) adult Asians

Table 3.7 Pooling allele effect of rs4588 on vitamin D deficiency/insufficiency

First Author, Year, (Reference No.)	Case		Control		OR (95% CI)
	C	A	C	A	
Caucasian					
Santos, 2013 [37]	87	45	203	61	1.721 (1.087, 2.726)
Touvier, 2014 [38]	1357	685	1120	386	1.465 (1.264, 1.698)
Suaini, 2014 [34]	108	68	582	210	1.745 (1.24, 2.456)
Pooled	1552	798	1905	657	1.522 (1.336, 1.733)
Asians					
Kitanaka, 2012 [33]	48	12	103	29	0.888 (0.417, 1.889)
Li, 2014 [21]	625	305	1064	404	1.285 (1.075, 1.536)
Suaini, 2014 [34]	88	118	488	416	1.127 (0.487, 2.61)
Pooled	761	435	1655	849	1.255 (1.058, 1.487)

CI; confidence interval, OR; odds ratio

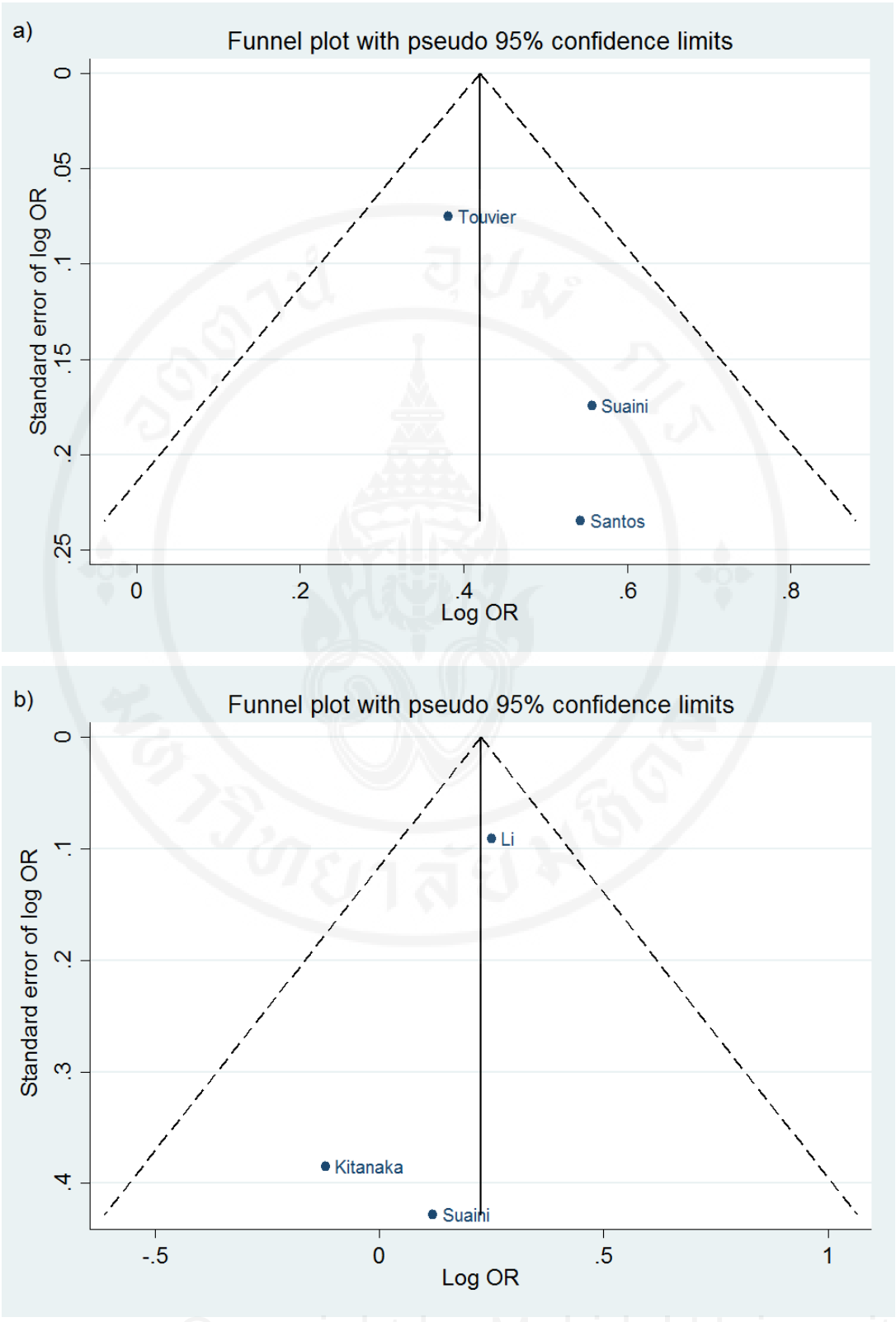

Figure 3.8 Funnel plots of rs4588 allele effect on vitamin D deficiency/insufficiency a) Log OR (A versus C) in Caucasians, b) Log OR (A versus C) in Asians

Table 3.8 Pooling genotype effect of rs4588 on vitamin D deficiency/insufficiency

First Author, Year, (Reference No.)	Case			Control			OR_1 (95% CI)	OR_2 (95% CI)
	CC	AC	AA	CC	AC	AA		
Caucasian								
Santos, 2013 [37]	29	29	8	79	45	8	2.724 (0.936, 7.929)	1.756 (0.933, 3.302)
Touvier, 2014 [38]	455	447	119	418	284	51	2.144 (1.505, 3.054)	1.446 (1.185, 1.765)
Suaini, 2014 [34]	33	42	13	221	140	35	2.487 (1.194, 5.183)	2.009 (1.215, 3.321)
Pooled	517	518	140	718	469	94	2.207 (1.629, 2.99)	1.529 (1.28, 1.826)
Asian								
Kitanaka, 2012 [33]	19	10	1	41	21	4	0.539 (0.056, 5.159)	1.028 (0.406, 2.602)
Li, 2014 [21]	211	203	51	386	292	56	1.666 (1.1, 2.523)	1.272 (0.995, 1.626)
Suaini, 2014 [34]	6	7	2	25	24	7	1.19 (0.196, 7.249)	1.215 (0.357, 4.141)
Pooled	236	220	54	452	337	67	1.578 (1.06, 2.348)	1.251 (0.991, 1.58)

CI; confidence interval, OR_1; odds ratio of AA versus CC, OR_2; odds ratio of AC versus CC

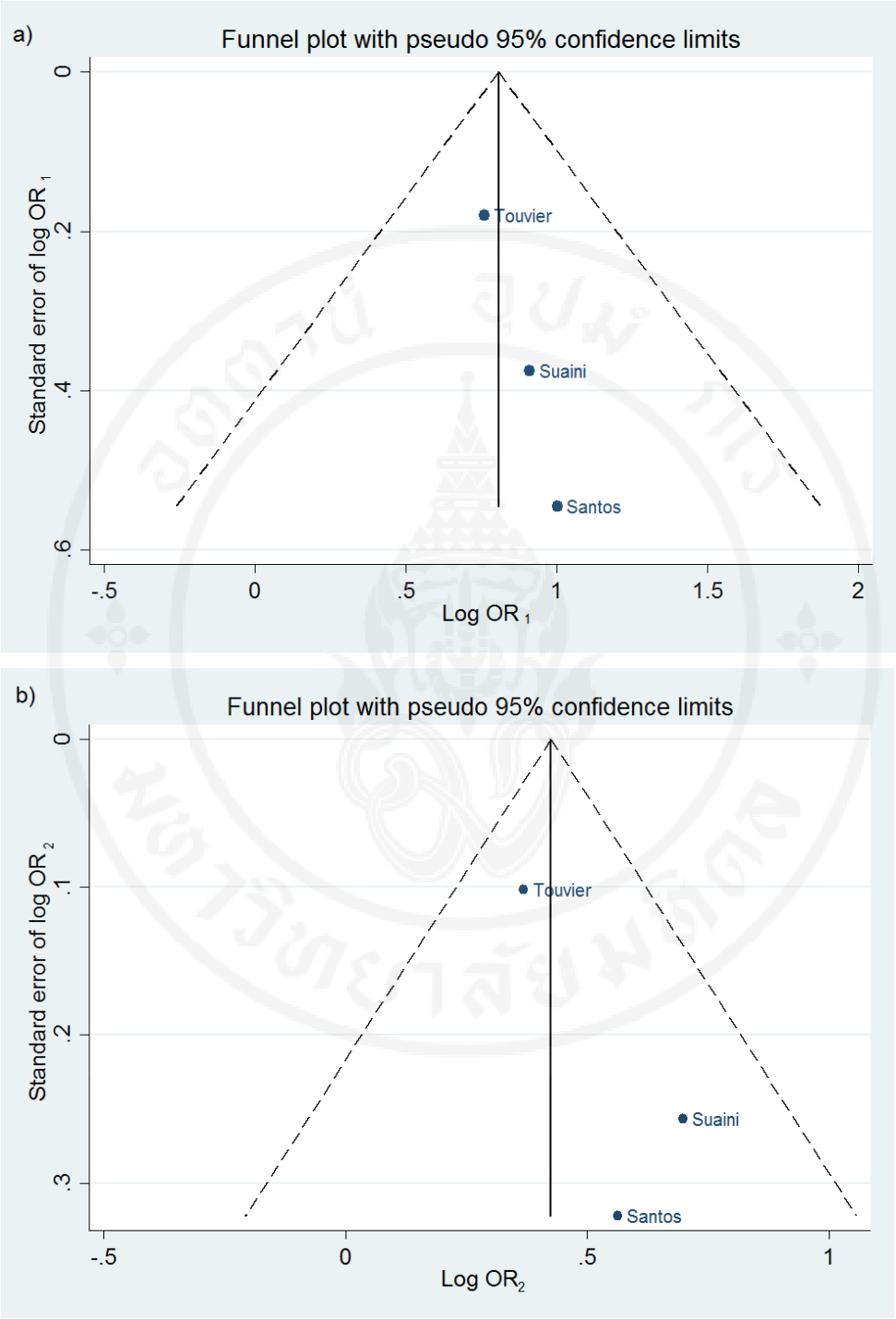

Figure 3.9 Funnel plots of rs4588 genotype effect on vitamin D deficiency/insufficiency in Caucasians a) Log OR_1 (AA versus CC), b) Log OR_2 (AC versus CC)

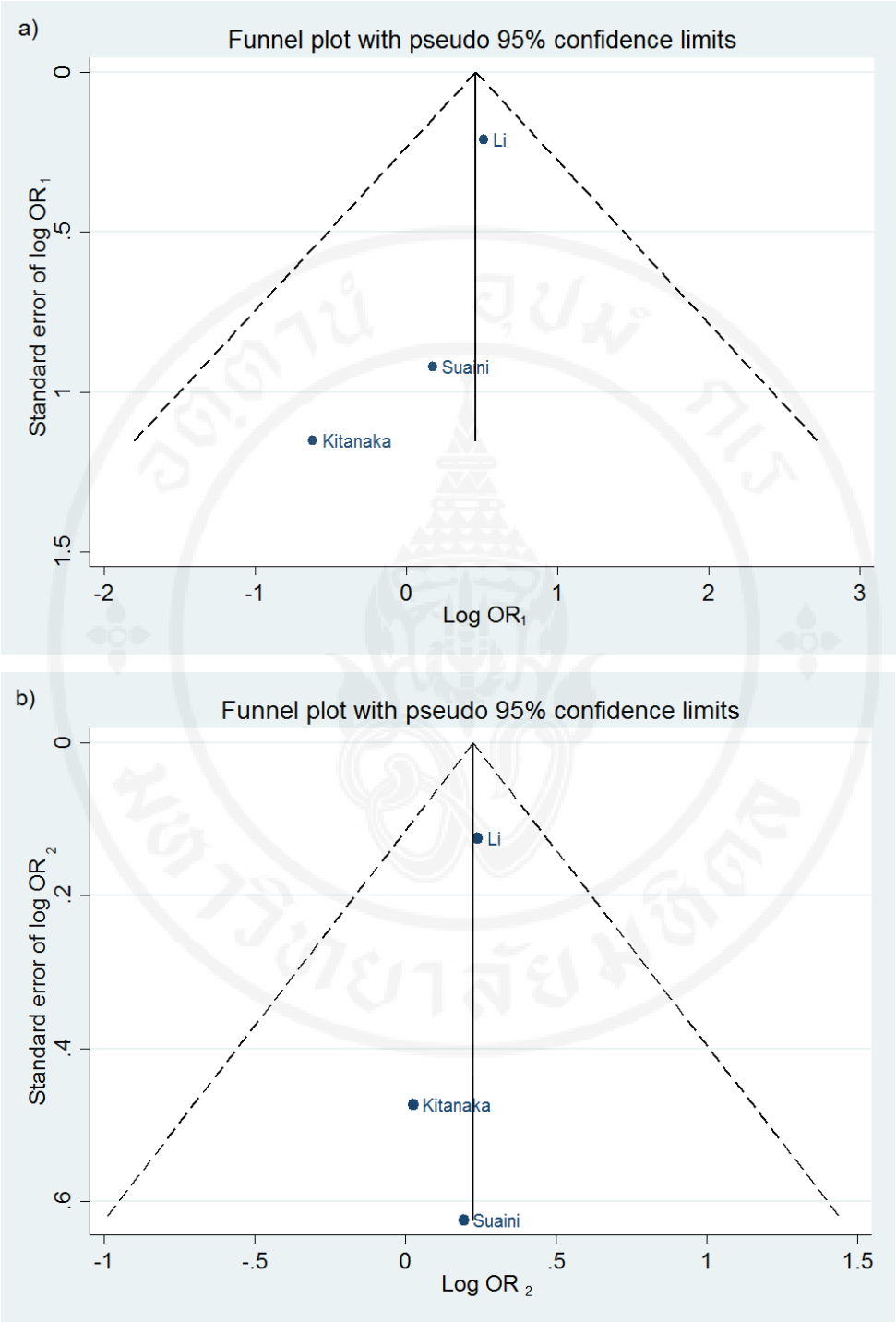

Figure 3.10 Funnel plots of rs4588 genotype effect on vitamin D deficiency/insufficiency in Asians a) Log OR_1 (AA versus CC), b) Log OR_2 (AC versus CC)

3.5 SNP rs7041

Twenty studies[20-22, 24, 27, 29, 33-35, 37, 38, 54, 56, 59-61, 63, 64, 68, 73] were included in the pooling for rs7041, 4 studies[33, 34, 37, 38] had the outcome of interests as vitamin D deficiency/insufficiency, 15 studies[20, 22, 24, 27, 29, 35, 54, 56, 59-61, 63, 64, 68, 73] had the outcome of interests as vitamin D level, and 1 study[21] reported for both data. Among these 16 studies, 7 studies[20, 22, 24, 27, 29, 61, 68] reported MDs among genotypes, 6 studies[21, 35, 54, 56, 63, 73] reported additive effects of minor genotype, 1 study[59] reported additive effect of major genotype, 1 study[64] had combined effect of TG and TT, and 1[60] study had MD for allele data.

3.5.1 Minor allele prevalence

The data for minor allele prevalence from 14 studies[20-22, 24, 27, 29, 33, 34, 37, 38, 56, 61, 68, 73] were described and for 6 studies[35, 54, 59, 60, 63, 64], the data was not available, see Table 3.9. HWE was checked for each study, and for studies where genotype data were not provided, we relied on assessing HWE from what was reported in the articles. Among 10 Caucasian studies, 3 studies[24, 34, 37] were conducted in children and 7 studies[20, 22, 27, 29, 38, 68, 73] were conducted in adults. The pooled minor allele T prevalence of these corresponding groups were respectively 0.442 (95%CI: 0.401, 0.483) and 0.436 (95%CI: 0.353, 0.519). Among 5 Asian studies, 2[33, 34] and 3 studies[21, 56, 61] were conducted in children and adults, with the pooled minor allele prevalence of 0.506 (95%CI: 0.049, 0.964) and 0.287 (95%CI: 0.258, 0.316), respectively.

3.5.2 Vitamin D level.

The MDs of 25(OH)D for TT versus GG (MD_1) and TG versus GG (MD_2) were described by ethnicity, see Table 3.10. There was only 1 Asian study[61], which was not included after a sensitivity analysis. Among 6 Caucasian studies, 5[20, 22, 27, 29, 68] and 1[24] studies were adults and children, respectively. There were not enough studies to pool by type of subject in Caucasians. Also the pooled prevalence among children and adult Caucasians were not much different, therefore, the genotype effects were combined regardless type of subjects. The MDs were high heterogeneous for MD_1 (I^2 = 71.4%) but not for MD_2 (I^2 = 0%); with the corresponding pooled MDs of -

2.707 (95%CI: -4.91, -0.504) and -1.407 (95%CI: -2.203, -0.612), see Figure 3.11 a-b. This suggested that Caucasians with TT genotype and TG genotype had 2.71 ng/ml and 1.41 ng/ml lower level of vitamin D than those who have GG genotype, respectively. Publication bias was assessed for pooling MD_1 and MD_2, which showed little asymmetry for MD_1 but symmetry for MD_2, see Figure 3.12. The Egger's tests were non-significant for both MD_1 and MD_2, with (Coefficient = 0.459, SE = 2.008, P = 0.831) and (Coefficient = -0.373, SE = 0.946, P = 0.713) respectively.

Six studies[21, 35, 54, 56, 63, 73] reported additive effects of minor genotype, 1 study[59] reported additive effect of major genotype. Among 6 studies reported additive effects, only 4 studies[35, 54, 63, 73] and 2 studies[21, 56] were conducted in Caucasians and Asians, with the pooled MDs of -0.023 (95%CI: (-0.031, -0.015) and, -0.015 (95%CI: -0.023, -0.008). Both poolings were high heterogeneities of (I^2 = 86.4%) and (I^2 = 93.4%), respectively.

3.5.3 Vitamin D deficiency/insufficiency

A total of 5 studies that assessed T vs G allele effect on vitamin D deficiency/insufficiency, 2[37, 38], 2[21, 33], and 1[34] studies were conducted in Caucasians, Asians, and both ethnicities, respectively. Their data were described in Table 3.11. Pooling these ORs stratified by ethnicity yielded the pooled ORs for Caucasians and Asians of 0.854 (95%CI: 0.487, 1.499) and 0.79 (95%CI: 0.662, 0.943), with high heterogeneity (I^2 = 91.3%) and homogeneity (I^2 = 0%), respectively, see Figure 3.13. This suggested that Caucasians and Asians, who have T allele, were approximately at 15% and 21% lower risk of developing vitamin D deficiency/insufficiency than those Caucasians and Asians who have G allele. For publication bias, funnel plots indicated asymmetry of allele-effect in Caucasian but not for in Asian, see Figure 3.14. However, the Egger's tests were non-significant for both Caucasians (Coefficient = 1.903, SE = 5.637, P = 0.793) and Asians (Coefficient = 0.699, SE = 0.503, P = 0.397).

Genotype data for TT versus GG (OR_1) and TG versus GG (OR_2) were described by ethnicity and type of subjects, i.e., 3[34, 37, 38] and 3 studies[21, 33, 34] were Caucasian and Asians, see Table 3.12. The ORs of Caucasians were OR_1 and OR_2, 0.761 (95%CI: 0.249, 2.329) and 0.963 (95%CI: 0.509, 1.822) with high heterogeneity

(I^2 = 90.5% and 78.5%, respectively), see Figure 3.15 a-b. The funnel plots showed asymmetry for both ORs in Caucasians (see Figure 3.16 a-b) but the Egger's tests were non-significant for both ORs, i.e., Coefficient = 1.871 (SE = 5.166, P = 0.779) and 1.344 (SE = 3.856, P = 0.787) respectively. The ORs of Asians were both homogenous

(I^2 = 0%) with OR_1 and OR_2 of 0.675 (95%CI: 0.452, 1.007) and 0.746 (95%CI: 0.589, 0.946), respectively, see Figure 3.17 a-b. The funnel plots also suggested a little deviation from asymmetry for both ORs in Asians, see Figure 3.18 a-b. The Egger's tests were non-significant for both OR_1 (coefficient = 0.375, SE = 0.674, P = 0.677) and OR_2 (coefficient = 0.802, SE = 0.254, P = 0.196), respectively.

Table 3.9 Pooling the prevalence of the minor T allele of rs7041

Author, Year, (Reference No.)	Ethnicity	Type of subjects	Total number of alleles	Minor T allele frequency	Prevalence of the minor allele (95% CI)	HWE
Navas-Nazario, 2014 [24]	Caucasian	Children	668	270	0.404 (0.367, 0.441)	0.086
Santos, 2013 [37]	Caucasian	Children	396	192	0.485 (0.436, 0.534)	0.326
Suaini, 2014 [34]	Caucasian	Children	968	429	0.443 (0.412, 0.474)	0.454
Pooled			2032	891	0.442 (0.401, 0.483)	
Gilbert, 2014 [73]	Caucasian	Adult	3750	1097	0.293 (0.278, 0.307)	0.287
Janssens, 2010 [20]	Caucasian	Adult	300	119	0.397 (0.341, 0.452)	0.837
Laursen, 2014 [29]	Caucasian	Adult	2982	1259	0.422 (0.404, 0.44)	0.851
Singh, 2013 [22]	Caucasian	Adult	354	166	0.469 (0.417, 0.521)	0.074
Sinotte, 2009 [27]	Caucasian	Adult	1466	633	0.432 (0.406, 0.457)	0.192
Touvier, 2014 [38]	Caucasian	Adult	3568	1985	0.556 (0.54, 0.573)	0.071
Wang, 2014 [68]	Caucasian	Adult	1746	843	0.483 (0.459, 0.506)	0.001[a]
Pooled			14166	6102	0.436 (0.353, 0.519)	
Kitanaka, 2012 [33]	Asian	Children	132	36	0.273 (0.197, 0.349)	0.573
Suaini, 2014 [34]	Asian	Children	142	105	0.739 (0.667, 0.812)	0.613
Pooled			274	141	0.506 (0.049, 0.964)	
Li, 2014 [21]	Asian	Adult	2398	675	0.281 (0.263, 0.299)	0.063
Lu, 2012 [56]	Asian	Adult	6366	1683	0.264 (0.254, 0.275)	0.218

Table 3.9 Pooling the prevalence of the minor T allele of rs7041 (continued)

Author, Year, (Reference No.)	Ethnicity	Type of subjects	Total number of alleles	Minor T allele frequency	Prevalence of the minor allele (95% CI)	HWE
Robien, 2013 [61]	Asian	Adult	982	318	0.324 (0.295, 0.353)	0.755
Pooled			9746	2676	0.287 (0.258, 0.316)	

[a] This study had Hardy-Weinberg disequilibrium but was included in the analysis after a sensitivity analysis

CI; confidence interval, HWE; Hardy-Weinberg Equilibrium

Table 3.10 Describes genotype data and pooled mean difference of vitamin D levels for rs7041

First Author, Year,	GG			TG			TT			MD_1	MD_2
(Reference No.)	n	mean	SD	n	mean	SD	n	mean	SD		
Caucasians											
Sinotte, 2009 [27]	228	26.96	7.86	377	26.04	7.78	128	24.12	7.7	-2.84 (-4.519, -1.161)	-0.92 (-2.207, 0.367)
Janssens, 2010 [20]	54	26.3	8.7	73	24.6	9.1	23	20.9	5	-5.4 (-8.492, -2.308)	-1.7 (-4.821, 1.421)
Singh, 2013 [22]	44	27.4	12.94	100	23.08	10.02	33	29.37	14.62	1.97 (-4.315, 8.255)	-4.32 (-8.618, -0.022)
Navas-Nazario, 2014 [24]	111	26.92	16.59	176	26.96	5.69	47	28.21	5.49	1.29 (-2.172, 4.752)	0.04 (-3.159, 3.239)
Laursen, 2014 [29]	496	33.57	9.96	731	31.53	12.71	264	27.72	18.66	-5.85 (-8.266, -3.434)	-2.04 (-3.312, -0.768)
Wang, 2014 [68]	258	24.12	7.78	387	22.64	7.8	228	22.03	7.32	-2.09 (-3.433, -0.747)	-1.48 (-2.707, -0.253)
Pooled MD										-2.707 (-4.91, -0.504)	-1.407 (-2.203, -0.612)
Additive model Caucasians											
Bu, 2010 [35]										1.18 (-0.643, 3.003)	

Table 3.10 Describes genotype data and pooled mean difference of vitamin D levels for rs7041 (continued)

First Author, Year, (Reference No.)	GG			TG			TT			MD_1	MD_2
	n	mean	SD	n	mean	SD	n	mean	SD		
Hibler, 2012 [54]										-2.22 (-3.533, -0.907)	
Strawbridge, 2014 [63]										-0.023 (-0.031, -0.015)	
Gilbert, 2014 [73]										-1.65 (-2.532, -0.768)	
Pooled MD										-0.023 (-0.031, -0.015)	
Asians											
Lu, 2012 [56]										-0.015 (-0.023, -0.007)	
Li, 2014 [21]										-1.42 (-2.126, -0.714)	
Pooled MD										-0.015 (-0.023, -0.008)	

MD_1; mean difference of TT versus GG, MD_2; mean difference of TG versus GG, n; number of subjects, SD; standard deviation

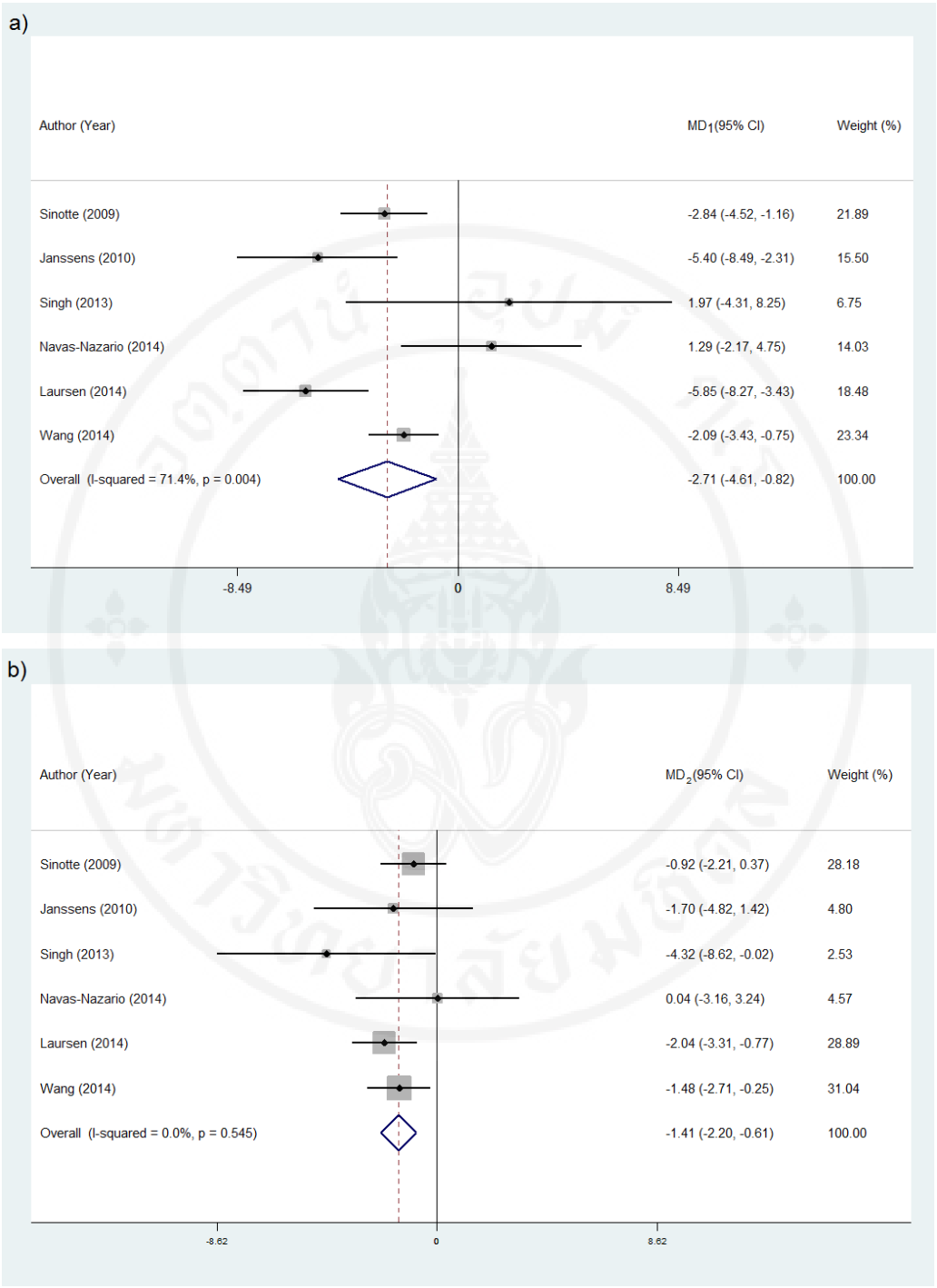

Figure 3.11 Forest plot of pooled MD in adult Caucasians for rs7041 a) MD_1 for TT versus GG, b) MD_2 for TG versus GG

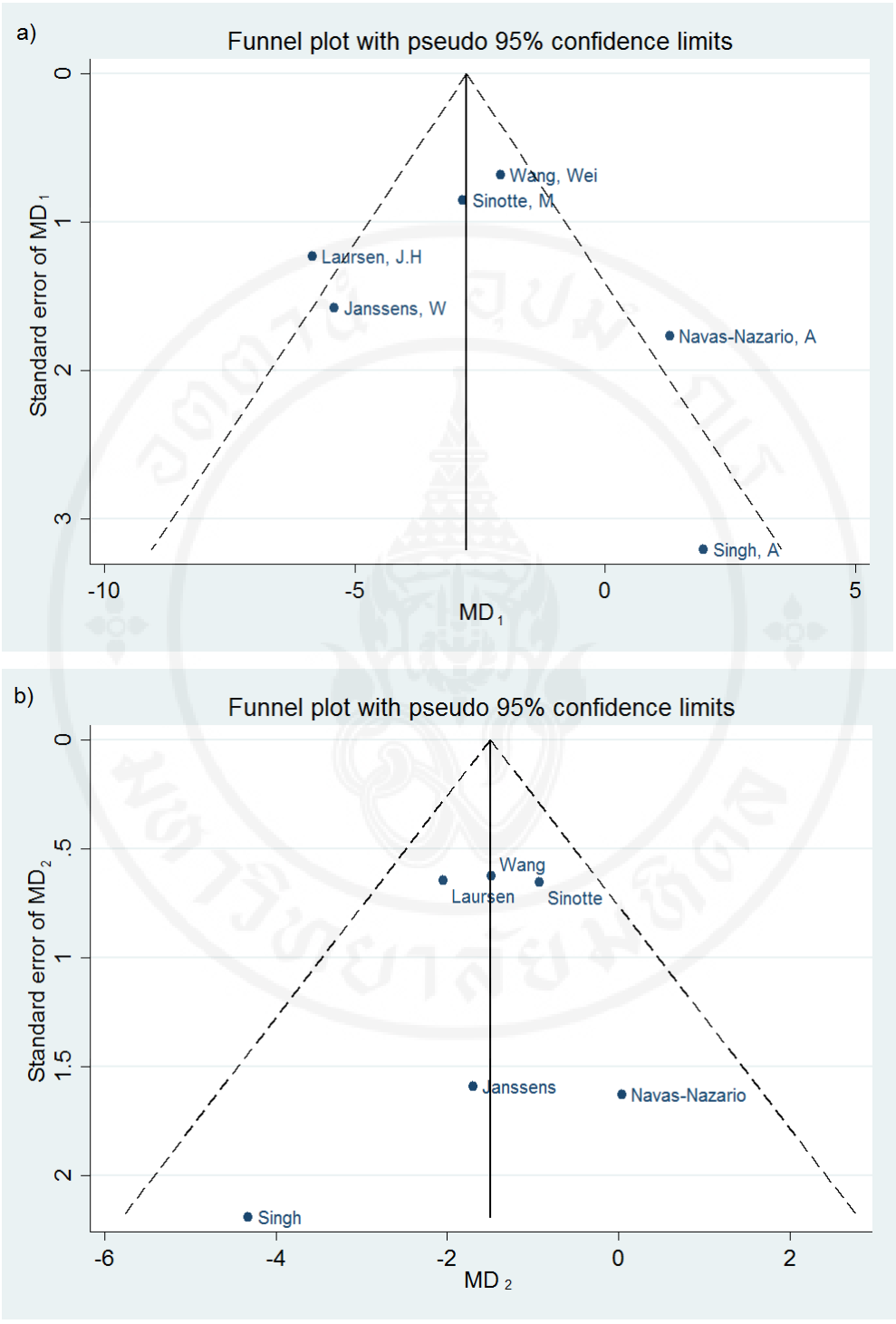

Figure 3.12 Funnel plots for pooling MD_1 and MD_2 for rs7041 in Caucasian studies a) MD_1 (TT versus GG), b) MD_2 (TG versus GG)

Table 3.11 Pooling allele effect of rs7041on vitamin D deficiency/insufficiency

First Author, Year, (Reference No.)	Case		Control		OR
	G	T	G	T	
Caucasians					
Santos, 2013 [37]	82	50	122	142	0.524 (0.342, 0.803)
Suaini, 2014 [34]	81	95	458	334	1.608 (1.158, 2.233)
Touvier, 2014 [38]	984	1080	599	905	0.726 (0.635, 0.831)
Pooled	1147	1225	1179	1381	0.854 (0.487, 1.499)
Asians					
Kitanaka, 2012 [33]	46	14	96	36	0.812 (0.399, 1.651)
Li, 2014 [21]	697	233	1026	442	0.776 (0.645, 0.934)
Suaini, 2014 [34]	7	23	30	82	1.202 (0.468, 3.089)
Pooled	750	270	1152	560	0.79 (0.662, 0.943)

CI; confidence interval, OR; odds ratio

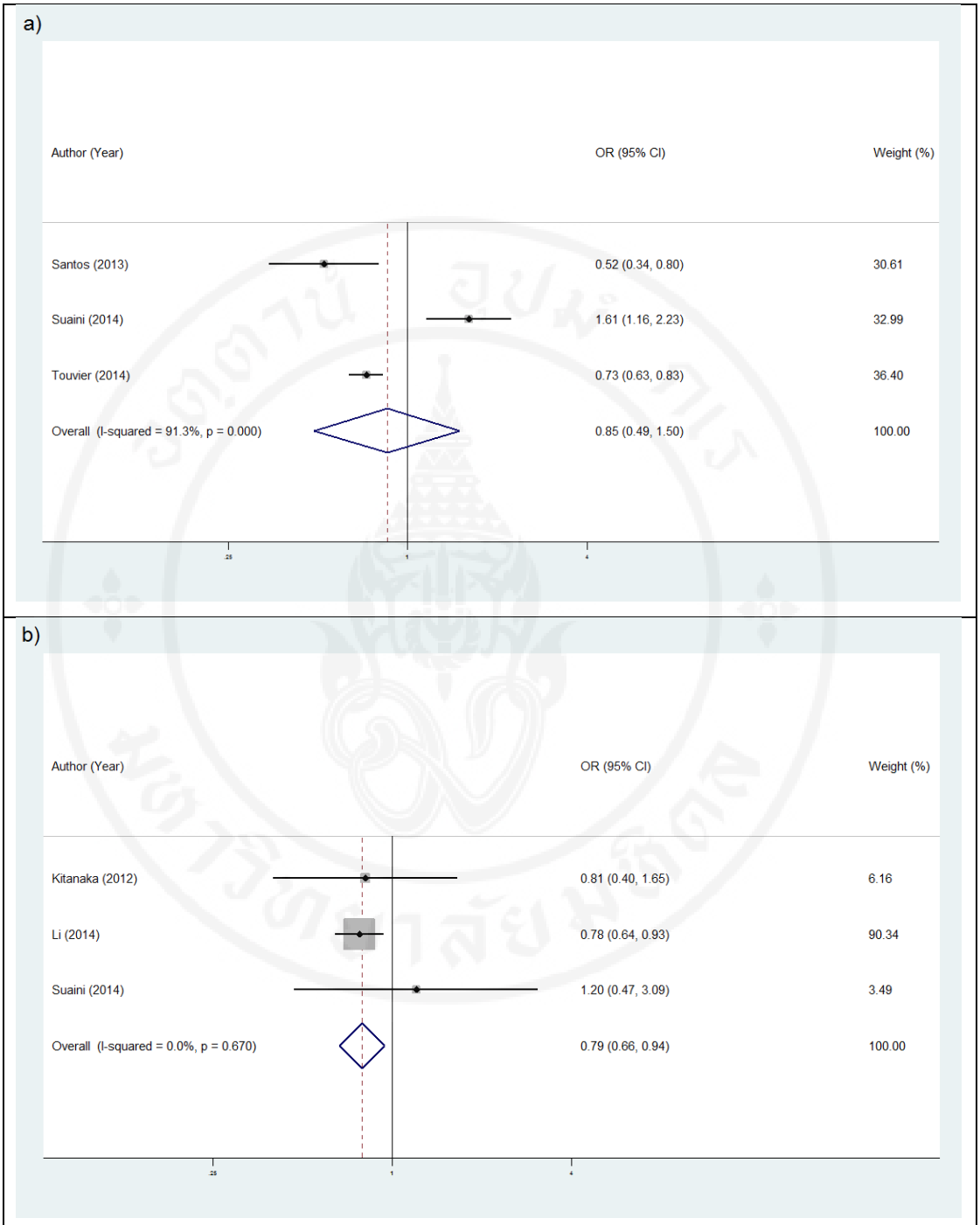

Figure 3.13 Forest plot of pooling ORs as allele effect on vitamin D deficiency/insufficiency a) Log OR (T versus G) in Caucasians, b) Log OR (T versus G) in Asians

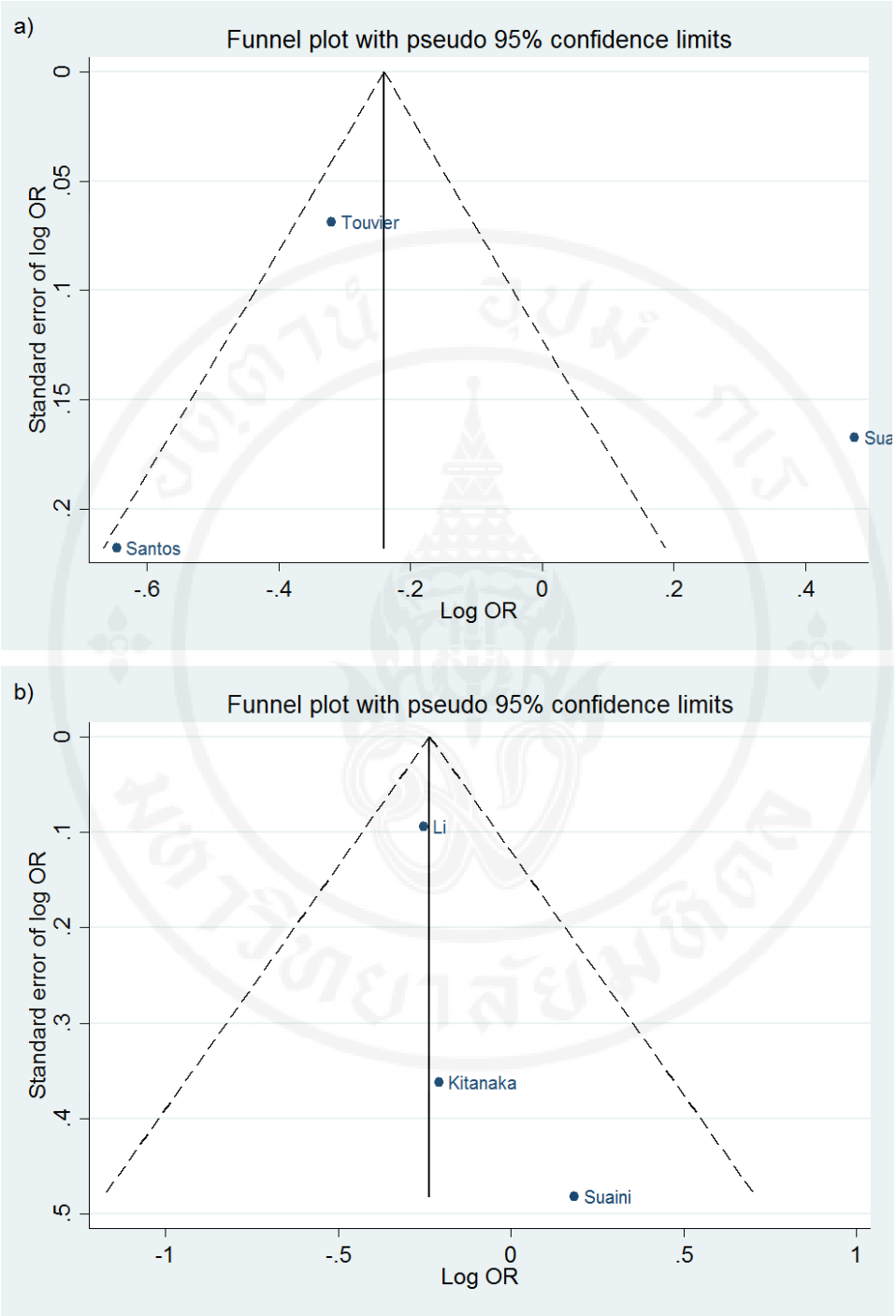

Figure 3.14 Funnel plots of rs7041 allele effect on vitamin D deficiency/insufficiency a) Log OR (T versus G) in Caucasians, b) Log OR (T versus G) in Asians

Table 3.12 Pooling genotype effect of rs7041on vitamin D deficiency/insufficiency

First Author, Year, (Reference No.)	Case	Case	Case	Control	control	Control	OR_1 (95% CI)	OR_2 (95% CI)
	GG	TG	TT	GG	TG	TT		
Caucasians								
Santos, 2013 [37]	26	30	10	30	62	40	0.288 (0.121, 0.688)	0.558 (0.282, 1.105)
Suaini, 2014 [34]	16	49	23	130	198	68	2.748 (1.362, 5.547)	2.011 (1.097, 3.686)
Touvier, 2014 [38]	240	504	288	130	339	283	0.551 (0.421, 0.722)	0.805 (0.625, 1.038)
Pooled OR	282	583	321	290	599	391	0.761 (0.249, 2.329)	0.963 (0.509, 1.822)
Asians								
Kitanaka, 2012 [33]	17	12	1	34	28	4	0.5 (0.052, 4.827)	0.857 (0.351, 2.092)
Li, 2014 [21]	268	161	36	364	298	72	0.679 (0.442, 1.044)	0.734 (0.572, 0.941)
Suaini, 2014 [34]	0	7	8	4	22	30	2.508 (0.123, 51.346)	3 (0.144, 62.488)
Pooled OR	285	180	45	402	348	106	0.675 (0.452, 1.007)	0.746 (0.589, 0.946)

CI; confidence interval, OR_1; odds ratio of TT versus GG, OR_2; odds ratio of TG versus GG

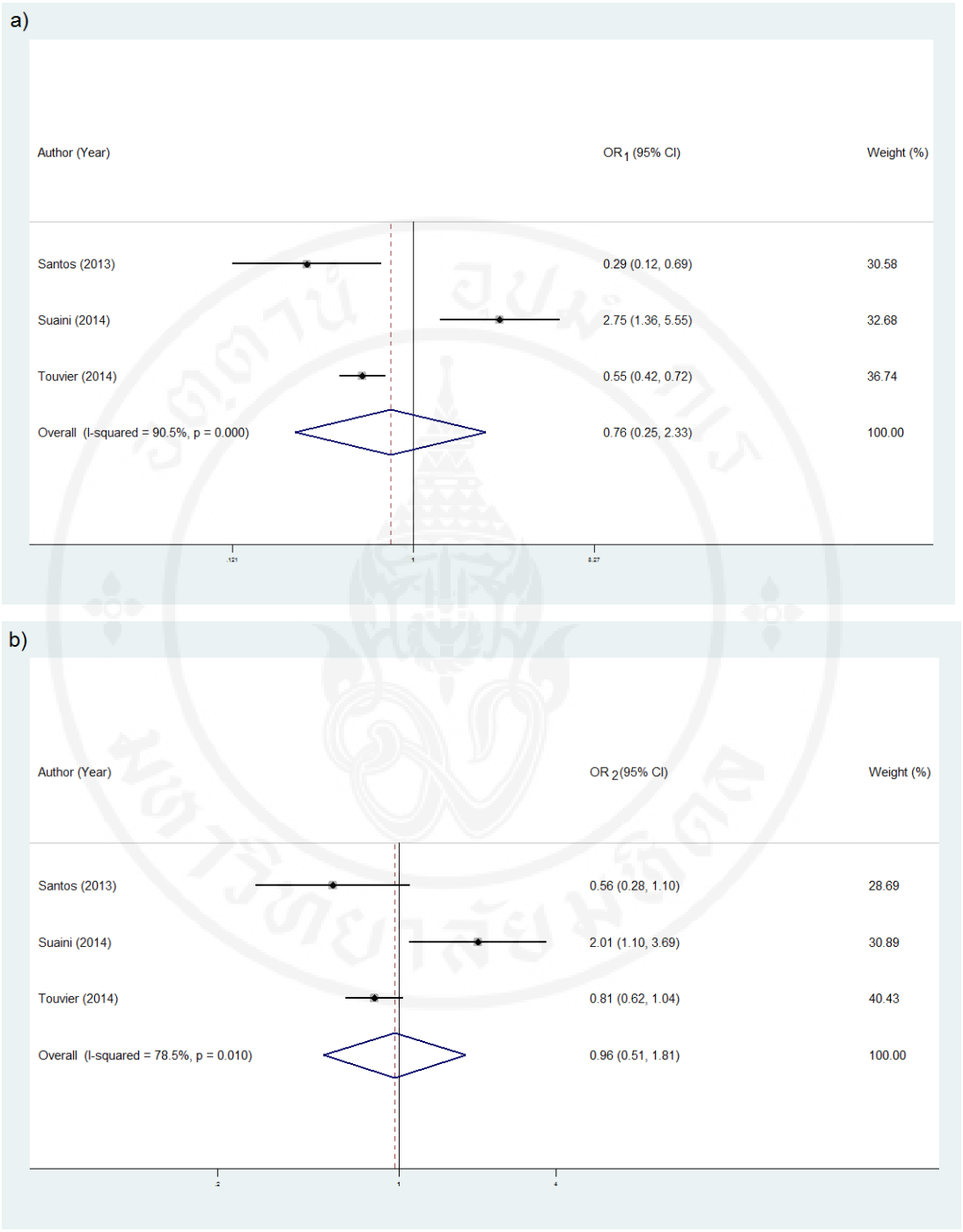

Figure 3.15 Forest plots of rs7041 genotype effect on vitamin D deficiency/insufficiency in Caucasians a) Log OR_1 (TT versus GG), b) Log OR_2 (TG versus GG)

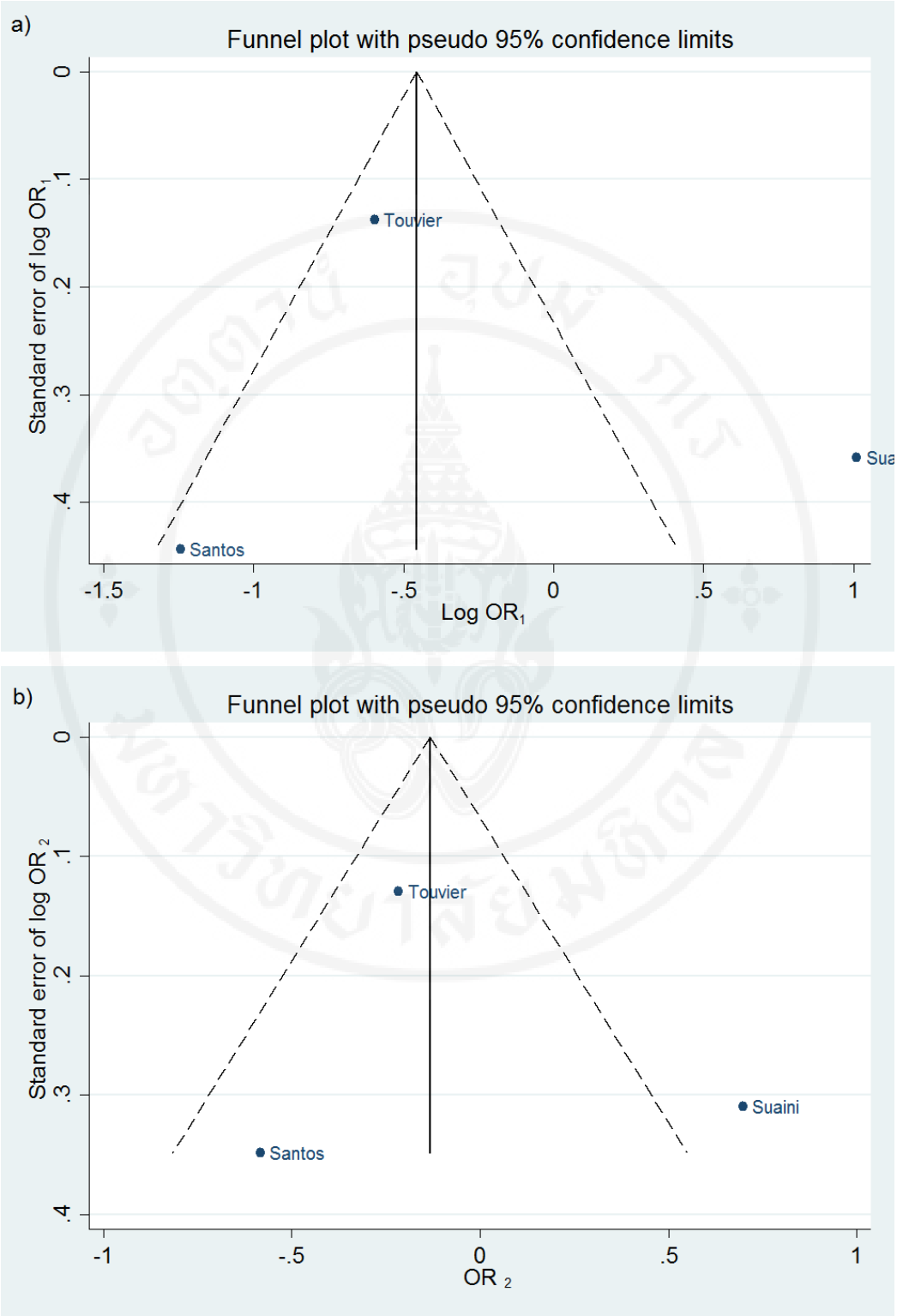

Figure 3.16 Funnel plots of rs7041 genotype effect on vitamin D deficiency/insufficiency in Caucasians a) Log OR_1 (TT versus GG), b) Log OR_2 (TG versus GG).

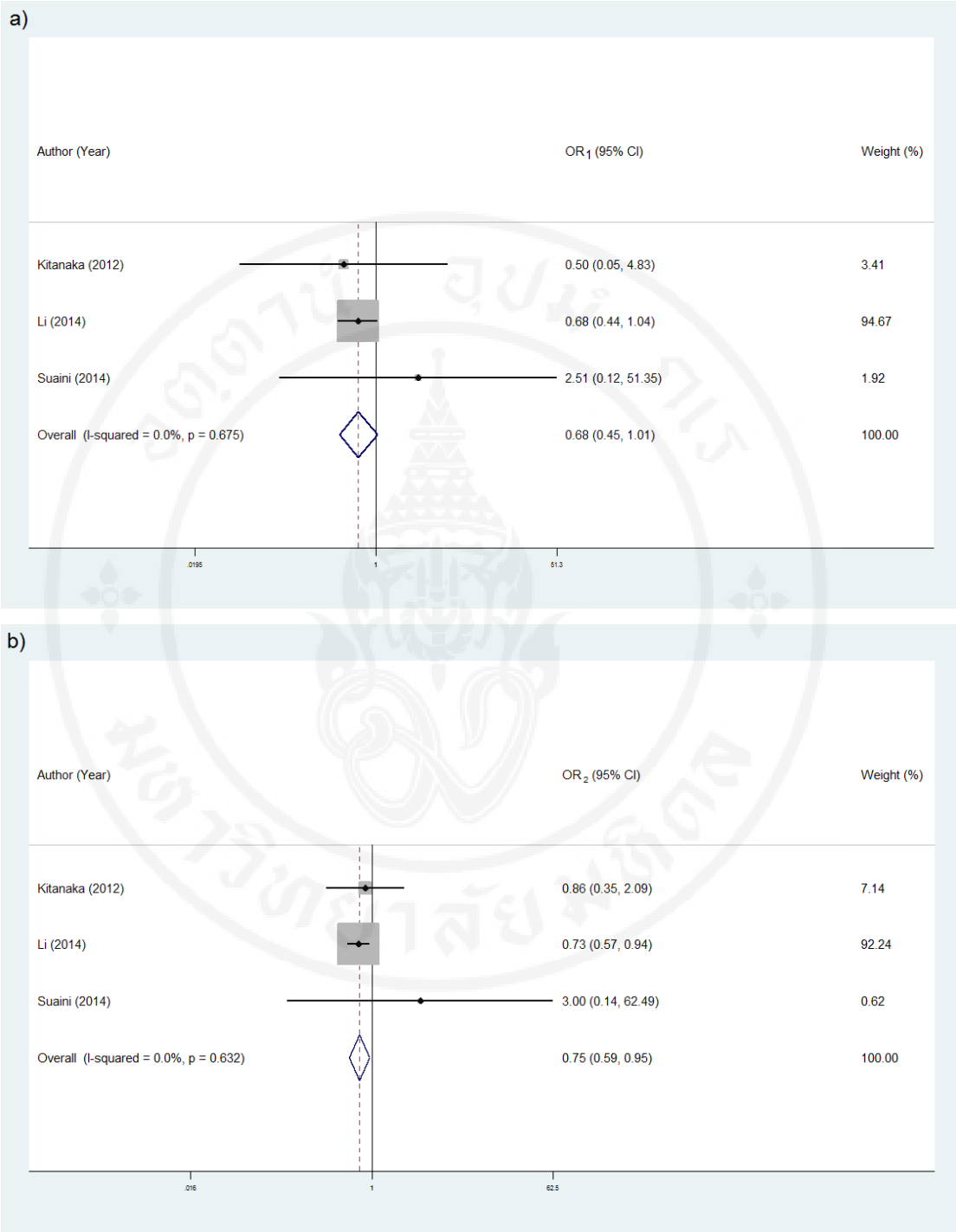

Figure 3.17 Forest plots of rs7041 genotype effect on vitamin D deficiency/insufficiency in Asians a) Log OR_1 (TT versus GG), b) Log OR_2 (TG versus GG)

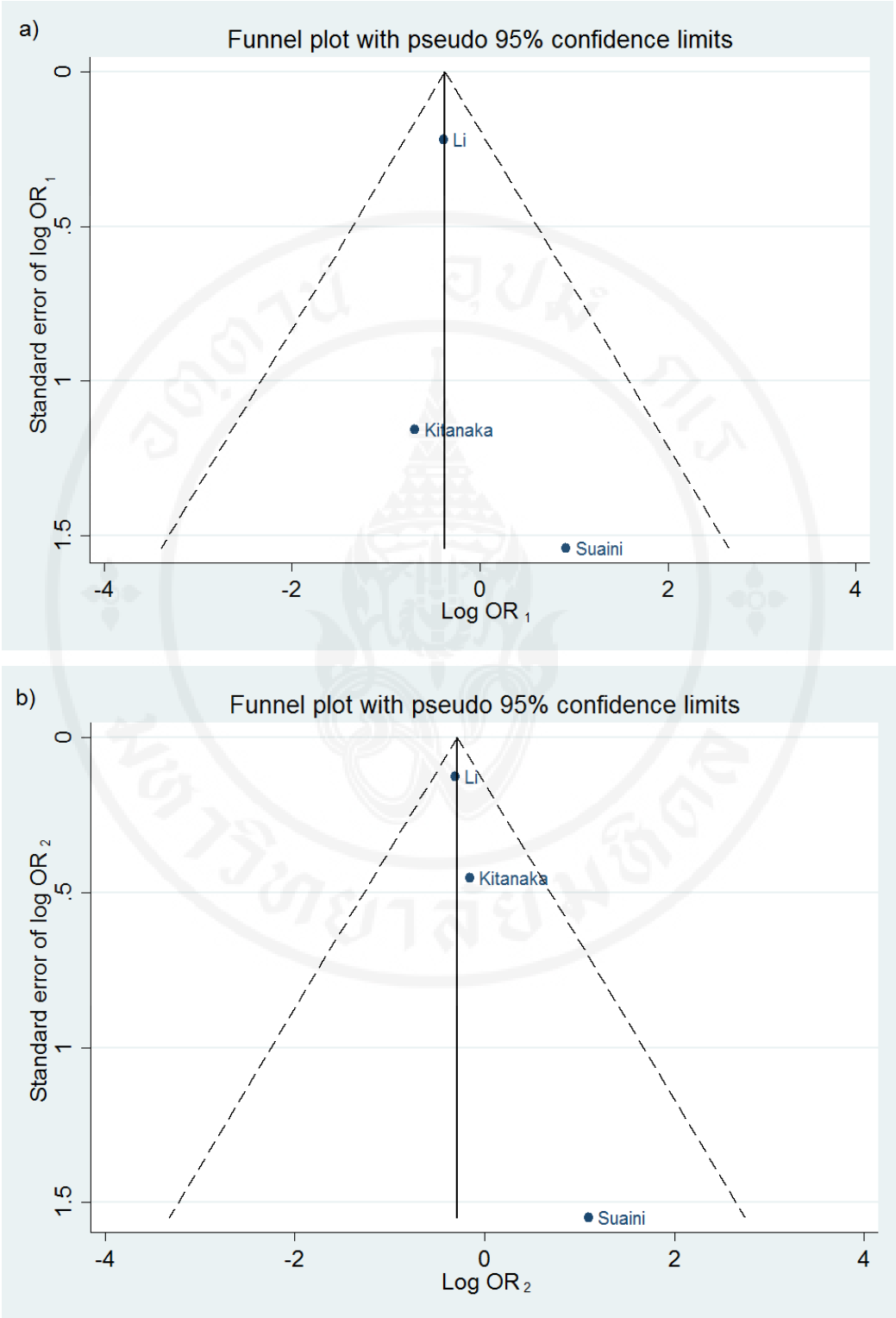

Figure 3.18 Funnel plots of rs7041 genotype effect on vitamin D deficiency/insufficiency in Asians a) Log OR_1 (TT versus GG), b) Log OR_2 (TG versus GG)

3.6 SNP rs2282679

Among 26 studies[10, 21, 25, 26, 28, 29, 33-35, 49, 51, 52, 55-57, 62, 63, 65-73] for rs2282679, 3[33, 34, 67], 18[10, 25, 26, 29, 35, 49, 55, 56, 62, 63, 65, 66, 68-73], and 5 studies[21, 28, 51, 52, 57] had vitamin D deficiency/insufficiency, vitamin D level, an both as the outcomes, respectively. From the 8 studies for pooling ORs, 5[21, 28, 33, 34, 52] and 2 studies[51, 57] had genotype frequencies and additive genotype effects, 1 study [67] had assessed allele effect.

From the 23 studies, 7 studies[25, 28, 29, 65, 66, 68, 71] reported means among genotypes, 1 study[26] reported the MDs, 10 studies[10, 21, 35, 49, 56, 57, 63, 69, 70, 73] reported additive genotype effects, 3 studies[52, 55, 62] had combined genotype effects, and 2 studies[51, 72] had MD for allele effect.

3.6.1 Minor allele prevalence

Among 26 studies the data from 16 studies[21, 25, 26, 28, 29, 33, 34, 52, 56, 62, 65, 66, 68, 70, 71, 73] were used for pooling minor allele prevalence (see Table 3.13), because 1 study[55] observe HWE, and 9 studies[10, 35, 49, 51, 57, 63, 67, 69, 72] did not have data for pooling minor allele prevalence. There were 9 studies in Caucasians, 6 studies in Asians and 2 studies in Africans. Among 9 Caucasian studies, 2[25, 34] and 8 studies[25, 26, 28, 29, 65, 66, 68, 73] were respectively children and adults with the pooled minor allele G prevalence of 0.279 (95%CI: 0.257, 0.300) and 0.292 (95%CI: 0.259, 0.326). Among 6 Asian studies, 2[33, 34] and 4 studies[21, 56, 70, 71] were respectively children and adults with the minor allele G prevalence of 0.285 (95%CI: 0.170, 0.400) and 0.311 (95%CI: 0.226, 0.396). Among 2 African studies, with the pooled minor allele G prevalence of 0.068 (95%CI: 0.047, 0.089).

3.6.2 Vitamin D level

The overall MDs for GG versus TT (MD_1) and GT versus TT (MD_2) from 7/8 studies[25, 26, 28, 29, 65, 66, 68] were described by ethnicity, see Table 3.14. Among them, 7 studies[25, 26, 28, 29, 65, 66, 68] were Caucasians, and 1 study[71] was Asian. Pooling in adult Caucasians yielded MD_1 and MD_2 of -3.407 (95%CI: -4.686, -2.127) and -1.673 (95%CI: -2.471, -0.875) with high heterogeneity for both (I^2 = 73.3% and 79.8%, respectively), see Figure 3.19 a-b. This suggested that Caucasians with GG and GT genotypes had 3.4 ng/ml and 1.67 ng/ml lower levels of 25(OH)D than those with TT

genotype. Publication bias was assessed, funnel plots for pooling MD_1 and MD_2, which showed asymmetry for both, see Figure 3.20 a-b. The Egger's tests were non-significant for both MD_1 and MD_2, with (Coefficient = -1.041, SE = 1.973, P = 0.620) and (Coefficient = -3.591, SE = 2.064, P = 0.142) respectively.

There were 9/10 studies that reported additive genotype effects and their data were available for pooling. There were 5 studies[35, 49, 57, 63, 73] with Caucasians and 4 studies[21, 56, 69, 70] with Asians, with the MDs of -0.037 (95%CI: -0.046, -0.027) and -0.031 (95%CI: -0.039, -0.023) and both had high heterogeneity of (I^2 = 92.9%) and (I^2 = 88.1%), respectively. Publication bias for studies which reported the MD for the additive model, funnel plots of SE of MD against MD for Caucasians and Asians showed asymmetry for both, respectively, see Figure 3.21 a-b. The Egger's tests were non-significant for Caucasians with (Coefficient = -3.379, SE = 1.248, P = 0.073) and significant for Asians with (Coefficient = -3.081, SE = 0.349, P = 0.013). A contour enhanced funnel plot showed that there was the possibility of publication bias, see Figure 3.21 c.

3.6.3 Vitamin D deficiency/insufficiency

Among 4/5 studies that assessed G vs T allele effect on vitamin D deficiency/insufficiency and their data were available for pooling, 2[28, 34] and 3[21, 33, 34] were respectively studies in Caucasians and Asians, 1 study[52] was African, see Table 3.15. The pooled ORs (G vs T) for Caucasians and Asians were 1.397 (95%CI: 0.874, 2.234) and 1.272 (95%CI: 1.073, 1.507), with the heterogeneity I^2 of 85.9% and I^2 0%, respectively, see Figure 3.22. This suggested that Caucasians and Asians carrying G allele were approximately at 40% and 27% higher risk of developing vitamin D deficiency/insufficiency than those Caucasians and Asians carrying T allele. Publication bias was assessed for the Asians studies for the allele effect, funnel plot which showed symmetry, see Figure 3.23. The Egger's tests was non-significant with (coefficient = 0.063, SE = 0.466, P = 0.915).

Genotype data from studies for GG versus TT (OR_1) and GT versus TT (OR_2) were described, i.e., 2[28, 34] and 3 studies[21, 33, 34] were respectively Caucasian and Asians, see Table 3.16, 1 study[52] was African which was not included in the pooling after a sensitivity analysis. There were not enough studies to stratify them by type of subject

(children and adults). The OR_1 and OR_2 of Caucasians were, 1.733 (95%CI: 0.788, 3.811) and 1.507 (95%CI: 0.846, 2.686), with the heterogeneities I^2 of 77.2% and 78.2%, respectively. The ORs of Asians, OR_1 and OR_2 were, 1.569 (95%CI: .055, 2.332) and 1.297 (95%CI: 1.028, 1.636), both homogenous with (I^2 = 0%) with respectively, see Figure 3.24 a-b. This suggested that Caucasians and Asians, who have GG genotype were approximately at 1.73 and 1.57 times higher risk of developing vitamin D deficiency/insufficiency than those Caucasians and Asians who have TT genotype. For publication bias for genotype effect of Asian studies, the funnel plots for both OR_1 and OR_2 showed symmetry, see Figure 3.25 a-b. The Egger's tests were non-significant for both OR_1 and OR_2, with coefficients of -0.010 (SE = 0.418, P = 0.984) and 0.051 (SE = 0.149, P = 0.790), respectively.

Table 3.13 Pooling the prevalence of the minor G allele of rs2282679

Author, Year, (Reference No.)	Ethnicity	Type of subjects	Total number of alleles	Minor G allele frequency	Prevalence of the minor allele (95% Confidence Interval)	HWE
Nissen, 2014 [25]	Caucasian	Children	680	180	0.265 (0.232, 0.298)	0.923
Suaini, 2014 [34]	Caucasian	Children	968	280	0.289 (0.261, 0.318)	0.060
Pooled			1648	460	0.279 (0.257 , 0.300)	
Theodoratou, (2012) [28]	Caucasian	Adult	5556	1596	0.287 (0.275, 0.299)	0.298
Trummer (2012) [66]	Caucasian	Adult	2540	721	0.284 (0.266, 0.301)	0.250
Perna (2013) [26]	Caucasian	Adult	7482	2083	0.278 (0.289, 0.268)	0.868
Trummer (2013) [65]	Caucasian	Adult	6260	1769	0.283 (0.271, 0.294)	0.330
Gilbert (2014) [73]	Caucasian	Adult	3750	1097	0.293 (0.278, 0.307)	0.287
Laursen (2014) [29]	Caucasian	Adult	2962	818	0.276(0.26, 0.292)	0.439
Nissen, 2014 [25]	Caucasian	Adult	818	224	0.274 (0.243, 0.304)	0.923
Wang (2014) [68]	Caucasian	Adult	1746	843	0.483 (0.459, 0.506)	0.001[a]
Pooled			23632	7068	0.291 (0.263, 0.320)	
Kitanaka, 2012 [33]	Asian	Children	132	30	0.227 (0.156, 0.299)	0.679
Suaini, 2014 [34]	Asian	Children	142	49	0.345 (0.267, 0.423)	0.774
Pooled			274	79	0.285 (0.170, 0.400)	
Lu (2012) [56]	Asian	Adult	6334	2613	0.413(0.4, 0.425)	0.124
Zhang (2013) [70]	Asian	Adult	5796	1797	0.31(0.298, 0.322)	0.127
Li (2014) [21]	Asian	Adult	2398	701	0.292 (0.274, 0.311)	0.830

Table 3.13 Pooling the prevalence of the minor G allele of rs2282679 (continued)

Author, Year, (Reference No.)	Ethnicity	Type of subjects	Total number of alleles	Minor G allele frequency	Prevalence of the minor allele (95% Confidence Interval)	HWE
Thongthai, 2014 [71]	Asian	Adult	8952	2035	0.227 (0.219, 0.236)	0.363
Pooled			23480	7146	0.311 (0.226, 0.396)	
Signorello, 2011 [62]	African	Adult	750	59	0.079 (0.059, 0.098)	0.347
Foucan, 2013 [52]	African	Adult	594	34	0.057 (0.039, 0.076)	0.610
Pooled			1344	93	0.068 (0.047, 0.089)	

[a] This study had Hardy-Weinberg disequilibrium

CI; confidence interval, HWE; Hardy-Weinberg Equilibrium

Table 3.14 Describes genotype data and pooled mean difference of vitamin D levels for rs2282679

First Author, Year, (Reference No.)	TT			GT			GG			MD_1	MD_2
	n	mean	SD	n	mean	SD	n	mean	SD		
Adult Caucasians											
Theodoratou (2012) [28]	1,400	14.01	8.65	1,160	13.29	8.24	218	12.59	8.45	-1.42 (-2.63, -0.21)	-0.72 (-1.376, -0.064)
Trummer (2012) [66]	643	16.9	9.56	533	13.77	7.75	94	11.66	5.75	-5.24 (-6.617, -3.863)	-3.13 (-4.119, -2.141)
Perna[a] (2013) [26]										-2.320 (-3.418, -1.222)	-0.880 (-1.429, -0.331)
Trummer (2013) [65]	1,622	18.05	10.19	1,247	16.86	9.34	261	14.33	7.76	-3.72 (-4.784, -2.656)	-1.19 (-1.907, -0.473)
Laursen (2014) [29]	770	33.21	5.29	604	29.89	14.07	107	28.08	36.3	-5.13 (-12.018, 1.758)	-3.32 (-4.503, -2.137)
Nissen (2014) [25]	219	29.57	9.68	156	28.08	9.19	34	25.48	8.58	-4.09 (-7.246, -0.934)	-1.49 (-3.42, 0.44)
Wang (2014) [68]	258	24.12	7.78	387	22.64	7.8	228	22.03	7.32	-2.09 (-3.433, -0.747)	-1.48 (-2.707, -0.253)
Pooled MD										-3.407 (-4.686, -2.127)	-1.673 (-2.471, -0.875)

Table 3.14 Describes genotype data and pooled mean difference of vitamin D levels for rs2282679 (continued)

First Author, Year, (Reference No.)	TT			GT			GG			MD_1	MD_2
	n	mean	SD	n	mean	SD	n	mean	SD		
Additive model											
Caucasians											
Ahn (2010) [49]										-0.14 (-0.179, -0.101)	
Bu (2010) [35]										-0.99 (-2.872, 0.892)	
Muindi (2012) [57]										-2.49 (-4.489, -0.491)	
Strawbridge (2014) [63]										-0.03 (-0.04, -0.02)	
Gilbert (2014) [73]										-2.27 (-3.23, -1.31)	
Pooled MD										-0.037 (-0.046, -0.027)	
Asians											
Lu (2012) [56]										-0.029 (-0.037, -0.021)	
Zhang (2013) [70]										-0.153 (-0.263, -0.043)	

Table 3.14 Describes genotype data and pooled mean difference of vitamin D levels for rs2282679 (continued)

First Author, Year, (Reference No.)	TT			GT			GG			MD_1	MD_2
	n	mean	SD	n	mean	SD	n	mean	SD		
Li (2014) [21]										-1.23 (-1.936, -0.524)	
Yoshida (2014) [69]										-0.13 (-0.195, -0.065)	
Pooled MD										-0.031 (-0.039, -0.023)	

[a] Perna et al reported MD_1 and MD_2

MD_1; mean difference of GG versus TT, MD_2; mean difference of GT versus TT, n; number of subjects, SD; standard deviation

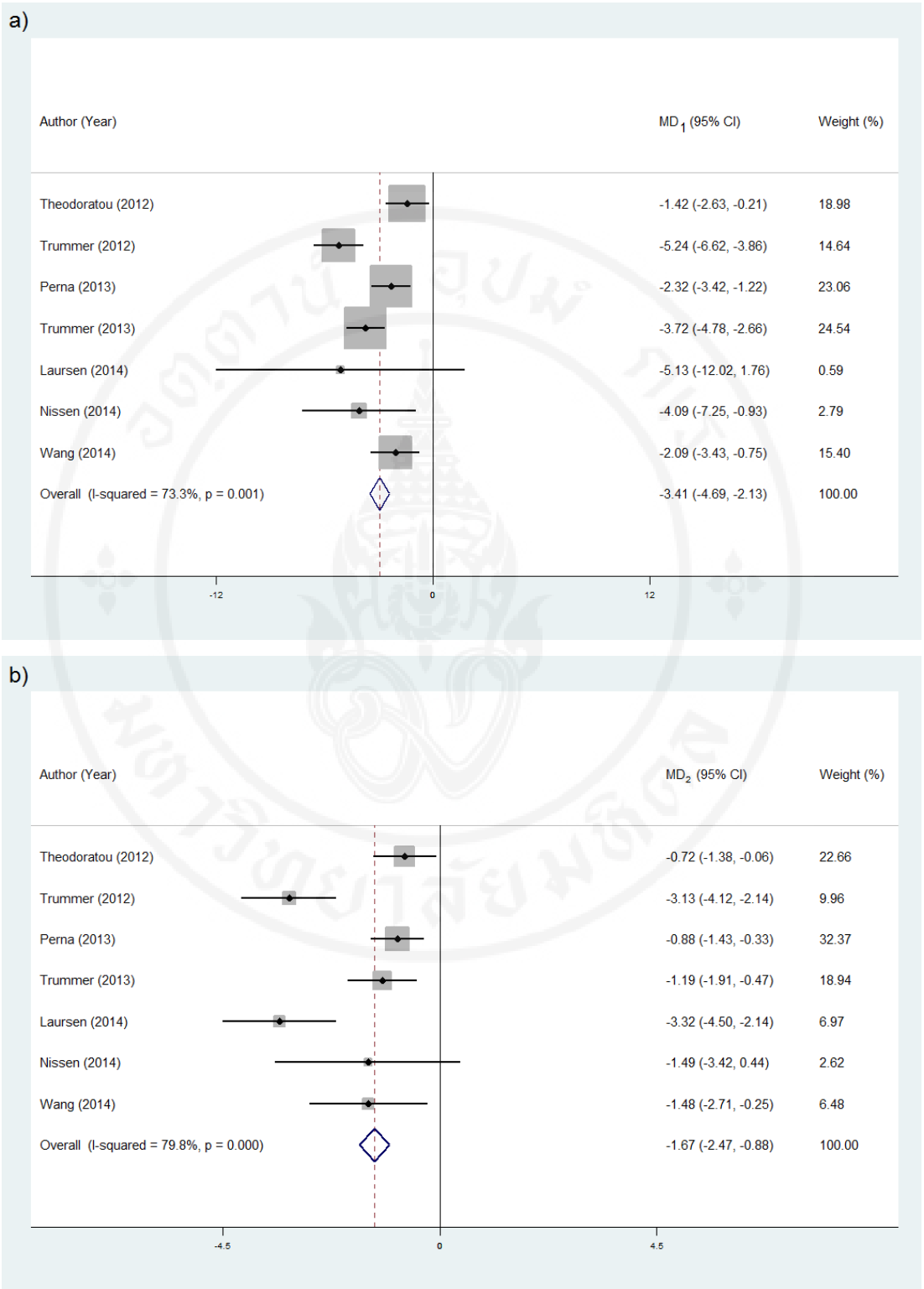

Figure 3.19 Forest plot of pooled MD in adult Caucasians for rs2282679 a) MD_1 for GG versus TT, b) MD_2 for GT versus TT

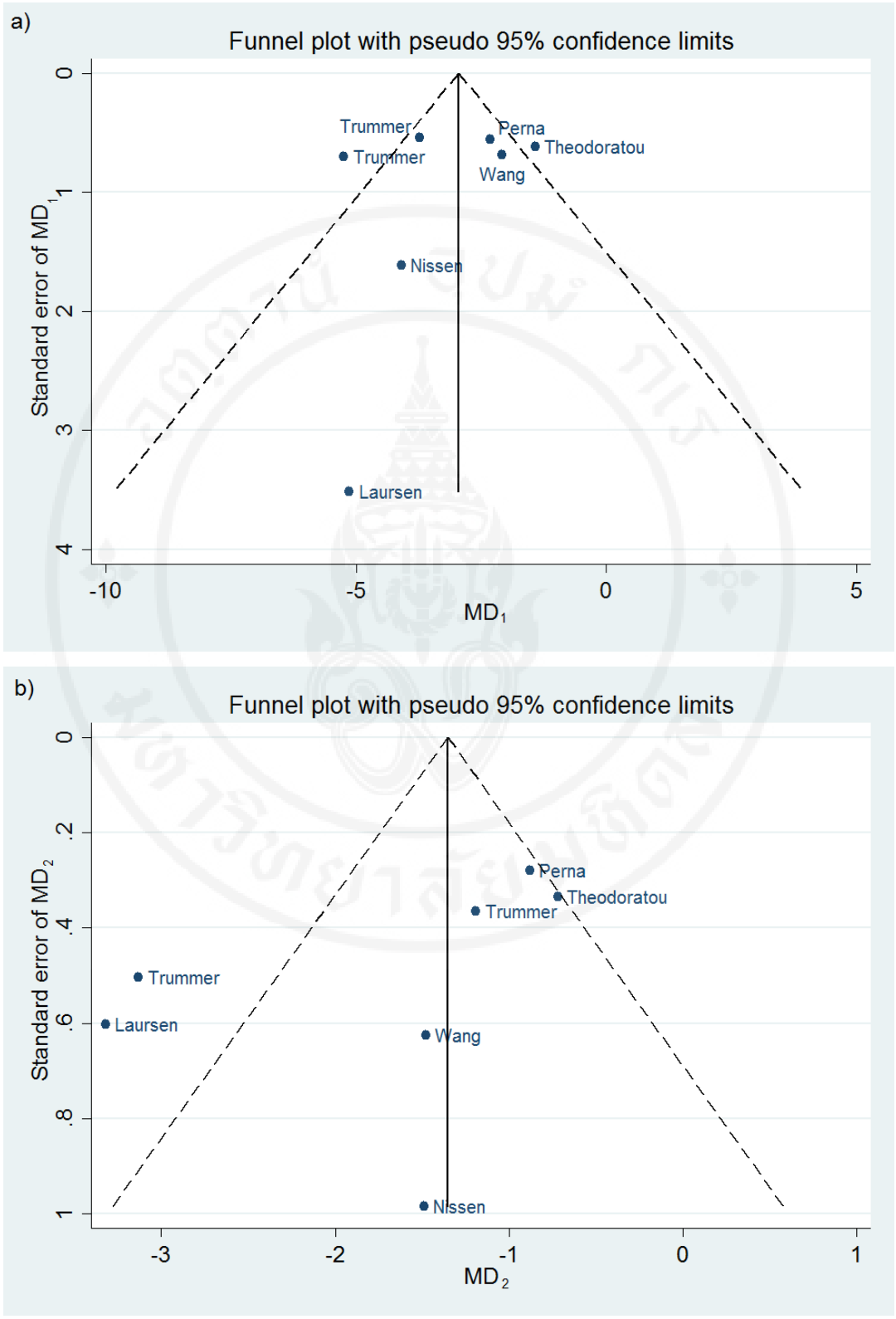

Figure 3.20 Funnel plots for pooling MD_1 and MD_2 for rs2282679 in adult Caucasian studies a) MD_1 (GG versus TT), b) MD_2 (TG versus TT)

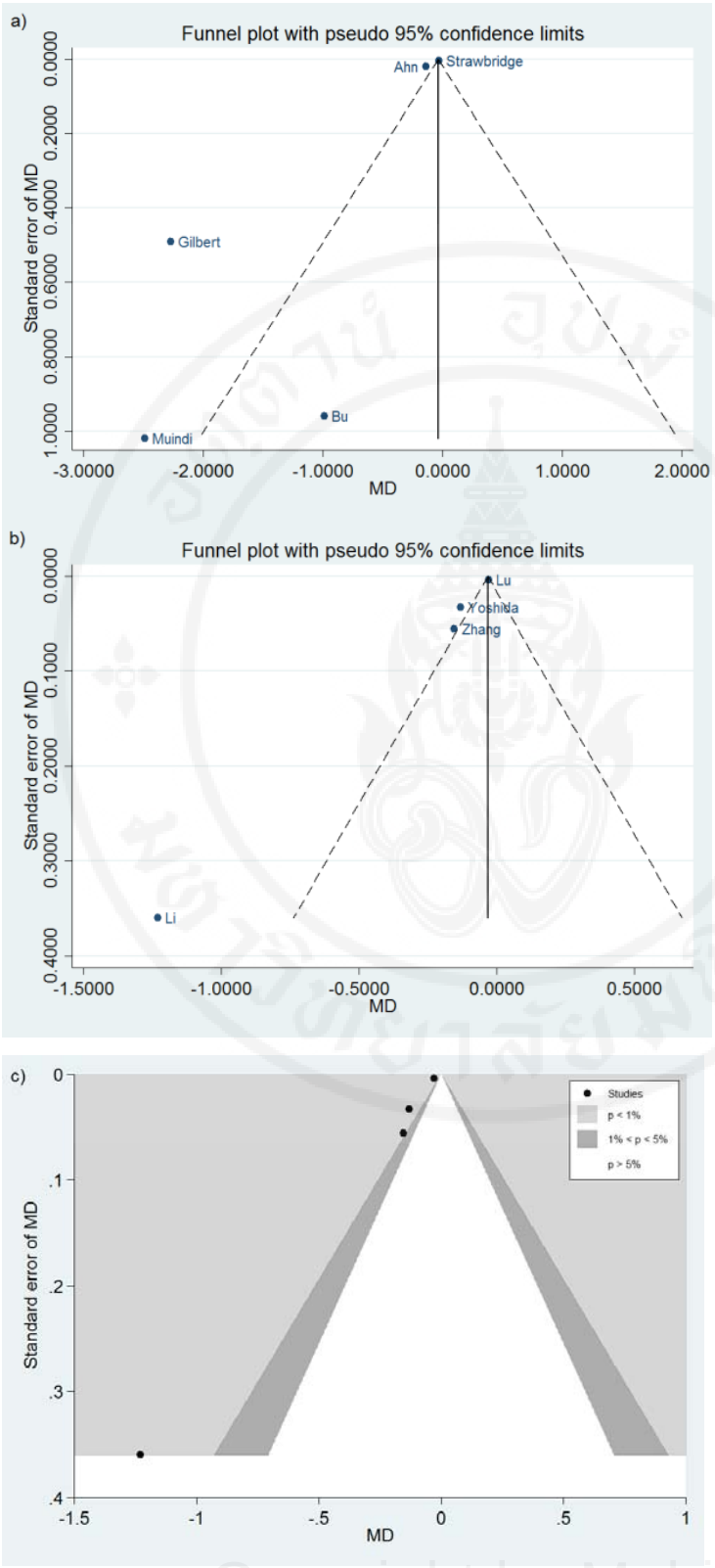

Figure 3.21 Funnel plot of pooling MDs as additive effect for rs2282679 a) Caucasians, b) Asians, c) Contour enhanced funnel plot for Asians

Table 3.15 Pooling allele effect of rs2282679 on vitamin D deficiency/insufficiency

First Author, Year, (Reference No.)	Case		Control		OR (95% CI)
	T	G	T	G	
Caucasians					
Theodoratou, 2012 [28]	964	2232	1235	3231	1.13 (1.022, 1.249)
Suaini, 2014 [34]	70	106	210	582	1.83 (1.302, 2.573)
Pooled	1034	2338	1445	3813	1.397 (0.874, 2.234)
Asians					
Kitanaka, 2012 [33]	18	42	30	102	1.457 (0.734, 2.894)
Li, 2014 [21]	300	630	401	1067	1.267 (1.059, 1.516)
Suaini, 2014 [34]	11	19	38	74	1.127 (0.487, 2.61)
Pooled	329	691	469	1243	1.272 (1.073, 1.507)

CI; confidence interval, OR; odds ratio.

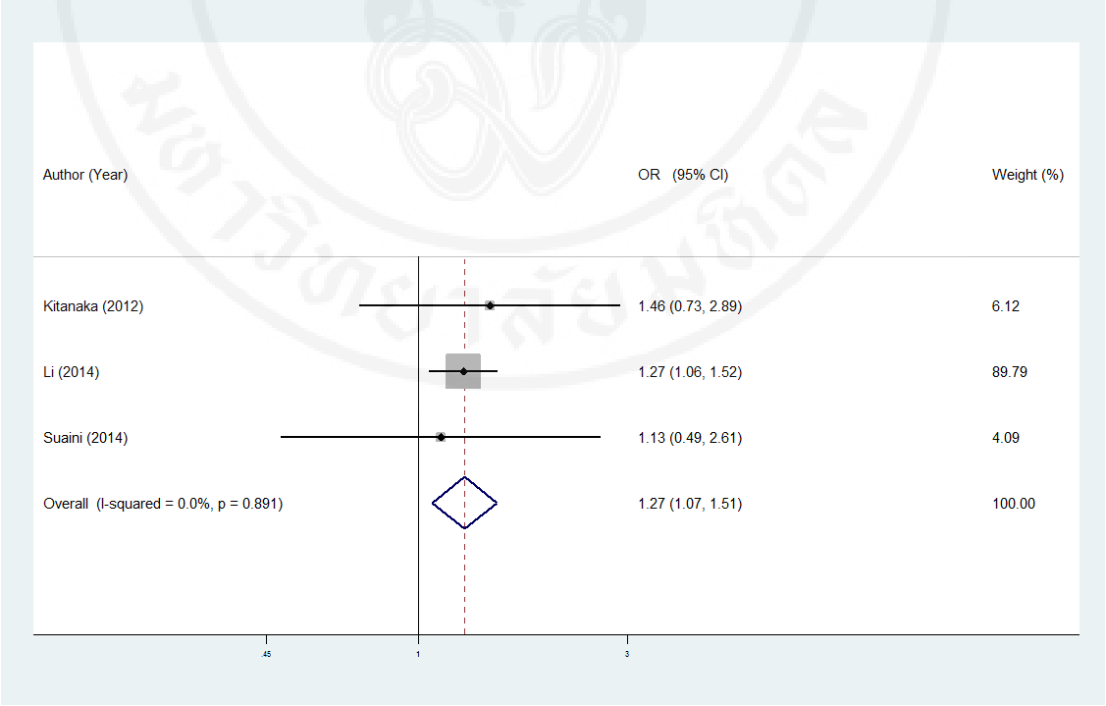

Figure 3.22 Forest plot of rs2282679 allele effect on vitamin D deficiency/insufficiency in Asians, Log OR (G versus T)

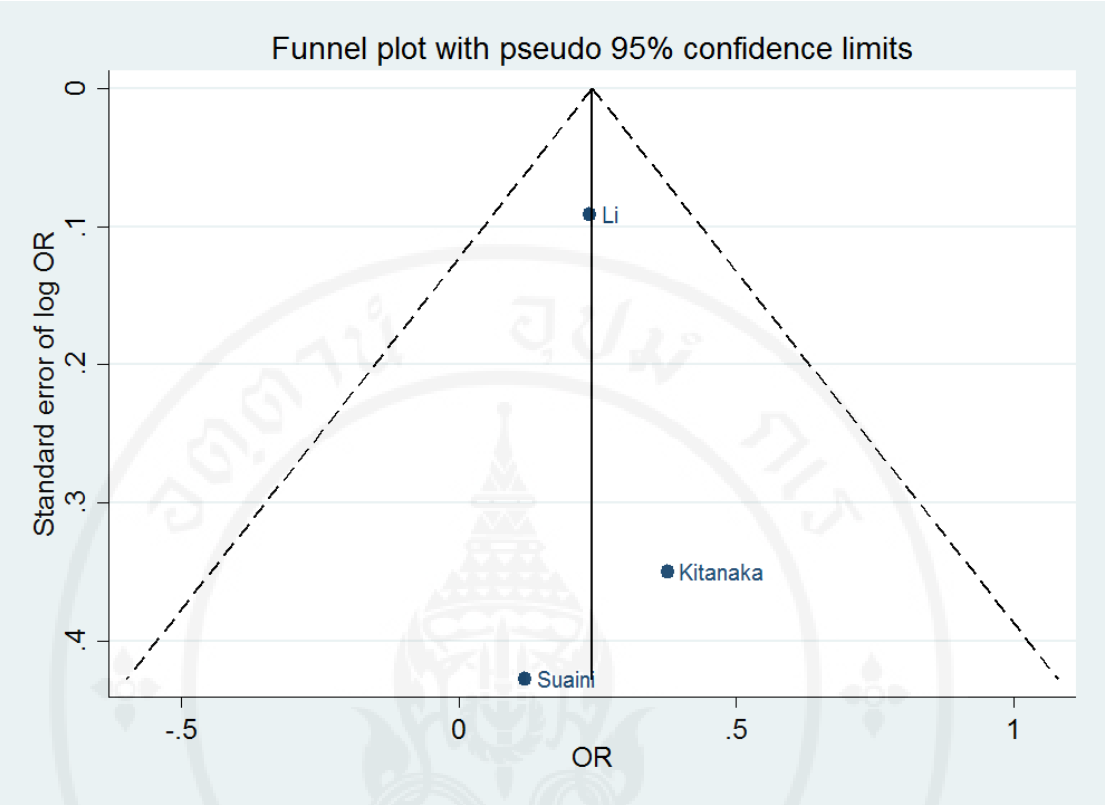

Figure 3.23 Funnel plot of rs2282679 allele effect on vitamin D deficiency/insufficiency in Asians, Log OR (G versus T)

Table 3.16 Pooling genotype effect of rs2282679 on vitamin D deficiency/insufficiency

First Author, Year, (Reference No.)	Case	Case	Case	Control	Control	Control	OR_1 (95% CI)	OR_2 (95% CI)
	TT	GT	GG	TT	GT	GG		
Caucasians								
Theodoratou, 2012 [28]	767	698	133	1163	905	165	1.222 (0.956, 1.563)	1.169 (1.022, 1.338)
Suaini, 2014 [34]	32	42	14	221	140	35	2.763 (1.342, 5.688)	2.072 (1.249, 3.438)
Pooled OR	799	740	147	1384	1045	200	1.733 (0.788, 3.811)	1.507 (0.846, 2.686)
Asians								
Kitanaka, 2012 [33]	15	12	3	40	22	4	2 (0.4, 10.008)	1.455 (0.58, 3.651)
Li, 2014 [21]	213	204	48	389	289	56	1.565 (1.028, 2.383)	1.289 (1.009, 1.647)
Suaini, 2014 [34]	6	7	2	25	24	7	1.19 (0.196, 7.249)	1.215 (0.357, 4.141)
Pooled OR	234	223	53	454	335	67	1.569 (1.055, 2.332)	1.297 (1.028, 1.636)

CI; confidence, OR_1; odds ratio of GG versus TT,OR_2; odds ratio of GT versus TT

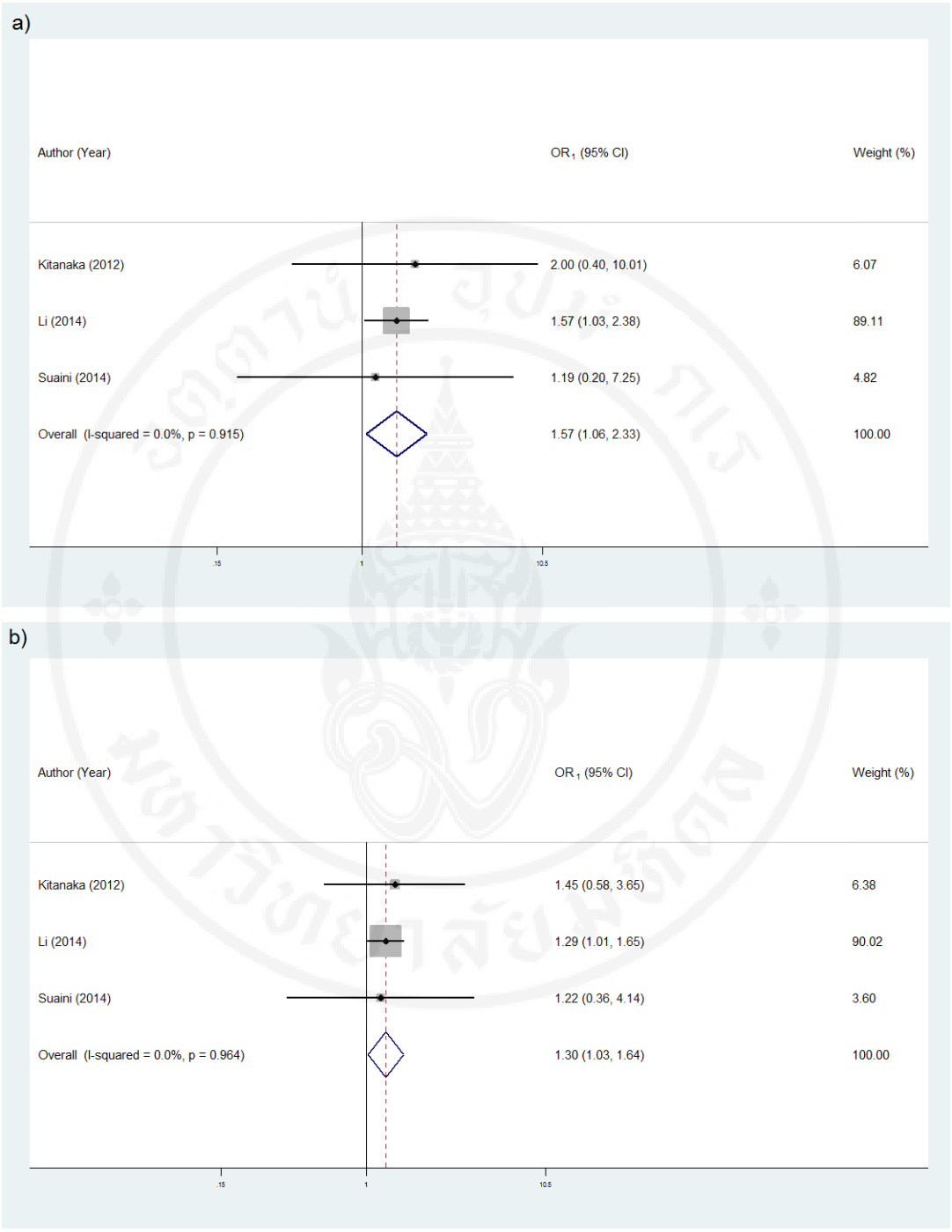

Figure 3.24 Forest plots of rs2282679 genotype effect on vitamin D deficiency/ insufficiency in Asians a) Log OR_1 (GG versus TT), b) Log OR_2 (TG versus TT)

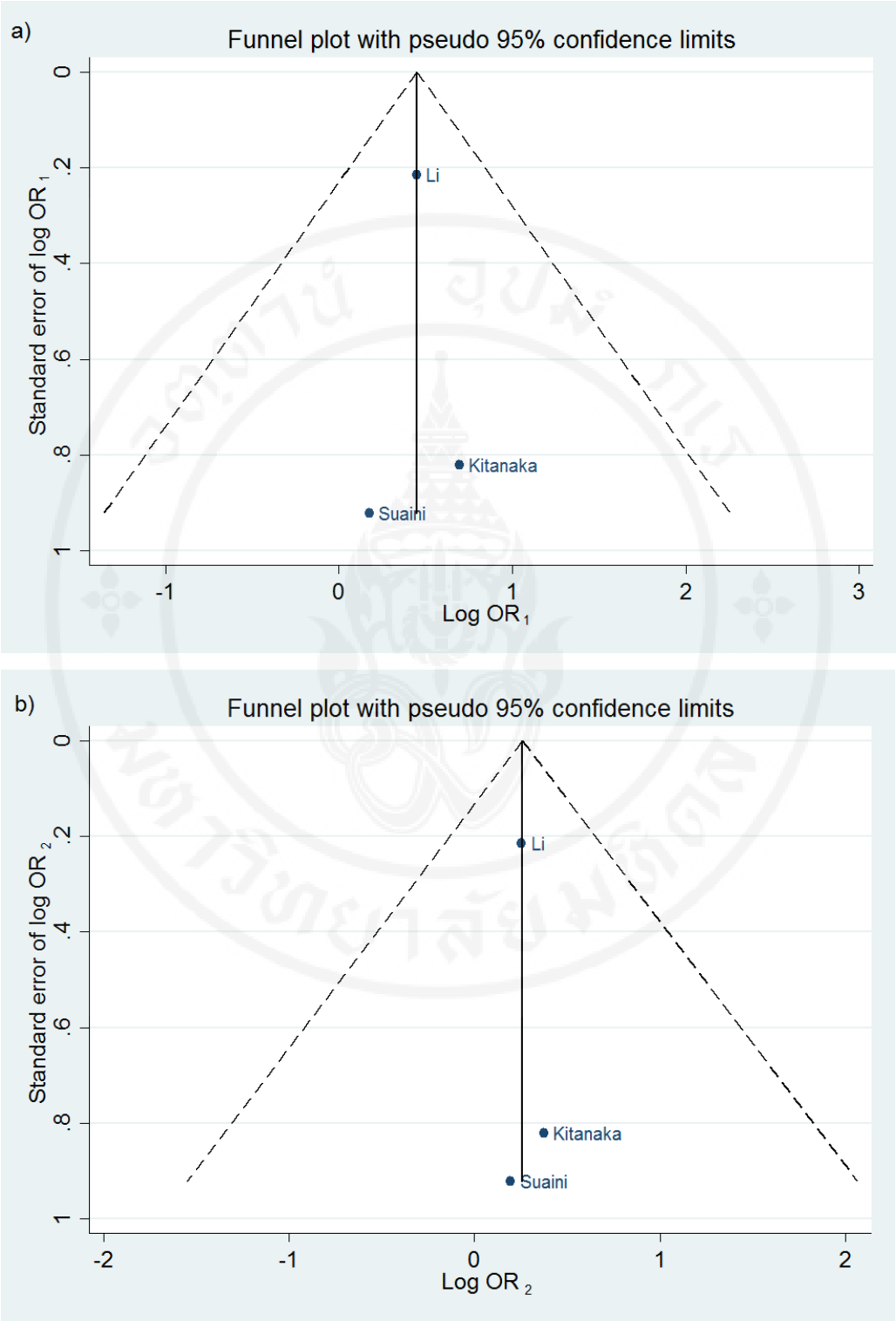

Figure 3.25 Funnel plots of rs2282679 genotype effect on vitamin D deficiency/insufficiency in Asians a) Log OR_1 (GG versus TT), b) Log OR_2 (TG versus TT)

CHAPTER IV
DISCUSSION

The pooled minor allele prevalence of the rs4588, was similar for Caucasians and Asians and did not differ much among adults and children; 0.232 (95% CI: 0.190, 0.274), 0.287 (95% CI: 0.278, 0.296), 0.281 (95% CI: 0.158, 0.404) and 0.293 (95% CI: 0.275, 0.312), for children Caucasian, adults Caucasian, children Asians and adults Asians respectively. The pooled minor allele prevalence of the rs7041, was similar for children Caucasian and children Asian, but it was twice as large among adult Caucasian compared to the adult Asians; 0.442 (95% CI: 0.401, 0.483), 0.436 (95% CI: 0.353, 0.519), 0.506 (95% CI: 0.049, 0.964) and 0.287 (95% CI: 0.258, 0.316), for children Caucasian, adults Caucasian, children Asians and adults Asians respectively. The pooled minor allele prevalence of the rs2282679 was similar for Caucasians and Asians and did not differ much among adults and children but it was remarkably small among the adult Africans; 0.279 (95% CI: 0.257, 0.300), 0.291 (95% CI: 0.263, 0.320), 0.285 (95% CI: 0.170, 0.400), 0.311 (95% CI: 0.226, 0.396) and 0.068 (95% CI: 0.047, 0.089), for children Caucasian, adults Caucasian, children Asians, adults Asians and adult Africans respectively, see Table 18. There were no studies that explored the prevalence of the SNPs rs4588 and rs7041 among Africans.

The GC gene polymorphism rs4588 produces a biochemically different form of vitamin D binding protein (DBP 2)[27]. This different form of the DBP may have lower affinities for binding with vitamin D[23]. Studies have also suggested that this different form of DBP may be metabolized faster and this causes faster metabolism of 25(OH)D and so results in a lower level of vitamin D in the blood[27]. This meta-analysis showed that there was a negative association between those who have the SNP rs4588 and the serum vitamin D level. This negative association was seen across all ages and found in both Caucasians and Asians, see Table 19. Those who have this SNP have 4.042 ng/ml lower levels of vitamin D (for adults Asian) and since vitamin D deficiency is defined as having vitamin D levels below 20ng/ml, a

Table 4.1 Summary of pooled prevalence

SNP	Ethnicity	Type of subjects	Pooled prevalence of the minor allele (95% CI)
rs4588	Caucasian	Children	0.232 (0.190, 0.274)
	Caucasian	Adult	0.287 (0.278, 0.296)
	Asian	Children	0.281 (0.158, 0.404)
	Asian	Adult	0.293 (0.275, 0.312)
rs7041	Caucasian	Children	0.442 (0.401, 0.483)
	Caucasian	Adult	0.436 (0.353, 0.519)
	Asian	Children	0.506 (0.049, 0.964)
	Asian	Adult	0.287 (0.258, 0.316)
rs2282679	Caucasian	Children	0.279 (0.257, 0.300)
	Caucasian	Adult	0.291 (0.263, 0.320)
	Asian	Children	0.285 (0.170, 0.400)
	Asian	Adult	0.311 (0.226, 0.396)
	African	Adult	0.068 (0.047, 0.089)

CI; confidence interval

Table 4.2 Summary of pooled mean difference

SNP	Ethnicity/Type of subject	Pooled MD_1 (95% CI)	Pooled MD_2 (95% CI)
rs4588	Caucasian children	-3.705 (-6.241, -1.169)	-2.211 (-2.930, -1.491)
	Caucasian adults	-3.619 (-4.978, -2.261)	-1.601 (-2.369, -0.833)
	Asian adults	-4.042(-5.621, -2.462)	-1.722 (-2.754, -0.69)
(Additive model)	Caucasian adults	-1.932 (-2.719, -1.146)	
	Asian adults	-0.03 (-0.037, 0.022)	
rs7041	Caucasians	-2.707 (-4.91, -0.504)	-1.407 (-2.203, -0.612)
(Additive model)	Caucasians	-0.023 (-0.031, -0.015)	
	Asians	-0.015 (-0.023, -0.008)	
rs2282679	Caucasian adults	-3.407 (-4.686, -2.127)	-1.673 (-2.471, -0.875)
(Additive model)	Caucasians	-0.037 (-0.046, -0.027)	
	Asians	-0.031 (-0.039, -0.023)	

CI; confidence interval, MD_1; mean difference between the homozygous risk genotype and the homozygous common genotype, MD_2; mean difference between the heterozygous genotype and the homozygous common genotype

lowering of vitamin D level by 4ng/ml could be clinically significant[41]. Fewer studies reported the additive model, but this too showed that there is a mild negative association between rs4588 and the serum vitamin D level. There was heterogeneity and the sources of this need to be explored to assess this. In this review sources of heterogeneity could not be assessed because there was no consistency in reporting the sources of heterogeneity across studies. The odds of developing vitamin D deficiency or insufficiency were greater in those who carried the SNP rs4588, although the odds were only increased slightly, see Tables 20-21. The assessment of publication bias among studies that reported vitamin D deficiency/insufficiency and vitamin D level showed that there was no publication bias.

The rs7041 SNP produces a different isoform of the DBP (DBP 1) which may have a lower affinity for binding with vitamin D and affect the delivery of Vitamin D to the tissues and DBP 1 may also be metabolized faster which in turn causes faster metabolism of 25(OH)D and subsequent lower levels of vitamin D in the blood[27 54]. This meta-analysis showed that those who had this SNP had 2.707 ng/ml

Table 4.3 Summary of Pooled allele effect of the SNP on vitamin D deficiency/insufficiency

SNP	Ethnicity	Pooled OR (95% CI)
rs4588	Caucasian	1.522 (1.336, 1.733)
	Asians	1.255 (1.058, 1.487)
rs7041	Caucasians	0.854 (0.487, 1.499)
	Asians	0.79 (0.662, 0.943)
rs2282679	Caucasians	1.397 (0.874, 2.234)
	Asians	1.272 (1.073, 1.507)

CI; confidence interval, OR; odds ratio between the risk allele and the common allele

lower levels of vitamin D. This negative association was seen in adults. There was only one study that reported the association in children. Fewer studies reported the additive model, but this too showed that there was a mild negative association between rs7041 and the serum vitamin D level. The assessment of the publication bias suggested none. The analysis of the association between the vitamin D deficiency/insufficiency and the SNP rs7041 showed a protective effect, that is, having

this SNP reduces the odds of developing vitamin D deficiency/insufficiency. Since having this SNP showed a negative association with the level of vitamin D, while at the same time the risk of having vitamin D deficiency/insufficiency is lowered, the association is giving a conflicting picture. Inability to explore the sources of heterogeneity, and the fewer number of studies could be the cause of this mixed picture of protective and harmful association. Publication bias assessment showed none.

Table 4.4 Summary of Pooled genotype effect of the SNP on vitamin D deficiency/insufficiency

SNP	Ethnicity	Pooled OR_1 (95% CI)	Pooled OR_2 (95% CI)
rs4588	Caucasian	2.207 (1.629, 2.99)	1.529 (1.28, 1.826)
	Asians	1.578 (1.06, 2.348)	1.251 (0.991, 1.58)
rs7041	Caucasians	0.761 (0.249, 2.329)	0.963 (0.509, 1.822)
	Asians	0.675 (0.452, 1.007)	0.746 (0.589, 0.946)
rs2282679	Caucasians	1.733 (0.788, 3.811)	1.507 (0.846, 2.686)
	Asians	1.569 (1.055, 2.332)	1.297 (1.028, 1.636)

CI; confidence interval, OR_1; odds ratio between the homozygous risk genotype and the homozygous common genotype, OR_2; odds ratio between the heterozygous genotype and the homozygous common genotype

This meta-analysis showed that those who had the SNP rs2282679 had 3.407 ng/ml lower levels of vitamin D (Caucasians), which suggests a negative association. There were not enough studies to assess this association among adults and children or among Asians. Studies which reported the additive model suggested a negative association as well but this association was very small and the publication bias for these studies suggested that there could be missing studies among the Asian studies.

The strength of this study was in the greater sample size (overall sample size (N) = 67652, rs4588 only (n = 7840), rs7041 only (n = 879), rs2282679 only (n = 34940), rs4588 and rs7041 (n = 6077), rs7041 and rs2282679 (n = 5844), rs4588 and rs2282679 (n = 5016), and (n = 7056) had all three SNPs. which gave a more reliable indication of the effect of these three SNPs on the levels of vitamin D. In addition, two

databases were searched for relevant studies and two reviewers selected the studies. Therefore selection bias was less likely occurred.

In this review, Hispanics were pooled along in with the Caucasians, but the some studies had treated these two ethnicities as different, similarly Middle Eastern populations were pooled along in with Africans and South Asians were pooled with South East Asians. These ethnicities might not have been similar enough to be pooled together, but since there were only few of these studies, the choice was to either not include them in the pooling or pool them together with other ethnicities.

Several potential confounders (e.g., mean age, mean BMI, gender, physical activity, alcohol consumption, vitamin D supplementation, kidney disease, liver disease, serum vitamin D level, sun exposure, and smoking) were collected from the studies, but only the mean age, mean BMI and gender were reported with any consistency. This made it difficult to assess for sources of heterogeneity. Some studies[27] showed that there may be seasonal variations to the level of vitamin D in the blood. This study did not take seasonal variations into account because this information was not available for all the studies. Future research needs to be more consistent in the collection of confounding factors.

In conclusion, the SNPs rs4588 and rs2282679 showed a negative association with the level of serum vitamin D and the development of vitamin D deficiency or insufficiency. The SNP rs7041 gave a mixed picture and its association was inconclusive. However more studies need to be done with a consistent method for collecting the potential confounding factors, especially environmental factors. More studies need to be done on other ethnicities (such as Hispanic, African, Mediterranean and Indian) to assess their association with vitamin D and these SNPs.

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APPENDICES

APPENDIX A

Search domains and search terms

Domain	Search terms
General domain	"allele"
	"genotype"
	"polymorphism"
	"polymorphisms"
	"SNPs"
	"single nucleotide polymorphisms"
Exposure	"rs4588"
	"rs7041"
	"rs2282679"
	"Thr420Lys"
	"Glu416Asp"
	"group specific component"
	"GC gene"
	"GC loci"
	"GC locus"
Outcome	"1, 25(OH)2D"
	"1, 25-dihydroxyvitamin D"
	"25(OH)D"
	"25 hydroxyvitamin D"
	"vitamin D"

APPENDIX B

Coding for the ineligibility criteria

	code	Definition
General	1	Does not have the full text of the articles available.
	2	Was not in English language.
	3	Was a narrative review or was not an observational studies (i.e. cohort, cross-sectional, or case-controls studies).
Population	4	Was not done on human subjects
Exposure	5	Did not study the effect of genotypes or alleles of any of the following SNPs of the group specific component (GC) gene; rs2282679, rs7041, or rs4588
Outcome	6	Did not measured the outcomes as mean serum 25 hydroxyvitamin D, or mean serum 1, 25 dihydroxyvitamin D levels for continuous outcomes, or vitamin D insufficiency or deficiency for dichotomous outcomes
	7	Did not have sufficient data for pooling a. For continuous outcome, reported the mean and standard deviation or the beta coefficients and the standard error of serum 25 hydroxyvitamin D or serum 1, 25 dihydroxyvitamin D levels between subjects who had or had not the interested SNPs. b. For categorical outcome; reported the contingency table of interested SNPs and vitamin D insufficiency or deficiency or the risk ratio and the 95% confidence interval or the odds ratio and the 95% confidence interval.

APPENDIX C

Data Extraction Form

Study ID _____

Data Extraction Form

Systematic review and meta-analysis of the association between vitamin D level and the single nucleotide polymorphisms, rs7041, rs4588, and rs2282679 of the group-specific component gene (GC).

Reviewer (name) ……..

Author: _____

Journal: _____

Year: _____ **Volume:** _____ **Pages:** _____

Type of Study Design [ ]

1) Cohort study
2) Case-control study
3) Cross-sectional study
8) Other
   If other, specify …….

Study age group [ ]

1) < 18 years 2) ≥ 18 years to <50 years 3) age ≥ 50 years 4) ≥ 18 years

Ethnicity [ ]

1) White
2) Black
3) Hispanic
4) Mediterranean
5) Indian
6) Asian
7) Mixed
8) Other

If other, specify ……

Country study was conducted in ……

Type of studied subjects [ ]

1) General subjects
2) Disease subjects specify ……..

Study factors

Single nucleotide polymorphisms (SNP)

Type of SNP

1) rs4588 [ ] 2) rs7041 [ ] 3) rs2282679 [ ]
Others_____

Method of genotyping
…….

Hardy-Weinberg Equilibrium (HWE) [ ]

1) Done
2) Not done
9) Unknown

Vitamin D supplement [ ]

1) Taken
2) Not taken
9) Unknown

Specify the amount taken, if given in the study
……
……
……
……
……
……

SNP data (tick in the brackets means yes)

[ ] Genotype data [ ] Haplotype

[ ] Allele data [ ] Interaction

Outcome

Continuous outcome [ ]

1) 25 (OH) D, (25, hydroxyvitamin D)
2) 1, 25 (OH)2 D, (1, 25 dihydroxyvitamin D)

Dichotomous outcome [ ]

1) Vitamin D deficient specify cut off
   ……
2) Vitamin D insufficient specify cut off
   ……

Method used to measure vitamin D
……

Baseline characteristics				
Mean Age				
Mean BMI				
%Male				
%Active Physical activity				
%Alcohol				
% vitamin D supplementation				
Kidney disease				
Liver disease				
Serum vitamin D level				
Sun exposure				
% smoking				

Categorical outcome

Group	Vitamin D deficiency [ ] Vitamin D insufficiency [ ]		OR	95%CI	
	Yes	No			
rs2282679 Genotype data					
TT					
GT					
GG					
Allele data					
T					
G					
rs7041 Genotype data					

GG					
TG					
TT					
Allele data					
G					
T					
rs4588 Genotype data					
CC					
AC					
AA					
Allele data					
C					
A					
*Used to denote the reference group					

group	n	mean	SD	Beta	SE	
rs2282679 Genotype data						
TT						
GT						
GG						

Allele data						
T						
G						
rs7041 Genotype data						
GG						
TG						
TT						
Allele data						
G						
T						
rs4588 Genotype data						

CC						
AC						
AA						
Allele data						
C						
A						

*Used to denote the reference group

APPENDIX D

Risk of bias form

Risk of bias assessment for genetic association studies of association between Vitamin D and the Single Nucleotide Polymorphisms, rs7041, rs4588, and rs2282679 of the group-specific complement gene (GC)

For case control study designs [39]

Domain	Item	How to grade	Low risk of bias
Selection bias	Representativeness of cases A. Consecutive/randomly with a random frame. B. Consecutive/randomly without a random frame. C. Not described.	 Consecutive/randomly selected from cases population with a clearly defined random frame. Consecutive/randomly selected from cases population without clearly defined random frame or with extensive inclusion criteria If the method of selection is not described.	 Yes Yes No
	Representativeness of controls D. Consecutive/randomly from same area E. Consecutive/randomly from different area F. Not describe	 Controls were consecutive/randomly drawn from same area (ward/community) as cases with the same criteria Controls were consecutively/randomly drawn from different areas as cases Not describe	Yes No No
	Differential participation in case and control A. Non-participant rate <10% B. Incomplete participant rates	 Non-participant rate is small (< 10%) and similar (to the rates) between case and control groups. The non-participant rate did not exceed 10% of the estimated sample size for the study. Incomplete participant rates are different - Refusal or inability to provide data - Refusal or inability to provide biological specimens - Insufficient amount quality of data/ quality of DNA	Yes No
Information bias	Ascertainment of vitamin D level		

	A. Method of vitamin D measurement and definition given. B. Not describe/unclear.	- If the method of vitamin D measurement was clearly given with a clear definition of vitamin D deficiency or insufficiency. - Not describe/unclear definition.	Yes No
	Ascertainment of control A. Method of measurement given. B. Only vitamin D level given. C. Not describe.	 - If the method of vitamin D measurement was clearly given. - Just mentioned the vitamin D levels of the controls. - Not describe.	 Yes No No
	Ascertainment of genotyping examination A. Genotyping done 'blind' for case and control groups. B. Genotyping error rate < 5%. C. Genotyping error arte not given. D. Quality control procedure if replicate sample. Analysis if replicate sample E. Not blinded. F. Not mention what was done G. No quality control check	 - Genotyping done under "blind" condition of case and control group's specimens - Genotyping error rate < 5%. - Genotyping error arte not given. - Quality control procedure e.g., reanalysis of random specimens, using different genotyping methods for analysis. Analysis if replicate sample. - Not blinded - Not mention what was done - No quality control check	 Yes Yes No Yes No No No
Confounding bias	Population stratification A. Same ethnicity of case and control B. Cases not related to controls C. Genomic controls D. Not report Other confounding bias E. Confounding variables reported F. Not controlled/not mentioned	 - No difference in ethnic origin between cases and controls - Use of controls who were not related to cases - Use of genomic controls - Not report what was done - Controls for at least 2 or 3 confounding variables (e.g., age, gender, obesity, physical activity, kidney disease, liver disease, sun exposure, alcohol intake, vitamin D supplement) in analysis - Not controlled /not mentioned (or, no control/ no mention)	 Yes Yes Yes No Yes No
Multiple testing	Multiple polymorphisms studied and multiple comparisons done. A. Adjusted for multiple	 - Adjustment for multiple tests such as for e.g., Bonferroni correction, Sidak correction or the Holm-	 Yes

	tests. B. No adjustments done. C. Regression analysis was done. D. Multiple pair wise comparison done for genetic model assessment. E. A genetic model was assumed and analyzed for many.	Bonferroni method has been done. - No adjustment for multiple testing was done - Regression analysis was done. - For genetic model assessment a multiple pair wise comparison was done. - A genetic model was assumed but analyzed for many models.	 No Yes Yes No
Selective reporting (for replication studies)	A. Report for all polymorphisms. B. Report selective	- Report results of all polymorphisms mentioned in objectives, non-significant or not - Report results of only significant polymorphisms	Yes No
HWE	A. has HWE B. has HWD C. not check HWE	- HWE in control group - HW disequilibrium in control group - Not check HWE	Yes No No
Yes=low/no risk of bias, No = possible/high risk of bias, or information is unclear or not given			

For cohort and cross-sectional study designs [39]			
Domain	Item	How to grade	Low risk of bias
Selection bias	Representativeness A. Consecutive/randomly with a random frame. B. Consecutive/randomly without a random frame. C. Not described.	Consecutive/randomly selected from target population with a clearly defined random frame. Consecutive/randomly selected from target population without clearly defined random frame or with extensive inclusion criteria If the method of selection is not described.	Yes Yes No
Information bias	Ascertainment of vitamin D level A. Method of vitamin D measurement and definition given B. Not describe/unclear	 - If the method of vitamin D measurement was clearly given with a clear definition of vitamin D deficiency or insufficiency. - Not describe/unclear definition	 Yes No
	Ascertainment of non-exposure group D. Method of measurement given	- If the method of vitamin D measurement was clearly given	Yes

	E. Only vitamin D level given F. Not describe	- Just mentioned the vitamin D levels of the controls - Not describe	No No
	Ascertainment of genotyping examination A. Genotyping done 'blind' for exposure and non-exposure groups. B. Genotyping error rate < 5%. C. Genotyping error arte not given. D. Quality control procedure if replicate sample. Analysis if replicate sample A. Not blinded. B. Not mention what was done C. No quality control check	- Genotyping done under "blind" condition of exposure and non-exposure group's specimens - Genotyping error rate < 5%. - Genotyping error arte not given. - Quality control procedure e.g., reanalysis of random specimens, using different genotyping methods for analysis. Analysis if replicate sample. - Not blinded - Not mention what was done - No quality control check	Yes Yes No Yes No No No
Confounding bias	Population stratification A. Same ethnicity of exposure and non-exposure groups. B. exposure group subjects not related to controls C. Genomic controls D. Not report Other confounding bias E. Confounding variables reported F. Not controlled/not mentioned	- No difference in ethnic origin between exposure and non-exposure groups. - Use of controls who were not related to exposure group subjects. - Use of genomic controls - Not report what was done - Controls for at least 2 or 3 confounding variables (e.g., age, gender, obesity, physical activity, kidney disease, liver disease, sun exposure, alcohol intake, vitamin D supplement) in analysis - Not controlled /not mentioned (or, no control/ no mention)	Yes Yes Yes No Yes No
Multiple testing	Multiple polymorphisms studied and multiple comparisons done. A. Adjusted for multiple tests. B. No adjustments done. C. Regression analysis was done. D. Multiple pair wise comparison done for	- Adjustment for multiple tests such as for e.g., Bonferroni correction, Sidak correction or the Holm-Bonferroni method has been done. - No adjustment for multiple testing was done	Yes No Yes

	genetic model assessment. E. A genetic model was assumed and analyzed for many.	- Regression analysis was done. - For genetic model assessment a multiple pair wise comparison was done. - A genetic model was assumed but analyzed for many models.	Yes No
Selective reporting (for replication studies)	A. Report for all polymorphisms. B. Report selective	- Report results of all polymorphisms mentioned in objectives, non-significant or not - Report results of only significant polymorphisms	Yes No
HWE	A. has HWE B. has HWD C. not check HWE	- HWE in control group - HW disequilibrium in control group - Not check HWE	Yes No No

Yes=low/no risk of bias, No = possible/high risk of bias, or information is unclear or not given

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