RESEARCH

CaSR gene A986S polymorphism contributes to the increased risk of primary hyperparathyroidism: A meta-analysis

Samrat Rakshit, [a] Lakkakuka Saikrishna,[b] and LVKS. Bhaskar[a]

[a] Department of Zoology, Guru Ghasidas Vishwavidyalaya, Bilaspur

[b] Department of Public health, Nellore Municipal Corporation, Nellore

*Corresponding author e-mail: lvksbhaskar@gmail.com

ABSTRACT

Primary hyperparathyroidism (PHPT) generally occurs due to mis-regulated secretion of parathyroid hormone. In humans, CaSR gene is responsible for calcium homeostasis, which regulates parathyroid hormone. By carefully evaluating published studies, the current meta-analysis assessed the association of *CaSR* gene R990G (rs1042636) and A986S (rs1801725) polymorphisms with the risk of primary hyperparathyroidism (PHPT). The meta-analysis includes five studies that focused on *CaSR* R990G and A986S polymorphisms. The effect size measures such as odds ratio (OR) and 95% confidence intervals (CI) were assessed for independent studies. The heterogeneity test showed no significant heterogeneity between studies; hence, pooled effects were assessed under fixed effect model. Meta-analysis of the *CaSR* polymorphisms demonstrated that only A986S polymorphism showed increased risk of PHPT in the dominant model (SS+AS vs. AA: OR = 1.40, 95% CI = 1.13-1.73, P = 0.002). Further, there is no evidence for publication bias for these polymorphisms. In conclusion, this meta-analysis supports that the *CaSR* A986S polymorphism correlates with an increased risk of PHPT.

KEYWORDS: Primary hyperparathyroidism, CaSR, R990G, A986S, rs1042636, rs1801725, Polymorphism, Meta-analysis.

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INTRODUCTION

Primary hyperparathyroidism (PHPT) generally occurs due to improper mineral metabolism, hypercalcemia and dis-regulated secretion of parathyroid hormone (PTH) (Corbetta et al., 2006, Han et al., 2013, Lakkakula & Neral, 2019). PHPT can also arise due to several familial endocrine disorders, such as multiple endocrine neoplasia type 1 and type 2A along with familial hyperparathyroidism. In western countries, PHPT is the third most common endocrine disease with 21.6 cases per 100,000 per-years. Almost 0.3 % of the general population and 1–3 % of the postmenopausal women were shown to have PHPT (Wang et al., 2016). Clinically, PHPT can cause neuromuscular disease, overt bone disease, hypercalcemia, nephrolithiasis and urolithiasis (Ghanta & Lakkakula, 2021). Increased serum PTH levels lead to increased serum calcium, which is an indication PHPT (Wang et al., 2016). It was well documented that PTH concentration in serum can be influenced by both genetic and environmental cues. Although irregular PTH concentration is genetically determined in 60% cases, genetic background of it is not yet completely known (Matana et al., 2018a; Matana et al., 2018b).

The human calcium sensing receptor (CaSR) gene is a G-protein coupled membrane receptor located on chromosome 3q13.3-21. *CaSR* has 8 exons with a coding region of 3234 base pairs (Vahe et al., 2017). In humans, the CaSR gene product is mainly localized in the distal kidney tubules and parathyroid glands. Upon activation, this protein inhibits PTH secretion and tubular calcium reabsorption to control serum calcium levels (Ding et al., 2017). Activating and inactivating mutations in the *CaSR* gene cause either hypo or hyper calciuria and calcemia, respectively. Though benign SNPs do not cause pathological phenotypes, but they can contribute to individual variability by influencing CaSR function (Rothe et al., 2008). In the parathyroid glands, binding of calcium to CaSR increased intracellular calcium via accumulation of inositol-1,4,5-phosphate through phospholipase C pathway. As, cyclic AMP gets reduced due to the inhibition of adenylate cyclase, secretion of PTH and subsequent gene expression is also reduced (Miedlich et al., 2001, Vezzoli et al., 2007). There are 3 clustered single nucleotide polymorphisms (SNPs) that have been identified in exon 7 of CaSR, i) A986S (Ala986Ser; rs1801725) protein variant where a guanine/thymine substitution occurred at codon 986; ii) R990G (Arg990Gly; rs1042636) protein variant where an adenine/guanine substitution occurred at codon 990; and iii) Q1011E (Gln1011Glu; rs1801726) protein variant where a cytosine/guanine substitution occurred at codon 1011 (Han et al., 2013, Liu et al., 2015). Previous reports suggested that the A986S and R990G polymorphisms are common in Caucasian and Asian populations, respectively (He et al., 2014). Several studies have analyzed the association between *CaSR* gene polymorphisms and PHPT, but the results are inconclusive. In the present study, a meta-analysis was carried out to assess the correlation between *CaSR* gene polymorphisms (R990G and A986S) and the risk of PHPT.

METHODS

Study design and search strategy

PubMed, Embase and GoogleScholar were searched to retrieve the papers related to *CaSR* gene polymorphisms and PHPT. Our search strategy included the following search terms or keywords: ("primary hyperparathyroidism" or "PHPT" or "hyperparathyroidism" or "pHPT"), ("calcium receptors" or "calcium-sensing receptor" or "CaSR" or "parathyroid calcium sensing

receptor"), rs1042636, rs1801725, CaSR R990G and CaSR A986S. Furthermore, same manual searches were carried out to retrieve potentially relevant studies from cross-references. Studies that met the following inclusion criteria were considered for meta-analysis: (1) study should have both case–control genotypes; (2) research should have been done to evaluate the correlation between CaSR gene polymorphisms and the risk of PHPT; (3) relevant complete data information: country, ethnicity, the number of cases for each genotype, and SNP site information must be available; (4) studies must have been published in English. Studies were directly rejected based on the following exclusion criteria: (1) case only studies; (2) unclear genotypic data; (3) studies involving unclear diagnostic criteria; (4) studies involving PHPT but other genes or SNPs.

Data extraction and Statistical analysis

By inclusion and exclusion criteria stated above, finally five studies were included in the meta-analysis (Miedlich et al., 2001, Cetani et al., 2002, Corbetta et al., 2006, Scillitani et al., 2007, Han et al., 2013). Two authors independently collected data from each study. To assess the relation between *CaSR* gene polymorphisms and PHPT risk, odds ratio (OR) and corresponding 95% confidence intervals (95% CI) were estimated. Forest plots were drawn using OR and 95% CI's of independent studies as well as pooled effects in the dominant model. Cochran's Q statistics and I^2 test were conducted to assess the heterogeneity among enrolled studies. To determine whether the results would be significantly influenced or not by deleting studies one by one, we implemented one-way sensitivity analysis. To confirm the reliability of original analysis results and to investigate publication bias, Begg's funnel plots and egger's linear regression test were conducted. Metagenyo web tool was used in the current meta-analysis (Martorell-Marugan et al., 2017).

RESULTS

Study characteristics

The selection process of articles for current meta-analysis is summarized in figure 1. After extensive search through different online databases, we identified total 37 records. After screening and evaluating their suitability using inclusion and exclusion criteria defined above, we selected 5 articles for meta-analysis. The present meta-analysis evaluated the association of *CaSR* gene R990G and A986S polymorphisms with the risk of PHPT. The genotype frequencies of *CaSR* R990G and A986S polymorphisms were documented in Table 1. The heterogeneity test showed no significant heterogeneity between studies of R990G (GG+RG Vs. RR: $P_{heterogeneity}$ = 0.381, I-squared = 4.5%) and A986S polymorphisms (SS+AS vs. AA: $P_{heterogeneity}$ = 0.605, I-squared = 0%).

CaSR polymorphisms and PHPT susceptibility

The association of *CaSR* R990G and A986S polymorphisms and the risk of PHPT was reported in five studies. The association for each individual study as well as the pooled effects for these polymorphisms are presented in Figure 2. Meta-analysis of the *CaSR* R990G polymorphism demonstrated no significant association with PHPT in the dominant genetic model (GG+RG Vs. RR: OR=0.82; 95% CI= 0.60-1.11; p=0.199) (Table 2). For *CaSR* A986S polymorphism, the pooled odds ratio for mutant genotypes in the dominant model showed increased risk of PHPT (SS+AS vs. AA: OR = 1.40, 95% CI = 1.13-1.73, P = 0.002).

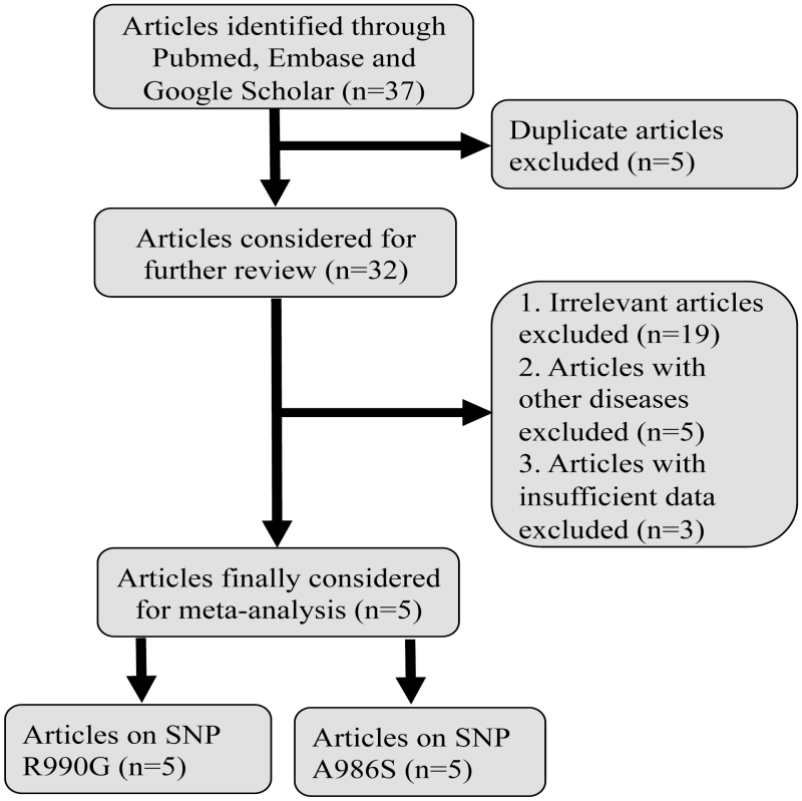

Figure 1: Flowchart of literature search and study selection.

Table 1: The distribution of CaSR gene R990G and A986S polymorphisms and risk of PHPT

Reference	Country	Ethnicity	PHPT			Controls			HW p value
CaSR R990G polymorphism			AA	AG	GG	AA	AG	GG	
Miedlich et al. 2001	Germany	Caucasian	46	4	0	102	11	3	0.001
Cetani et al. 2002	Italy	Caucasian	146	7	0	182	16	0	0.554
Corbetta et al. 2006	Italy	Caucasian	83	9	2	128	8	1	0.047
Scillitani et al. 2007	Rome	Caucasian	217	19	0	391	41	0	0.301
Han et al. 2013	China	Asian	44	93	27	50	119	61	0.573
CaSR A986S polymorphism			GG	GT	TT	GG	GT	TT	
Miedlich et al. 2001	Germany	Caucasian	30	20	0	73	26	3	0.714
Cetani et al. 2002	Italy	Caucasian	92	61	0	140	59	0	0.014
Corbetta et al. 2006	Italy	Caucasian	58	28	8	91	42	4	0.748
Scillitani et al. 2007	Rome	Caucasian	133	81	22	282	130	20	0.319
Han et al. 2013	China	Asian	215	15	0	151	12	1	0.180

Table 2: Results of the meta-analysis of CaSR R990G and A986S polymorphisms and PHPT.		
	CaSR R990G	CaSR A986S
Dominant Model	GG+RG Vs. RR	SS+AS vs. AA
Number of studies	5	5
I^2 %	4.5%	0
Heterogeneity p value	0.381	0.605
OR	0.82	1.40
95% CI	(0.60-1.11)	(1.13-1.73)
Association p value	0.199	0.002
Egger's test p-value	0.831	0.391

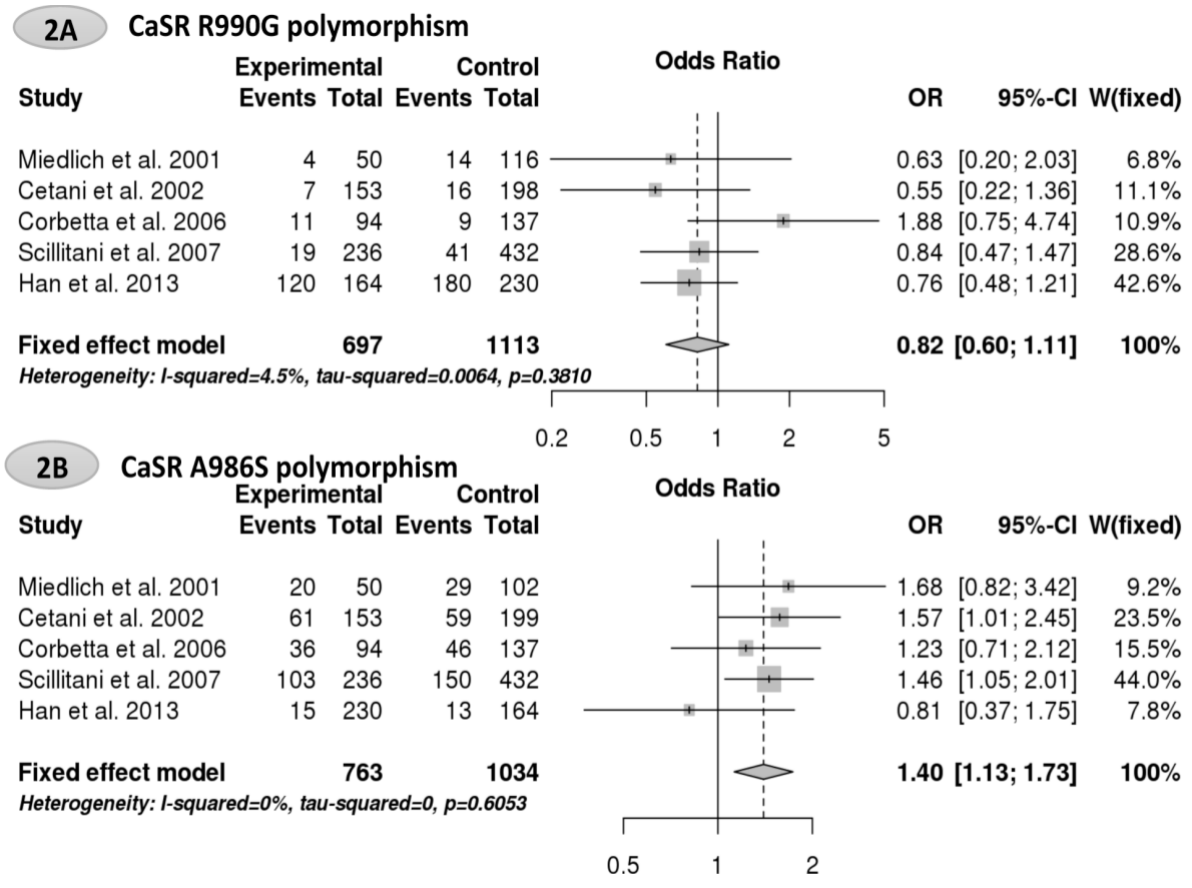

Figure 2. Forest plot of summarized results of meta-analysis.

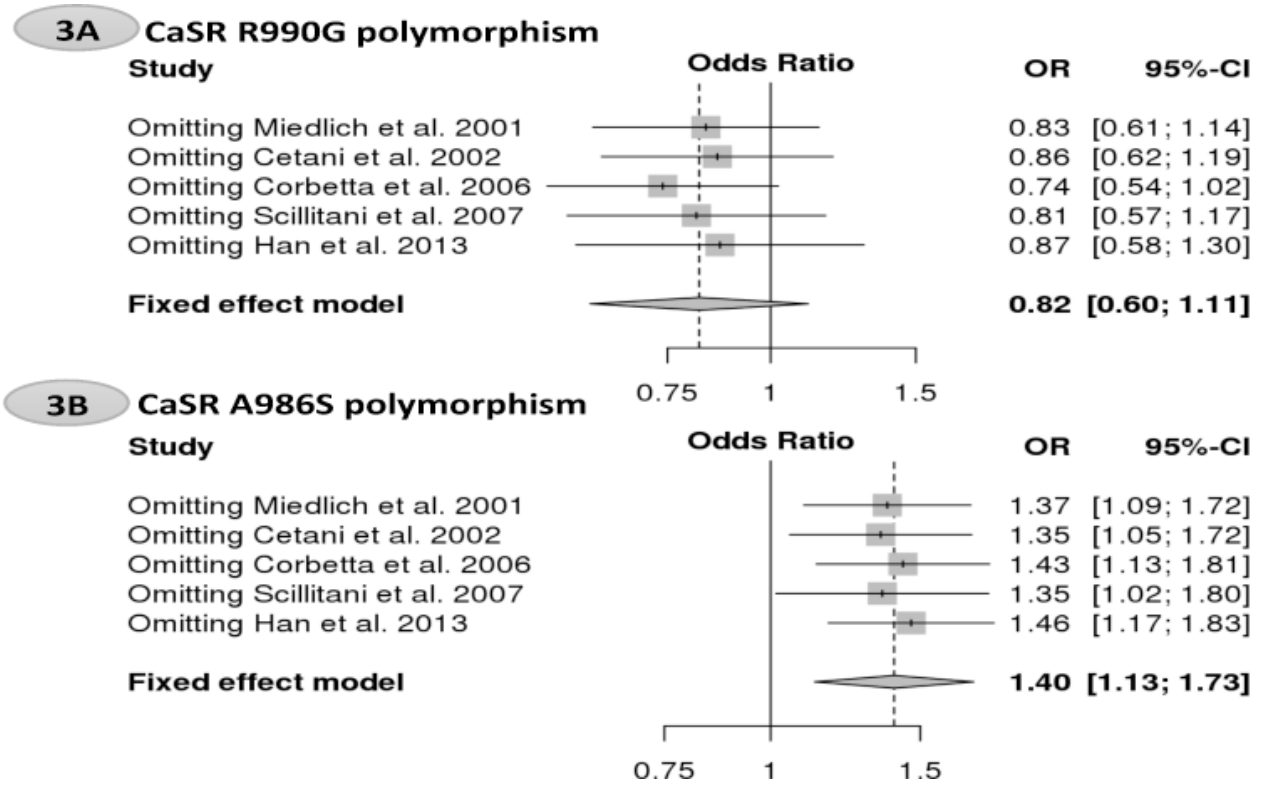

Figure 3. Pooled OR of sensitivity analysis.

Sensitivity analysis and publication bias

Each time by excluding one study, we performed sensitivity analysis to check robustness of the analysis. Sensitivity analysis demonstrated no significant changes in the pooled OR of both *CaSR* R990G and A986S polymorphisms (Figure 3), which indicated the statistical robustness of the results. Symmetry in the shape of the Begg's funnel plot for both CaSR R990G and A986S polymorphisms (Figure 4) indicated no publication bias. Egger's test further supported that there was no publication bias for both CaSR R990G (p=0.831) and A986S (0.391) polymorphisms.

DISCUSSION

The current meta-analysis was performed to investigate the correlations between *CaSR* gene polymorphisms (R990G and A986S) and the risk of PHPT. For the present study, we have collected data from five different studies published on Asian and Caucasian populations. For R990G polymorphism, 697 PHPT and 1113 controls met the inclusion criteria, and for A986S polymorphism, 763 PHPT patients with and 1034 controls met the inclusion criteria. The data obtained from the current meta-analysis suggested that the *CaSR* R990G polymorphism is not associated with the risk of PHPT. In contrast to this, *CaSR* A986S polymorphism increased the risk of PHPT. Hence, it could be an important biological marker for early diagnosis of PHPT.

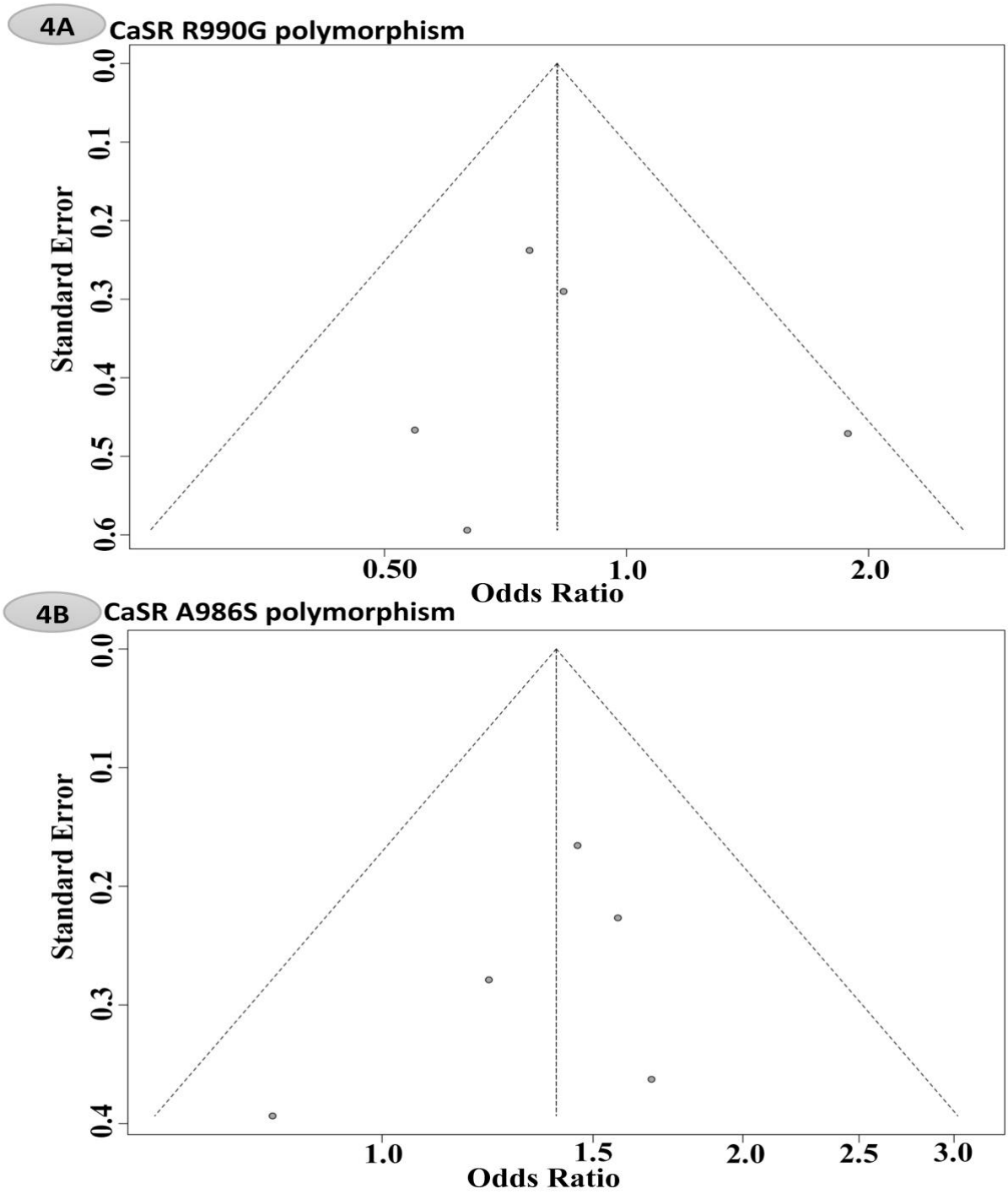

Figure 4. Begg's funnel plot for publication bias.

CaSR, a multifunctional receptor is found mainly in the tissues related to calcium homeostasis, but can be found in brain, pancreas, esophagus, stomach, heart, skin, lens epithelium, pituitary gland, ovary, breast, testis and prostate (Magno et al., 2011, Wang et al., 2016). Several lines of evidence demonstrated that the calcium-sensing receptor variants are known to influence the serum calcium

concentration (O'Seaghdha et al., 2010, Majumdar et al., 2020). Other than calcium homeostasis, *CaSR* gene also involves in several biological processes, such as cytoskeletal organization, entero-endocrine secretion, various ion channel activities, ion transport regulation, gene expression control and cell fate. By regulating PTH secretion and renal tubular calcium reabsorption, CaSR maintains calcium homeostasis (Breitwieser, 2012, Wang et al., 2016). Several other disorders were observed after loss-of-function mutations in *CaSR* gene, such as autosomal dominant familial hypocalciuric hypercalcemia, characterized by elevated parathyroid (PTH) levels with increased bone turnover (Wang et al., 2016). General populations significantly differ from PHPT patients in terms of the distributions of SNPs in the *CaSR* gene. Studies on *CaSR* R990G polymorphism indicated that this polymorphism is not associated the increased risk of PHPT (Miedlich et al., 2001, Cetani et al., 2002, Scillitani et al., 2007, Han et al., 2013). However, this polymorphism showed increased risk of PHPT and associated with disease parameters of PHPT (Corbetta et al., 2006). Studies related to *CaSR* R990G polymorphism showed that this polymorphism showed correlation with elevated risk of PHPT (Miedlich et al., 2001, Cetani et al., 2002, Corbetta et al., 2006, Scillitani et al., 2007). In contrast with this, there is no association between this polymorphism and PHPT in Chinese patients (Han et al., 2013).

We would like to acknowledge the limitations of our meta-analysis. Relatively small sample size in some studies may reduce the reliability of our conclusions. We did not consider studies published in languages other than English. The individual studies used in the meta-analysis are relatively small. Therefore, a greater number of studies with larger sample size and accurate genotyping method would provide a more reliable statistical analysis. In summary, this meta-analysis supports that the CaSR A986S polymorphism increases the risk of PHPT. This polymorphism in the CaSR gene can be beneficial and serve as a marker for the diagnosis of PHPT.

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Conflict of interest statement

The authors have declared to have no conflict of interest.

Authors' contributions

SR, LS performed literature search; SR, LS and BLVKS performed meta-analysis; SR, LS wrote the first draft; BLVKS critically revised the manuscript and all authors approved the final draft.

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Declaration of originality

The author declares that the work presented in this manuscript is original and no text/figure has been copied from elsewhere without appropriate citation.

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