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Original article

The associations between CYP24A1 polymorphisms and cancer susceptibility: A meta-analysis and trial sequential analysis

Man Zhu[a,1], Shili Qiu[a,1], Xianwei Zhang[a,1], Yingchao Wang[a], Tapara D.M. Souraka[a], Xue Wen[b], Chunzi Liang[a,*], Jiancheng Tu[a,*]

[a] Department of Clinical Laboratory Medicine and Center for Gene Diagnosis, Zhongnan Hospital of Wuhan University, Wuhan, Hubei 430071, PR China
[b] Center of Reproductive Medicine, Zhongnan Hospital of Wuhan University, Wuhan, Hubei 430071, PR China

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ABSTRACT

Purpose: Published data have shown that vitamin D may have a protective effect on cancer development. CYP24A1, the main enzyme responsible for the degradation of active vitamin D, plays an important role in many cancer related cellular processes. Up to now, relationships between CYP24A1 polymorphisms and cancer susceptibility have been widely investigated, whereas the results are inconsistent. The aim of present meta-analysis was to explore the associations between CYP24A1 polymorphisms and cancer susceptibility.

Methods: We searched on EMBASE, Web of Science, PubMed and China National Knowledge Infrastructure (CNKI) electronic databases (up to July 1, 2017) for relevant studies. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to make the evaluation clear.

Results: Twenty-nine studies published in eight publications involving 20,593 cases and 25,458 controls were included. Five CYP24A1 gene polymorphisms were evaluated: rs2181874, rs2585428, rs4809960, rs6022999, and rs6068816. Our analyses suggested that rs2585428 and rs4809960 polymorphisms were significantly associated with overall cancer risk. Stratification analyses of ethnicity indicated that rs2585428 and rs4809960 polymorphisms decreased the risk of cancer among Caucasians. When studies were stratified by cancer type, our results indicated that rs2585428 significantly decreased the risk of pancreas cancer, while rs4809960 significantly decreased the risk of breast cancer. There were no associations of rs2181874, rs6022999, or rs6068816 with overall cancer risks.

Conclusion: Associations between CYP24A1 polymorphisms and cancer risks were examined, and additional multi-center studies with large samples are necessary to validate our results.

1. Introduction

Cancer is still a major public health problem. It was estimated that there were approximately 14 million new cancer cases and 8 million deaths occurred in 2012 worldwide [1]. As a multifactorial disease, various etiologies involving multiple environmental and genetic factors contribute to cancer's development. In addition, genetic factors play important roles in carcinogenesis, and many genes have been described as cancer-susceptible genes [2], although the exact mechanism of carcinogenesis has not been fully understood.

Vitamin D, from sun exposure (accounting for up to 90%) and diet, was found to be associated with reduced risk of several cancers, including colorectal cancer, prostate cancer and breast cancer. It has become increasingly clear that vitamin D not only has a function in bone metabolism, but it also has a protective effect against malignant neoplasms due to its role in regulating cell differentiation, proliferation and apoptosis [3,4]. These biological functions demonstrated that vitamin D might be treated as an ideal therapeutic agent to resist the development of malignancy. The serum 25-hydroxyvitamin D (25(OH)D) is a widely accepted biomarker of vitamin D status. Up to now, a number of studies have been published and implied a possible association between serum 25(OH)D and cancer risk. Unfortunately, some studies have presented contradictory results. For instance, Stolzenberg et al. [5] indicated that high levels of circulating 25(OH)D was significantly associated with a high risk for pancreas cancer. However, Wolpin et al. [6] found an inverse association between 25(OH)D and

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval; 25(OH)D, 25-hydroxyvitamin D; GWAS, genome-wide association study; CNKI, China National Knowledge infrastructure; HWE, Hardy-Weinberg Equilibrium; TSA, trial sequential analysis; ER, estrogen receptor

* Corresponding authors.
E-mail addresses: cliang1873@outlook.com (C. Liang), jianchengtu@whu.edu.cn (J. Tu).
[1] These authors contributed equally to this work.

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pancreas cancer. Besides, such contradictions also existed in some other studies [7–9]. The possible reason is that serum 25(OH)D levels may not correspond to vitamin D exposure levels.

Several genes are involved in vitamin D metabolism. 1α-hydroxylase (encoded by CYP27B1 gene) converts 25(OH)D to $1,25(OH)_2D_3$ in the kidney, then it will be released into the blood circulation. $1,25(OH)_2D_3$ plays an important role in the regulation of cell functions and metabolic pathway. Finally, circulating 25(OH)D and $1,25(OH)_2D_3$ are degraded by 25-hydroxyvitamin D 24-hydrolase (encoded by CYP24A1 gene). It is evident that CYP24A1 is the main enzyme responsible for the degradation of vitamin D. Of note, the relationship between the mRNA expression levels of CYP24A1 and cancer risk has been investigated by some researchers in depth. Zhalehjoo et al. [10] demonstrated that the expression of CYP24A1 was significantly up-regulated in breast cancer. Moreover, Bokhari et al. [11] found that endometrial cancer expressed higher levels of CYP24A1 than normal tissues. Therefore, we believe that CYP24A1 may possess potential clinical value in cancer.

Recently, genome-wide association studies (GWASs) have identified CYP24A1 polymorphisms significantly associated with 25(OH)D concentrations. Up to now, five common CYP24A1 SNPs, rs2181874, rs2585428, rs4809960, rs6022999 and rs6068816, were found to be associated with cancer risks, including prostate cancer, breast cancer, colon cancer and pancreas cancer. However, the results are inconsistent, possibly because of limited sample sizes. To better explore the precise relationship, we performed a meta-analysis using currently published data to characterize the associations of rs2181874, rs2585428, rs4809960, rs6022999 and rs6068816 in CYP24A1 with cancer risks.

2. Material and methods

2.1. Literature search

We systematically searched on EMBASE, Web of Science, PubMed and China National Knowledge Infrastructure (CNKI) electronic databases (up to July 1, 2017) for relevant studies exploring the relationships between CYP24A1 polymorphisms and cancer risks. The detailed search strategy is described in Supplementary Table 1. The literature covered was limited to human. Three independent authors (Shili Qiu, Xianwei Zhang and Xue Wen) conducted the search. Finally, we also searched the references lists of all retrieved articles for potential studies manually.

2.2. Inclusion and exclusion criteria

Studies were included if they met the following criteria: (1) case-control/cohort studies; (2) investigating the associations between CYP24A1 polymorphisms (at least one of the five polymorphisms) and cancer risks; (3) providing sufficient data to calculate the OR and 95% CI, and *P* value; (4) genotype frequencies in controls were in agreement with Hardy-Weinberg Equilibrium (HWE). In addition, the exclusion criteria were: (1) non-human research; (2) not concerned with cancer risk; (3) did not study CYP24A1 polymorphisms (rs2181874, rs2585428, rs4809960, rs6022999 or rs6068816); (4) only a case population.

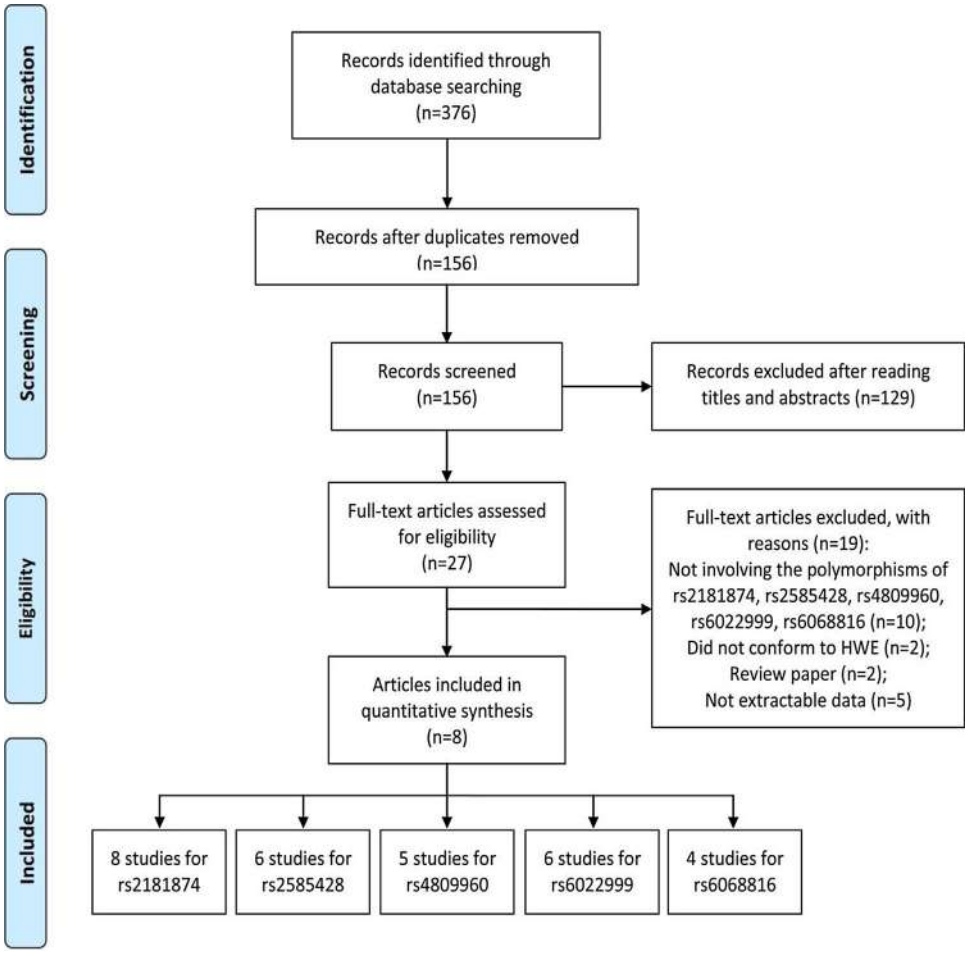

Fig. 1. Flow chart of the process for study identification and selection.

2.3. Data extraction and quality assessment

Two authors (Man Zhu and Shili Qiu) independently reviewed and extracted the detailed information from all eligible studies. The following information was collected: surname of first author, publication year, country of origin, ethnicity, cancer type, source of controls, genotyping method, genotype counts of cases and controls. The quality of each eligible study was assessed by two authors (Man Zhu and Shili Qiu) based on the quality assessment criteria (Supplementary Table 2). The modified criteria cover the ascertainment of cancer, the representativeness of case, the credibility of control, genotyping examination, control selection and total sample size. Total quality scores ranged from 0 to 12. A score ranging 9–12 points is classified as high quality.

2.4. Statistical analysis

All analyses were performed using the Stata software, version 12.0 (Stata, College Station, TX, USA). The strength of associations between CYP24A1 polymorphisms and cancer risks were estimated by OR and 95% CI. We measured the associations based on five different genetic models: dominant (BB + BA vs. AA) model, recessive (BB vs. BA + AA) model, homozygote (BB vs. AA) model, heterozygote (BB vs. BA) model, and allele (B vs. A) model (A: wild type allele; B: mutated type allele). Statistical heterogeneity among studies was assessed using Cochrane Q-test and P-values. If heterogeneity was present ($P \leq 0.10$ or $I^2 \geq 50\%$), random-effect model was used. If not, the fixed-effect model was more appropriate. Stratification analyses were performed by ethnicity, cancer type and genotyping method. Sensitivity analyses were conducted by sequentially excluding a study at each time to evaluate the stability of the overall results. Publication bias was evaluated using the funnel plot with Begg's test and Egger's test. A P value < 0.5 was considered statistically significant.

2.5. Trial sequential analysis (TSA)

Systematic or random errors can mislead results in meta-analyses. The risk of these errors may also increase remarkably due to sparse data and repeated significance testing. To obtain more comprehensive results, trial sequential analysis (TSA, Copenhagen Trial Unit, Center for Clinical Intervention Research, Denmark, 2011) was introduced in our meta-analysis. TSA is used to estimate the required sample size by adjusting threshold for significance level with sparse data and to confirm statistical reliability of systematic review and meta-analysis. In our study, TSA was performed by setting an overall type–I error of 5%, a statistical test power of 80% and a 20% relative risk reduction.

3. Results

3.1. Characteristics of the eligible studies

A total of 376 articles were preliminarily identified after our initial search. After removing duplicates and scanning the abstracts, there were 27 articles that conformed to our inclusion criteria. The following

Table 1
Characteristics of included studies.

First Author	Year	Country	Ethnicity	Cancer type	Control source	Genotyping method	Cases (AA/AB/BB)	Controls (AA/AB/BB)	HWE	Score
rs2181874										
Anderson	2011	Canada	Caucasian	Breast cancer	PB	PCR-RFLP	869/584/98	959/575/93	0.579	9
Holick	2007	USA	Caucasian	Prostate cancer	PB	SNPlex assay	339/199/36	314/201/36	0.617	10
Holt-1	2009	USA	Caucasian	Prostate cancer	PB	PCR-RFLP	406/249/48	400/273/41	0.531	12
Holt-2	2009	USA	African	Prostate cancer	PB	PCR-RFLP	37/56/22	22/31/14	0.616	10
Dong	2009	Mixed	Caucasian	Colon cancer	PB	iPLEX Gold	921/576/98	1105/713/123	0.579	11
Reimers	2015	USA	Caucasian	Breast cancer	PB	TaqMan	508/359/79	560/366/66	0.555	9
Anderson	2013	Canada	Caucasian	Pancreas cancer	PB	iPLEX Gold	338/239/51	665/460/68	0.320	9
Clendenen	2015	Sweden	Caucasian	Breast cancer	PB	Illumina	486/217/30	888/470/75	0.216	10
rs2585428										
Yao	2012	USA	Caucasian	Breast cancer	PB	Illumina	26/22/11	90/175/105	0.313	9
Holick	2007	USA	Caucasian	Prostate cancer	PB	SNPlex assay	186/283/114	161/258/125	0.270	10
Holt-1	2009	USA	Caucasian	Prostate cancer	PB	PCR-RFLP	218/348/132	203/353/141	0.579	12
Holt-2	2009	USA	African	Prostate cancer	PB	PCR-RFLP	33/48/33	19/29/15	0.548	10
Reimers	2015	USA	Caucasian	Breast cancer	PB	TaqMan	264/442/243	261/487/243	0.596	9
Anderson	2013	Canada	Caucasian	Pancreas cancer	PB	iPLEX Gold	212/278/137	346/578/264	0.443	9
rs4809960										
Holick	2007	USA	Caucasian	Prostate cancer	PB	SNPlex assay	329/230/27	323/184/37	0.129	10
Holt-1	2009	USA	Caucasian	Prostate cancer	PB	PCR-RFLP	432/220/45	387/260/46	0.794	12
Holt-2	2009	USA	African	Prostate cancer	PB	PCR-RFLP	93/18/1	46/17/0	0.216	10
Reimers	2015	USA	Caucasian	Breast cancer	PB	TaqMan	522/342/84	512/395/82	0.637	9
Clendenen	2015	Sweden	Caucasian	Breast cancer	PB	Illumina	479/218/36	861/496/76	0.679	10
rs6022999										
Holick	2007	USA	Caucasian	Prostate cancer	PB	SNPlex assay	324/225/37	298/208/42	0.498	10
Holt-1	2009	USA	Caucasian	Prostate cancer	PB	PCR-RFLP	413/253/41	419/266/32	0.208	12
Holt-2	2009	USA	African	Prostate cancer	PB	PCR-RFLP	23/47/45	7/32/28	0.627	10
Dong	2009	Mixed	Caucasian	Colon cancer	PB	iPLEX Gold	933/538/120	1112/692/128	0.150	11
Reimers	2015	USA	Caucasian	Breast cancer	PB	TaqMan	494/366/86	554/361/73	0.184	9
Clendenen	2015	Sweden	Caucasian	Breast cancer	PB	Illumina	479/229/26	888/481/66	0.933	10
rs6068816										
Holick	2007	USA	Caucasian	Prostate cancer	PB	SNPlex assay	454/118/11	443/93/8	0.227	10
Holt-1	2009	USA	Caucasian	Prostate cancer	PB	PCR-RFLP	558/135/6	580/127/5	0.493	12
Reimers	2015	USA	Caucasian	Breast cancer	PB	TaqMan	778/164/6	784/189/17	0.158	9
Clendenen	2015	Sweden	Caucasian	Breast cancer	PB	Illumina	590/136/7	1149/264/19	0.389	10

Abbreviation: PB: publication-based controls; HWE, Hardy-Weinberg Equilibrium; A: wild type; B: mutated type.

Table 2
Meta-analysis of associations between the rs2585428 polymorphism and cancer risk.

Comparison	Overall and Stratification analyses	Studies	OR (95% CI)	*P*-value	Random/Fixed effect model	*P* for heterogeneity	I^2 (%)
AA + AG vs. GG	Overall	6	**0.86(0.78, 0.96)**	**0.007**	Fixed	0.141	39.6
	Caucasian	5	**0.84(0.71, 0.98)**	**0.026**	Random	0.095	49.4
	African	1	1.06(0.54, 2.08)	0.865	Fixed	–	–
	Prostate cancer	3	0.91(0.77, 1.07)	0.262	Fixed	0.900	0
	Breast cancer	2	0.64(0.29, 1.43)	0.280	Random	0.007	86.1
	Pancreas cancer	1	**0.80(0.65, 0.99)**	**0.040**	Fixed	–	–
	PCR-RFLP	2	0.92(0.74, 1.14)	0.449	Fixed	0.663	0
	Other methods	4	0.81(0.62, 1.03)	0.086	Random	0.055	60.6
AA vs. AG + GG	Overall	6	0.95(0.85, 1.07)	0.425	Fixed	0.411	0.8
	Caucasian	5	0.94(0.84, 1.06)	0.350	Fixed	0.370	6.5
	African	1	1.30(0.64, 2.64)	0.462	Fixed	–	–
	Prostate cancer	3	0.90(0.74, 1.08)	0.309	Fixed	0.463	0
	Breast cancer	2	0.86(0.49, 1.51)	0.600	Random	0.101	62.9
	Pancreas cancer	1	0.98(0.78, 1.24)	0.856	Fixed	–	–
	PCR-RFLP	2	0.96(0.75, 1.23)	0.751	Fixed	0.365	0
	Other methods	4	0.95(0.83, 1.09)	0.463	Fixed	0.239	28.9
AA vs. GG	Overall	6	**0.87(0.76, 0.99)**	**0.038**	Fixed	0.200	31.4
	Caucasian	5	**0.86(0.75, 0.98)**	**0.028**	Fixed	0.166	38.2
	African	1	1.27(0.55, 2.91)	0.577	Fixed	–	–
	Prostate cancer	3	0.86(0.69, 1.06)	0.161	Fixed	0.577	0
	Breast cancer	2	0.64(0.24, 1.70)	0.370	Random	0.014	83.5
	Pancreas cancer	1	0.85(0.65, 1.11)	0.224	Fixed	–	–
	PCR-RFLP	2	0.91(0.68, 1.21)	0.525	Fixed	0.408	0
	Other methods	4	0.81(0.63, 1.04)	0.101	Random	0.091	53.6
AA vs. AG	Overall	6	1.01(0.89, 1.15)	0.857	Fixed	0.645	0
	Caucasian	5	1.00(0.88, 1.14)	0.952	Fixed	0.582	0
	African	1	1.33(0.62, 2.86)	0.466	Fixed	–	–
	Prostate cancer	3	0.92(0.75, 1.12)	0.401	Fixed	0.506	0
	Breast cancer	2	1.08(0.87, 1.33)	0.485	Fixed	0.491	0
	Pancreas cancer	1	1.08(0.84, 1.39)	0.533	Fixed	–	–
	PCR-RFLP	2	0.99(0.76, 1.29)	0.932	Fixed	0.418	0
	Other methods	4	1.02(0.88, 1.17)	0.801	Fixed	0.446	0
A vs. G	Overall	6	**0.92(0.86, 0.99)**	**0.026**	Fixed	0.112	44.1
	Caucasian	5	**0.90(0.81, 1.00)**	**0.045**	Random	0.089	50.4
	African	1	1.14(0.74, 1.76)	0.567	Fixed	–	–
	Prostate cancer	3	0.93(0.83, 1.03)	0.162	Fixed	0.588	0
	Breast cancer	2	0.76(0.43, 1.36)	0.355	Random	0.006	87.0
	Pancreas cancer	1	0.90(0.79, 1.04)	0.146	Fixed	–	–
	PCR-RFLP	2	0.95(0.83, 1.10)	0.500	Fixed	0.403	0
	Other methods	4	0.88(0.77, 1.02)	0.087	Random	0.046	62.6

Abbreviation: OR: Odds ratio; CI: Confidence interval. Bold values are statistically significant ($P < 0.05$).

articles were excluded: ten publications that did not describe CYP24A1 polymorphisms (rs2181874, rs2585428, rs4809960, rs6022999 and rs6068816) and cancer risk, two that did not conform to HWE [12,13], two were review papers [14,15], and five that not provide detailed genotyping data [16–20]. Finally, we identified eight eligible publications [21–28] including 29 studies (20,593 cases and 25,458 controls) in our meta-analysis. Fig. 1 describes the specific search process for our study. Among these studies, 25 studies were carried out among Caucasian populations and four studies were carried out among African populations. Fourteen studies reported the effects of CYP24A1 polymorphisms in prostate cancer, eleven reported in breast cancer, two in colon cancer and two in pancreas cancer. The quality scores of all included studies ranged from 9 to 12 points, suggesting that they were studies of high quality. The baseline characteristics of these included studies are summarized in Table 1.

3.2. Meta-analysis of rs2585428

Five publications including six studies with 3030 cases and 3853 controls examined rs2585428 polymorphism. As shown in Table 2, we found that rs2585428 polymorphism significantly decreased cancer risk in three models: dominant (AA + AG vs. GG, OR = 0.86, 95% CI = 0.78–0.96, $P = 0.007$, Fig. 2a), homozygote (AA vs. GG, OR = 0.87, 95% CI = 0.76–0.99, $P = 0.038$), and allele (A vs. G, OR = 0.92, 95% CI = 0.86–0.99, $P = 0.026$) models. Stratification analyses were conducted according to cancer type, ethnicity and genotyping method. Our data indicated that rs2585428 polymorphism significantly decreased cancer risk in Caucasians (AA + AG vs. GG, OR = 0.84, 95% CI = 0.71–0.98, $P = 0.026$; AA vs. GG, OR = 0.86, 95% CI = 0.75–0.98, $P = 0.028$, Fig. 2b; A vs. G, OR = 0.90, 95% CI = 0.81–1.00, $P = 0.045$), but not in Africans. When studies were stratified in to cancer type, significant associations were found in pancreas cancer, but not in prostate cancer and breast cancer. However, stratification analyses of genotyping method suggested rs2585428 was not related with the risks of PCR-RFLP and other genotyping methods.

Outcomes of trial sequential analysis (TSA) were concordant with our results. As shown in Fig. 4a, although the number of cases did not exceed the required information size (O'Brien-Fleming boundary), the cumulative Z-curve surpassed the trial sequential monitoring boundary, which verified the reliability of our results and revealed that rs2585428 polymorphism was significantly associated with cancer risk.

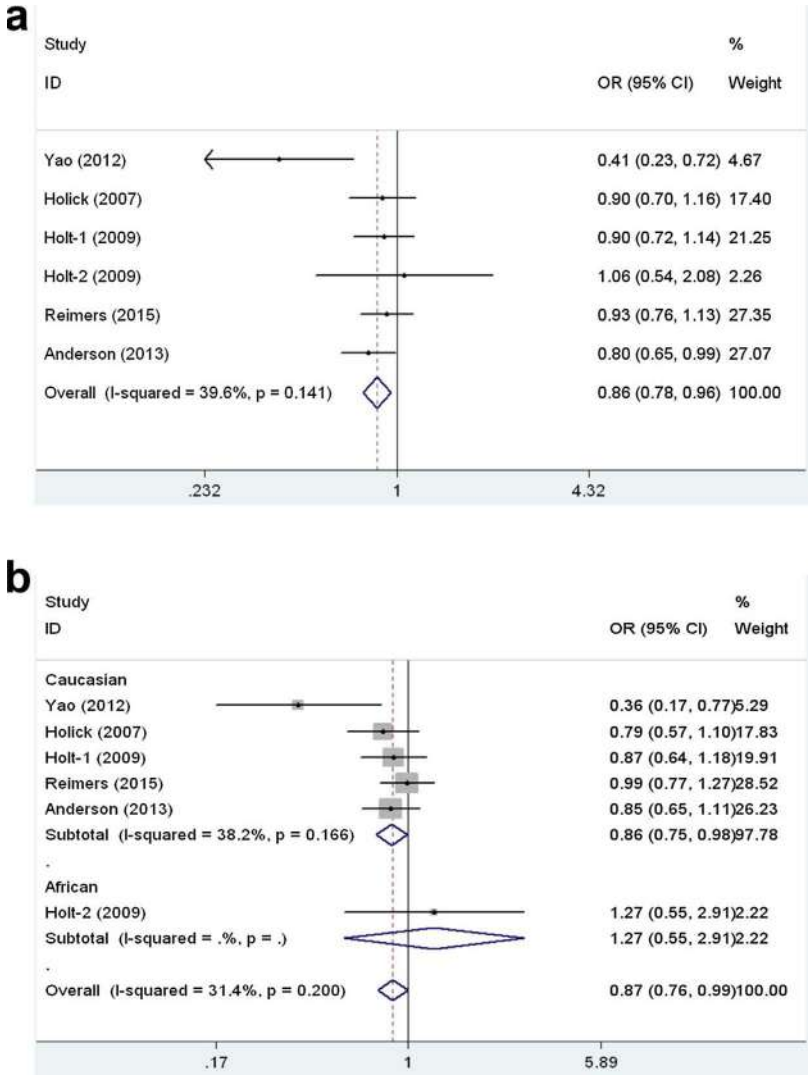

Fig. 2. Meta-analysis for the association between rs2585428 polymorphism and cancer risk. **a** overall comparison (dominant model: AA + AG vs. GG); **b** stratification analysis by ethnicity (homozygote model: AA vs. GG).

3.3. Meta-analysis of rs4809960

Four publications including five studies with 3076 cases and 3722 controls examined rs4809960 polymorphism. As shown in Table 3, we found that rs4809960 polymorphism significantly decreased cancer risk in two models: dominant (CC + CT vs. TT, OR = 0.86, 95% CI = 0.78–0.95, $P = 0.003$, Fig. 3a) and allele (C vs. T, OR = 0.90, 95% CI = 0.83–0.97, $P = 0.009$) models. When studies were stratified by ethnicity, significant associations were found in Caucasians (C vs. T, OR = 0.90, 95% CI = 0.83–0.98, $P = 0.014$). Stratification analyses of cancer type indicated that rs4809960 polymorphism decreased the risk of breast cancer (CC + CT vs. TT, OR = 0.84, 95% CI = 0.74–0.95, $P = 0.007$; C vs. T, OR = 0.89, 95% CI = 0.80–0.99, $P = 0.032$, Fig. 3b). Moreover, our data indicated that rs4809960 polymorphism was also significantly associated with a decreased risk of cancer in the studies with PCR-RFLP.

As shown in Fig. 4b, actually accrued number of cases did not meet the required information size and the cumulative Z curve did not cross the trial sequential monitoring boundary. More studies are demanded to get a solid conclusion.

3.4. Meta-analysis of rs2181874, rs6022999 and rs6068816

Seven publications including eight studies with 6845 cases and 8518 controls examined rs2181874 polymorphism; five publications including six studies with 4679 cases and 5687 controls examined rs6022999 polymorphism; four publications with 2963 cases and 3687 controls examined rs6068816 polymorphism. As shown in Supplementary Table 3, we found these three SNPs were not associated with cancer risk. Similar results were observed by subgroup analyses.

As for rs2181874, actually accrued number of cases met the required information size, however, TSA showed that there was insufficient evidence to show a reduction of cancer risk, the cumulative Z-curve did not cross the trial sequential monitoring boundary (Fig. 4c). With respect to the rs6022999 and rs6068816 polymorphisms, actually accrued number of cases did not exceed the information size and the cumulative Z curve did not cross the trial sequential monitoring boundary (Fig. 4d and e). Therefore, more studies are demanded for these two polymorphisms to get a solid conclusion.

Table 3
Meta-analysis of associations between the rs4809960 polymorphism and cancer risk.

Comparison	Overall and Stratification analyses	Studies	OR (95% CI)	*P*-value	Random/Fixed effect model	*P* for heterogeneity	I^2 (%)
CC + CT vs. TT	Overall	5	**0.86(0.78, 0.95)**	**0.003**	Fixed	0.111	46.8
	Caucasian	4	0.87(0.76, 1.01)	0.066	Random	0.105	51.1
	African	1	0.55(0.26, 1.16)	0.118	Fixed	–	–
	Prostate cancer	3	0.86(0.62, 1.20)	0.373	Random	0.036	69.8
	Breast cancer	2	**0.84(0.74, 0.95)**	**0.007**	Fixed	0.479	0
	PCR-RFLP	2	**0.76(0.62, 0.93)**	**0.008**	Fixed	0.391	0
	Other methods	3	0.91(0.76, 1.08)	0.287	Random	0.090	58.5
CC vs. CT + TT	Overall	5	0.94(0.77, 1.15)	0.563	Fixed	0.616	0
	Caucasian	4	0.94(0.77, 1.15)	0.547	Fixed	0.470	0
	African	1	1.71(0.07, 42.57)	0.744	Fixed	–	–
	Prostate cancer	3	0.84(0.61, 1.16)	0.277	Fixed	0.480	0
	Breast cancer	2	1.01(0.79, 1.30)	0.913	Fixed	0.560	0
	PCR-RFLP	2	0.98(0.64, 1.50)	0.930	Fixed	0.733	0
	Other methods	3	0.93(0.75, 1.17)	0.543	Fixed	0.286	20.2
CC vs. TT	Overall	5	0.89(0.73, 1.09)	0.262	Fixed	0.854	0
	Caucasian	4	0.89(0.73, 1.09)	0.254	Fixed	0.742	0
	African	1	1.49(0.06, 37.34)	0.808	Fixed	–	–
	Prostate cancer	3	0.81(0.58, 1.13)	0.217	Fixed	0.787	0
	Breast cancer	2	0.94(0.73, 1.22)	0.644	Fixed	0.538	0
	PCR-RFLP	2	0.89(0.58, 1.36)	0.578	Fixed	0.748	0
	Other methods	3	0.89(0.71, 1.12)	0.330	Fixed	0.538	0
CC vs. CT	Overall	5	1.04(0.84, 1.27)	0.745	Fixed	0.224	29.6
	Caucasian	4	1.03(0.84, 1.27)	0.781	Fixed	0.150	43.5
	African	1	2.84(0.11, 74.42)	0.531	Fixed	–	–
	Prostate cancer	3	0.87(0.48, 1.59)	0.654	Random	0.123	52.3
	Breast cancer	2	1.14(0.88, 1.49)	0.325	Fixed	0.737	0
	PCR-RFLP	2	1.18(0.76, 1.84)	0.467	Fixed	0.593	0
	Other methods	3	0.95(0.64, 1.40)	0.779	Random	0.082	60.0
C vs. T	Overall	5	**0.90(0.83, 0.97)**	**0.009**	Fixed	0.354	9.2
	Caucasian	4	**0.90(0.83, 0.98)**	**0.014**	Fixed	0.341	10.5
	African	1	0.63(0.32, 1.25)	0.185	Fixed	–	–
	Prostate cancer	3	0.91(0.80, 1.03)	0.140	Fixed	0.177	42.3
	Breast cancer	2	**0.89(0.80, 0.99)**	**0.032**	Fixed	0.344	0
	PCR-RFLP	2	**0.83(0.70, 0.98)**	**0.027**	Fixed	0.423	0
	Other methods	3	0.92(0.84, 1.01)	0.079	Fixed	0.285	20.3

Abbreviation: OR: Odds ratio; CI: Confidence interval. Bold values are statistically significant ($P < 0.05$).

3.5. Publication bias and sensitivity analysis

We utilized Begg's and Egger's tests to assess the publication bias. As illustrated in Fig. 5, the Begg's funnel plots seemed symmetrical. Meanwhile, Egger's test indicated that there is no evidence of significant publication bias (dominant model: $P_{Egger} = 0.672$ for rs2181874, $P_{Egger} = 0.675$ for rs4809960, $P_{Egger} = 0.380$ for rs2585428, $P_{Egger} = 0.351$ for rs6022999, and $P_{Egger} = 0.118$ for rs6068816) in our meta-analysis. In addition, sensitivity analysis was performed to evaluate whether the individual studies affected the overall results. As a result, we found that none of the single research significantly changed the final conclusions (Fig. 6).

4. Discussion

It has long been established that genetics determines future cancer risk over the past few decades. Because SNP is the major cause of human genetic variation, the relation between SNP and cancer risk has attracted considerable attention. With the development of medical technology, genetic susceptibility has aroused great interest, and the study of tumor genetic polymorphism is also increasing. Among the polymorphisms widely researched for risk factors associated with malignancies, CYP24A1 has become an important gene.

CYP24A1, a member of the cytochrome P450 family, is an enzyme that can degrade 25(OH)D and $1,25(OH)_2D_3$. Nowadays accumulating evidence suggested that CYP24A1 may play a significant role in carcinogenesis. Elevated CYP24A1 gene expression levels or a reduced rate of CYP24A1 gene silencing has been found in various tumors, including prostate cancer [29], breast cancer [10], pancreas cancer [22], lung cancer [30], endometrial cancer [11], and colorectal cancer [31]. As suggested by Sun et al. [31] in 2016, higher CYP24A1 gene expression was detected in colorectal cancer tissues than in adjacent normal colorectal tissues. Furthermore, elevated CYP24A1 expression was also correlated with a poorer prognosis [31]. Thus, CYP24A1 might be a potential diagnostic and prognostic indicator in cancer. Based on the above researches, we hypothesize that CYP24A1 may play a pivotal role in the pathogenesis of cancer. Currently, epidemiological studies have investigated the associations between CYP24A1 gene polymorphisms and cancer risk, while the results were inconsistent. Hence, we conducted a meta-analysis of all available studies.

In the current study, our data found that rs2585428 polymorphism was significantly associated with a decreased risk of overall cancer, and this result was confirmed by TSA. Among these included studies, there were three studies on prostate cancer, two on breast cancer and one on pancreas cancer. Stratified analyses by cancer type revealed a significant association between rs2585428 and pancreas cancer, but not

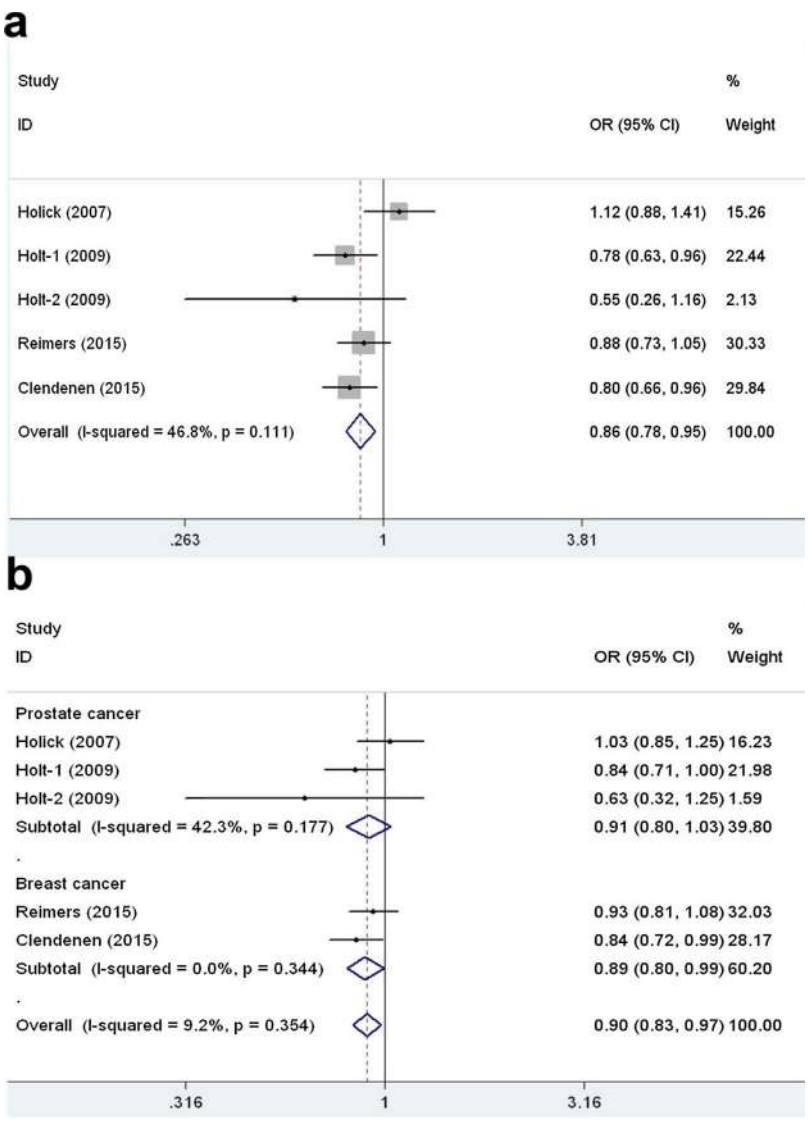

Fig. 3. Meta-analysis for the association between rs4809960 polymorphism and cancer risk. **a** overall comparison (dominant model: CC + CT vs. TT); **b** stratification analysis by cancer type (allele model: C vs. T).

prostate cancer and breast cancer. Our results were partially consistent with the consequence of the study by Reimers et al. [27], which reported that there was no significant association between rs2585428 and breast cancer in Caucasians. However, study by Yao et al. [28] suggested that rs2585428 was associated with a reduced risk of breast cancer in Caucasians. It is noteworthy that Yao et al. [28] indicated that rs2585428 polymorphism may be related to the higher prevalence of estrogen receptor (ER)-negative but not ER-positive breast cancer. At present, a large number of researches indicated that there were important differences in genetic susceptibility between ER-negative and ER-positive breast cancer [32]. Therefore, it is reasonable to hypothesize that rs2585428 polymorphism may have a specific effect on the susceptibility to ER-negative breast cancer. Of note, due to limited data, lack of further evaluation between rs2585428 and ER-negative and ER-positive breast cancer prevented our comprehensive understanding. Further large-cohort and well-designed studies are necessary to identify the possible association between them. In addition, stratification analyses of ethnicity revealed a significant association between rs2585428 and cancer risk in Caucasians, but not in Africans. However, we included only one study (114 cases and 63 controls) in Africans, and we also do not know whether these conclusions can also be adopted in other populations. Further multi-center and large-cohort studies are necessary to validate our findings.

As for rs4809960, we found that this polymorphism significantly decreased cancer risk. Stratification analyses of ethnicity suggested rs4809960 polymorphism decreased the risk of cancer in Caucasians, but not in Africans. Possible reasons can be explained as follows: (1) the different genetic backgrounds of cancer across ethnicities. In this meta-analysis, the pooled rs4809960C allele frequency of the controls showed a large difference across ethnicities (Caucasians: 25.0%; Africans: 13.5%), which may possibly affect the relationships between rs4809960 polymorphism and cancer risk among different racial subgroups. (2) the limited sample size. Only 175 subjects (112 cases and 63 controls) were included in our study, which may not be sufficient to support or deny an association. Moreover, when studies were stratified by cancer type and genotyping method, we also found that rs4809960

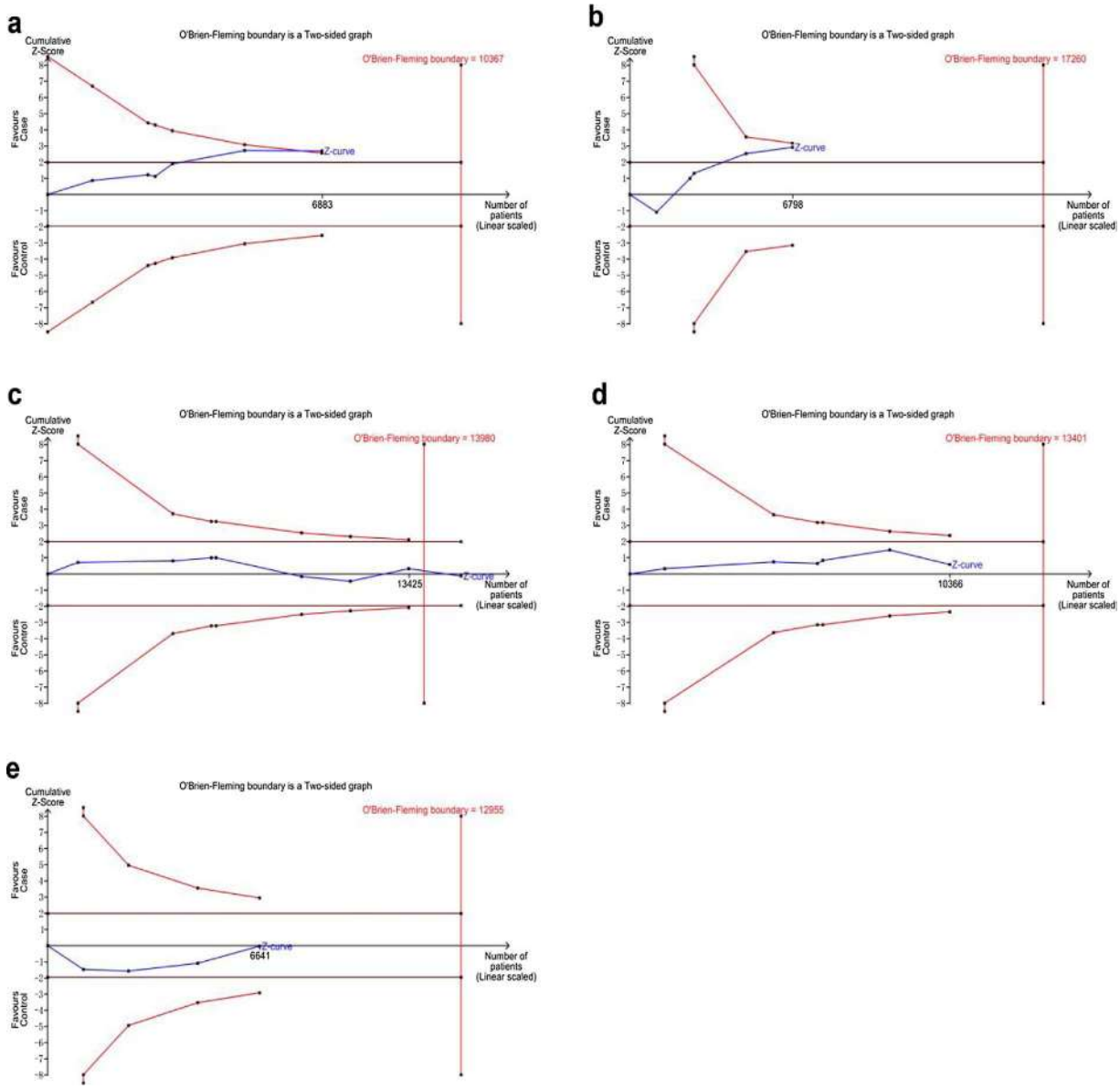

Fig. 4. Trial sequential analyses of the association between rs2585428, rs4809960, rs2181874, rs6022999, and rs6068816 polymorphisms (dominant model) and cancer risk. **a** rs2585428; **b** rs4809960; **c** rs2181874; **d** rs6022999; **e** rs6068816.

polymorphism was significantly associated with a decreased risk in the breast cancer subgroup and PCR-RFLP subgroup. However, most subgroups had insufficient numbers, which may attenuate the statistical power. With respect to the remaining three SNPs, we failed to find any associations between rs2181874, rs6022999 and rs6068816 and cancer risk. Given the limited sample size, our results should be interpreted with caution.

The current analysis might have several advantages: (1) our results were based on sufficient evidence, which were proved by TSA for the first time; (2) all of the eligible studies in our current study were in agreement with HWE, which may improve the reliability of the conclusions; (3) our study is the first systematical meta-analysis of reviewing the relationships between five CYP24A1 polymorphisms (rs2181874, rs2585428, rs4809960, rs6022999 and rs6068816) and cancer susceptibility. However, several drawbacks should also be noted. First, in the subgroup analysis, we found that our analysis was limited on Caucasians and Africans, and most populations were Caucasians, which may cause publication bias. Future studies on other ethnic populations are necessary. Second, the number of studies on rs2585428, rs4809960, rs6022999, and rs6068816 included in some subgroups was relatively small, which might create insignificant or significant results by chance due to insufficient statistical power. Third, our study is a summary of multiple data sources. In some included studies, detailed information (e.g., drinking status, smoking, radiation exposure, carcinogen, and other risk factors) was not gathered, which further prevented the stratification analyses. Thus, more studies by standardized unbiased methods are required to offer more detailed data.

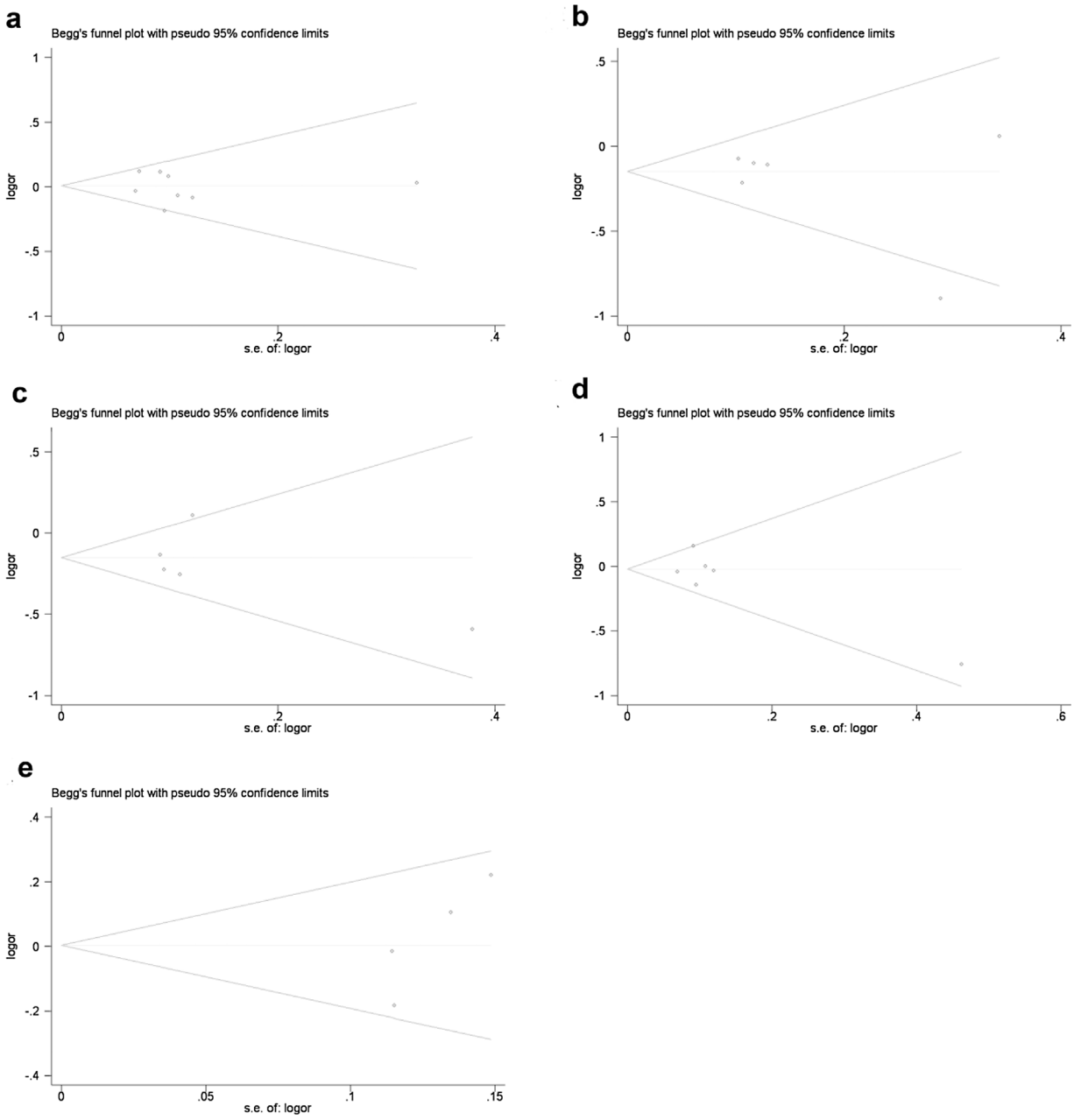

Fig. 5. Begg's test for publication bias (dominant model). **a** rs2181874; **b** rs2585428; **c** rs4809960; **d** rs6022999; **e** rs6068816.

5. Conclusions

In conclusion, this systematical meta-analysis indicated that rs2585428 polymorphism plays important roles in cancer pathogenesis, especially in pancreas cancer and Caucasians. Moreover, we also found that rs4809960 polymorphism significantly decreased the risk of cancer, especially in breast cancer and Caucasians. However, the other three SNPs (rs2181874, rs6022999 and rs6068816) are not associated with cancer risk. Further multi-center and well-designed studies are necessary to validate our findings.

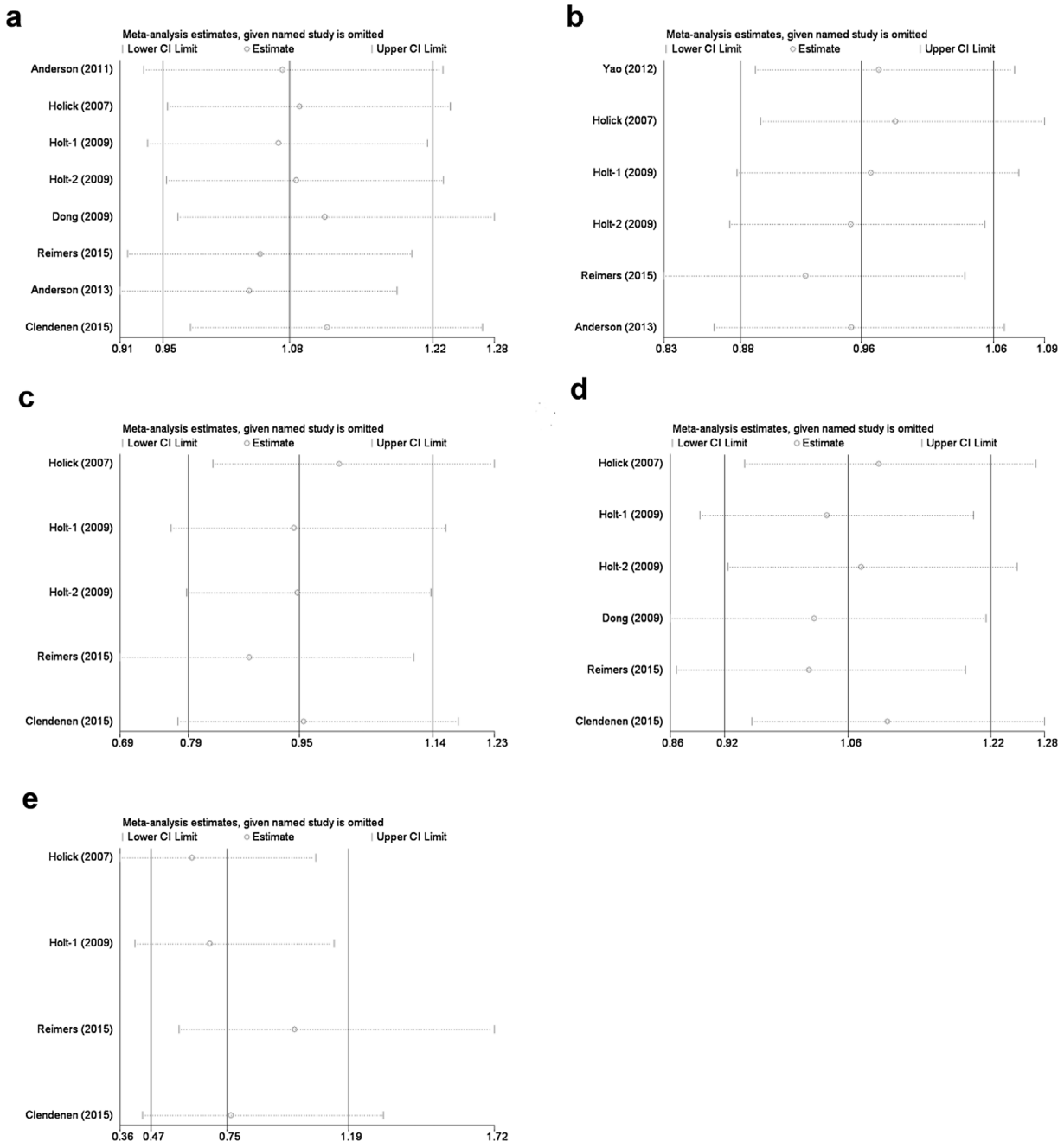

Fig. 6. Sensitivity analyses of the studies (recessive model). **a** rs2181874; **b** rs2585428; **c** rs4809960; **d** rs6022999; **e** rs6068816.

Conflict of interest

None.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

For this type of study, formal consent is not required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.prp.2017.11.014.

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