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# A case-control study and systematic review of the association between glutathione S-transferase genes and chronic kidney disease

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#### ABSTRACT

*Background:* GSTM1 deletion was reported to be associated with CKD progression in cohort studies. However, the results of case-control studies were conflicting. The association between GST genes and CKD progression needs to be studied in China. Therefore, we conducted this case-control study and systematic review for Southwest China to outline the association between GST genes and CKD.

*Methods*: CKD patients and healthy controls were enrolled from June 1, 2022 to 1 August 2022. Reported case–control studies were identified by searching databases until 1 September 2022 for meta-analysis.

*Results*: Significant associations were found between deletions of GSTM1 and GSTT1 and CKD risk (all P < 0.01) but not in GSTP1 rs1695 (all P > 0.05) in Southwest China. Then, we conducted a meta-analysis on 30 studies and found positive associations between deletions of GSTM1 and GSTT1 and CKD risk (all P < 0.01) but failed to find associations in GSTP1 rs1695 (all P > 0.05). Stratification analysis for ethnicity only showed a significant association in Southern Asia (P < 0.05) but not in Eastern Asia or other populations. This was different from our case–control results. The current evidence was influenced by study quality and PCR method but not by control selection. Given the different stages of CKD patients, a subanalysis of disease stages was performed, and the results remained positive. Interestingly, we found no significant associations between DM-CKD and GST genes, which should be interpreted with caution.

*Conclusion:* We found that GSTM1 and GSTT1 null genotypes were risk factors for CKD in China. The results of the meta-analysis were somewhat different from our results. We considered that antioxidant therapy might be useful for the treatment of these patients.

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#### 1. Introduction

Chronic kidney disease (CKD) has a marked racial disparity and affects more than 10 % of the United States population [1,2]. Environmental (such as nephrotoxic compound exposure) and clinical factors (such as diabetes and hypertension) increase the burden of CKD [3,4]. The treatment therapy for CKD is control of blood pressure and proteinuria using angiotensin-converting enzyme inhibitor (ACEI) drugs, which were introduced more than 20 years ago. However, many individuals with CKD still progress to end-stage renal disease (ESRD), and hemodialysis and renal replacement therapy are required [1]. Some studies have reported that individuals with glutathione-S-transferase M1 (GSTM1) deletion have a high risk for CKD, ESRD, allograft dysfunction, and all-cause mortality [5–7]. These results indicated that GST deletions might affect CKD progression and have a high risk of adverse renal outcomes.

In 2013, GSTM1 deletion was first demonstrated to be a risk factor for adverse clinical outcomes, such as kidney failure, in an AASK cohort study with 731 participants [2]. In 2016, GSTM1 null was reported to affect CKD progression among the black AASK population [8]. In 2017, the ARIC cohort study with 5700 participants also showed that GSTM1 deletion was associated with incidents of kidney failure and a higher level of oxidative stress (OS) but not CKD events [5]. In 2020, Gstm1 knockout mice displayed kidney injury in a CKD model, and the results of the ARIC study also proved that high consumption of cruciferous vegetables was associated with fewer kidney failure events in participants with homozygous GSTM1 deletion [9]. In 2020, a case–control study in southern India confirmed that GSTM1 deletion increased the risk for rapid CKD progression to ESRD among non-dialysis patients and caused high mortality rates among ESRD patients [10]. This finding provided an explanation for the smaller effect sizes in the 2020 study with a larger sample size than those of the 2017 ARIC study. In addition, in 2021, GSTM1 null was also reported to be associated with rapid progression of CKD in children using a CKD cohort study with 674 participants [11]. However, in 2019, Zhang failed to find any significant results in a large cohort study with 46983 participants [12].

The effects of GSTT1 null and GSTP1 rs1695 were ignored in all cohort studies [2,5,8,9,11,12], whereas many case-control studies delineated a delicate position of GSTT1 and GSTP1 in CKD patients. Glutathione-S-transferases (GSTs) are multifunctional enzymes, including GSTM1, GSTT1 and GSTP1, and their function is to neutralize endogenous oxidants and play a detoxifying role [10]. Defense against oxidative stress (OS) might be impaired due to reduced GST expression, and OS could be an alarming factor in the progression of CKD [13]. Deletions of GSTM1 and GSTT1 result in a lack of enzyme activities, and the G allele of GSTP1 (rs1695, p. Ile105Val) reduces its enzymatic activity by 50%–70 % compared to the wild-type [11–14]. Many case-control studies have been reported, and some of them showed significant associations between GST polymorphisms and CKD risk [10,15–44]. To extend our prospective study, this study was warranted. Furthermore, this was the first study to outline the associations between GST genes and CKD in a Chinese population.

## 2. Patients and methods

#### 2.1. Patients

The sample size included 1036 individuals who were recruited from June 2022 to August 2022 at the First Affiliated Hospital of Chengdu Medical College, located in Sichuan Province, in the Southwest region of China. This study was approved by the Ethics Committee of the First Affiliated Hospital of Chengdu Medical College. Written informed consent was obtained from all participants. The study was carried out following the Helsinki Declaration. The diagnosis of CKD was determined by the nephrologist based on the estimated glomerular filtration rate (eGFR) levels. Stages of CKD are classified by eGFR: stage  $1 \ge 90$  mL/min/1.73 m<sup>3</sup>, stage 2 = (60-89) mL/min/1.73 m<sup>3</sup>, stage 3 = (30-59) mL/min/1.73 m<sup>3</sup>, stage 4 = (15-29) mL/min/1.73 m<sup>3</sup>, and stage 5 < 15 mL/min/1.73 m<sup>3</sup> or dialysis. Patients under hemodialysis were also recruited from the Center of Hemodialysis. All patients underwent hemodialysis for 12–15 h per week for more than 3 months before the study, and they used a single–use dialyzer equipped with low– and high– flux polysulfone membranes. Kt/V (K, clearance of urea, t, time of dialysis and V, volume of distribution of urea) was calculated to evaluate the efficiency of dialysis. Baseline information, including age, sex, hemodialysis duration, smoking habits, systolic and diastolic pressure, and body mass index (BMI), was collected. Patients with infectious diseases, acute kidney injury, cancers, nephrotoxic compound exposure, IgA nephropathy and lupus nephritis were excluded from this study. Accordingly, the final case population consisted of 511 patients with CKD (261 males and 250 females; mean age  $\pm$  SD, 59.3  $\pm$  15.1 years old). A total of 525 healthy controls (260 males and 265 females; mean age  $\pm$  SD, 58.6  $\pm$  15.8 years old) were recruited from the health management center, and all individuals had no history of kidney disease.

## 2.2. Genotype analysis

Genomic DNA was extracted using phenol/chloroform methods from the peripheral blood. The genetic polymorphisms of GSTM1 and GSTT1 were measured using multiplex PCR, and CYP1A1 was used as an internal control [3]. GSTP1 rs1695 was analyzed by PCR–restriction fragment length polymorphism (PCR–RFLP) as described previously [3]. Serum urea and creatinine were measured in the clinical laboratory of the First Affiliated Hospital. The forward primer for GSTM1 was F: 5'-GAACTCCCTGAAAAGCTAAAGC-3', and the reverse primer for GSTM1 was R: 5' GTTGGGCTCAAATATACGGTGG-3'. The forward primer for GSTT1 was F: 5'-TTCCTTACTGGTCCTCACATCTC-3', and the reverse primer for GSTT1 was R: 5' TTCCTTACTGGTCCTCACATCTC-3', and the reverse primer for GSTT1 was R: 5'-TCACGGGATCATGGCCAGCA-3'. The presence of GSTM1 and GSTT1 was detected at 215 bp and 480 bp, respectively, and this method did not distinguish between heterozygous genotypes or homozygous wild-type genotypes, and linkage disequilibrium analysis could not be conducted. Primers of CYP1A1, F:

5'-GAACTGCCACTTCAGCTGTCT-3' and R: 5'-CAGCTGCATTTGGAAGTGCTC-3'. GSTP1 rs1695 (Ile105Val) polymorphism was measured using PCR-RFLP. The primers were F: 5'-ACCCCAGGGCTCTATGGGAA-3' and R: 5'-TGAGGGCACAAGAAGCCCCT-3'. The presence of 91 bp and 85 bp indicated the Val/Val allele (GG genotype), and 176 bp resulted from Ile/Val (GA genotype) and Ile/Ile (AA genotype). The dominant and recessive models were used for further analysis. GG + GA + AA were compared in the dominant model. GG and GG + GA + AA were compared in the recessive model.

## 2.3. Search strategy and inclusion criteria

A systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement. PubMed and Chinese National Knowledge Infrastructure (CNKI) databases were searched for eligible studies from inception to 1 September 2022. The search keywords that we used were "kidney disease", "GSTM1", "GSTP1", "glutathione S-transferase M1", "glutathione S-transferase P1" "renal disease", "end-stage renal disease", "diabetic nephropathy", "nephropathy", "renal transplant recipients", "chronic kidney disease", "dialysis", "hemodialysis", "renal transplantation", and "glomerulonephritis". Reference lists of the included studies were also reviewed to retrieve additional studies that were not identified through the search strategy.

## 2.4. Study selection

We included case-control studies and cross-sectional studies examining the associations between GST polymorphisms and the risk of kidney disease (consisting of CKD, ESRD, DN, hemodialysis, kidney transplantation, and allograft function). Animal studies, case reports, kidney cancer, acute kidney injury, nephrotoxic compound exposure, and studies with data not shown were excluded, as shown in Fig. 1. Risk estimates (OR) with 95 % CI had to be provided in the studies. All references of identified studies were reviewed to screen additional studies. In this meta-analysis, we expanded the boundary of the included studies and reported studies with CKD, diabetic nephropathy, ESRD or dialysis, kidney transplantation or allograft function, and survival rate of ESRD. The flow chart of study selection is shown in Fig. 1. Three authors J.P., P.M., and X-Q. W. performed screening separately. Titles, abstracts and keywords of all studies were screened for relevance to GST polymorphisms and kidney disease. The full text of studies was then selected to assess their eligibility and data extraction. Any discrepancies were resolved by rechecking the full text T-R. Y.

## 2.5. Data extraction

The following information was extracted: first author's last name, published year, country where the research was conducted and ethnicity, study design and control population type (population-based or selected sample), sample size (number of cases, number of controls), criteria of kidney disease, OR and 95 % CIs, male/female and age in Supplementary Table 1 (Table S1). S.L. performed the



Fig. 1. A flow diagram of the study selection process.

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data extraction, and the accuracy of the extractions was double-checked by Y-T. H. and J.P. Any discrepancies were resolved by rechecking the full text T-R. Y.

## 2.6. Quality assessment

All included studies were assessed using the Newcastle–Ottawa quality assessment scale (NOS), was rewritten by us and is shown in Table S2. The domain of "Exposure" was deleted, and domains of "Methodology" and "Deductions" were added in the rewritten NOS (reNOS). The PCR method, PCR control, subgroup analysis, Hardy–Weinberg equilibrium (HWE), and age of onset years were included in the domain of "Methodology". Deductions were defined by inconsistent data or incorrect descriptions, which would affect the quality score of studies. Studies with 6–10 stars or no deductions were considered high–quality studies. The detailed information and number of stars of the included studies were assessed by S.L. and J.P. and are shown in Table S3. Any discordance was rechecked by the third reviewer T-R. Y.

## 2.7. Risk of bias

The potential risk of bias was assessed using the risk of bias in nonrandomized studies of interventions (ROBINS–I) tool scale [45]. This tool includes 7 aspects: bias due to confounding; bias due to misclassification of exposure during follow-up; bias in selection of study participants; bias due to missing data; bias in exposure measurement; bias in selection of reported results and bias in measurement of outcomes, and the results are shown in Table S4. On this scale, a study that is rated 'low' in all aspects is considered at 'low risk of bias'; a study that is rated 'probably at risk' for one aspect is considered at 'low–to–moderate risk of bias'; a study is considered at 'serious risk of bias', if it is rated as 'high risk' for more than one aspect; and 'critical risk of bias' is considered if it is rated as 'critical risk' in at least one aspect. A study that is missing information in at least two aspects is classified as 'no information' for evaluation. Assessment was performed by two reviewers independently P.M. and X-Q. W. Any discordance was rechecked by the third reviewer T-R. Y.

## 2.8. Data synthesis and meta-analysis

OR and 95 % CIs were calculated based on detailed information of data extraction in Table S5. Data extraction was performed by two reviewers independently H-Z.L. and J.P. Any discordance was rechecked by the third reviewer T-R. Y.

The Q test and I<sup>2</sup> statistics were used to assess the heterogeneity in Review Manager 5.4. Potential sources of heterogeneity were investigated using subgroup analyses, such as geographic location, study group, star of NOS, and risk of bias. The study sample size accounted for the weight of the overall results and the width of the 95 % CI in the statistical modeling. In the sensitivity analysis, studies were removed one by one, and whether the results were stable was assessed. Publication bias was assessed using funnel plots in Review Manager 5.4 and Egger's test and Begg's test in Stata 12. The association between genetic frequencies and CKD was detected using Fisher's exact test. Multiple logistic regression was used to adjust for confounding factors (such as sex and years) to obtain odds ratios (ORs) and 95 % confidence intervals (95 % CIs). P < 0.05 was considered to be statistically significant.

# 3. Results

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## 3.1. GST gene polymorphisms in Chinese CKD patients

The genetic frequencies of GST genes are shown in Table 1. We found significant associations between null genotypes of GSTM1 and GSTT1 and CKD risk (all P < 0.01) in Southwest China but failed to find associations in GSTP1 (all P > 0.05).

Distribution of GS1	genes and risk analys	SIS IOF CKD.				
Genotype	CKD n (%)	Control n(%)	F	Pa value	OR (95 % CI)	Pb value
GSTM1						
Positive (+)	296 (57.9)	389 (74.1)			reference	
Null (–)	215 (42.1)	136 (25.9)	4.46	< 0.01	1.93 (1.37-2.71)	< 0.01
Total	511	525				
GSTT1						
Positive (+)	408 (79.8)	466 (88.8)			reference	
Null (–)	103 (20.2)	59 (11.2)	3.92	< 0.01	1.45 (1.22-2.36)	< 0.01
Total	511	525				
GSTP1						
Ile/Ile	289 (56.6)	261(49.7)			reference	
Ile/Val	187 (36.6)	234 (44.6)	2.41	0.031	0.87 (0.52-1.36)	>0.05
Val/Val	35 (6.8)	30 (5.7)	0.99	>0.05	1.14 (0.71–1.93)	>0.05
Total	511	525				

Table 1							
Distribution of GST	genes	and	risk	analy	/sis	for	CK

Pa: Analysis by Fisher's extract test; Pb: Multiple Logistic Regression to obtain of OR (95% CI).

Study or Subgroup	Events	e Total	Cont	trol Tota	Weight	Odds Ratio M-H, Random, 95% (	a	Odds Ratio M-H. Random. 95% CI
Agrawal 2007 Akoul 2012	86	184	215	569	4.5%	1.44 [1.03, 2.02		
Albeladi 2022	48	64	20	64	2.5%	6.60 [3.04, 14.32		
Azmandian 2017 Chen 2012	42	173	45	173	3.8%	0.91 [0.56, 1.48 0.97 [0.58, 1.63		-
Datta 2010 del ima 2018	48	100	35	100	3.4%	1.71 [0.97, 3.03		
Fujita 2000	51	105	38	69	3.2%	0.77 [0.42, 1.42		
Gutierrez -Amavizca 2013 Hashemi-Soteh 2020	67	110	24	263	2.5%	2.05 [1.22, 3.45 2.38 [1.09, 5.20		
Hovnik 2009	15	37	36	87	2.5%	0.97 [0.44, 2.11		
Klen 2015	58	130	21	51	3.0%	1.15 [0.60, 2.22		
Lin 2009 Makuc 2012	308	488	224	372	4.8%	1.13 [0.86, 1.49 0.76 [0.39, 1.47		_ <u></u>
Nomani 2016 Rotor 2002	95	133	64	137	3.7%	2.85 [1.72, 4.72		
Purkait 2014	31	84	44	275	3.5%	3.07 [1.78, 5.31		
Reliic 2013 Sayanthooran 2016	92 17	207	63	138	4.0%	0.95 [0.62, 1.47 0.90 [0.33, 2.49		
Sayanthooran 2017	17	48	3	12	1.1%	1.65 [0.39, 6.90		- <u>-</u>
Singh 2009	140	273	90	223	4.4%	1.56 [1.09, 2.22		-
Suvakov 2013 Toncheva 2004	119	199 95	97	199	4.2%	1.56 [1.05, 2.33 0.77 [0.44, 1.33		
Vasudevan 2020	136	392	31	202	4.0%	2.93 [1.90, 4.53		
Yang 2004	123	220	261	485	4.6%	1.09 [0.79, 1.50		+
Zaki 2015	8	18	43	97	1.8%	1.00 [0.37, 2.76		
Total (95% CI)	0040	4578	0000	5037	100.0%	1.32 [1.12, 1.56]		•
Total events Heterogeneity: Tau <sup>2</sup> = 0.13;	2212 Chi <sup>2</sup> = 88	53, df =	2038 = 28 (P <	0.000	01); I² = 68	%	-	
Test for overall effect: Z = 3	.31 (P = 0	.0009)					0.01	Favours [case] Favours [control]
Study or Subaroup	Cas Events	Total	Cont	trol Tota	Weight	Odds Ratio M-H, Random, 95% (	31	Odds Ratio M-H. Random, 95% Cl
Agrawal 2007	108	184	135	569	5.5%	4.57 [3.22, 6.49	1	
Albeladi 2022	46	64	24	64	3.9%	2.97 [1.45, 6.10	1	
Azmandian 2017 Chen 2012	56 57	192	50	182	5.0%	1.09 [0.69, 1.71	1	<b>T</b>
Datta 2010	56	100	24	100	4.3%	4.03 [2.20, 7.38	i	
Gutiérrez -Amavizca 2013	32 9	101 110	11	87	3.7%	3.20 [1.50, 6.84 2.70 [0.81, 9.01	1	
Hashemi-Soteh 2020 Hovnik 2009	14	57 37	59	263	4.0%	1.13 [0.58, 2.20	1	
Klen 2015	36	130	15	51	3.9%	0.92 [0.45, 1.88		1
Makuc 2012 Nomani 2016	56 70	88 133	70	109	4.4%	0.97 [0.54, 1.75 1.82 [1.12, 2.95	1	
Peter 2002 Relic 2013	40	228	11	103	3.9%	1.78 [0.87, 3.63	1	
Siddarth 2014	84	270	60	270	5.3%	1.58 [1.07, 2.32	1	-
Singh 2009 Suvakov 2013	57 67	273	44	223	5.1%	1.07 [0.69, 1.67	1	Ŧ
Toncheva 2004	19	95	18	112	3.9%	1.31 [0.64, 2.66		
Yan 2003	67	328	57	303	5.3%	1.11 [0.75, 1.64	1	+
Yang 2004 Zaki 2015	110	220	180	485	5.6%	1.69 [1.23, 2.34	1	
Tatal (OER/ CI)		2620		4000	100.05/	4 53 14 34 4 00		•
Total events	1156	2023	1083	4099	100.0%	1.52 [1.21, 1.90]		
Heterogeneity: Tau <sup>2</sup> = 0.21;	Chi <sup>2</sup> = 85	76, df =	= 22 (P <	0.000	01); l <sup>2</sup> = 74	%	0.01	0.1 1 10 100
C			Con	Ind		Odda Batio		Favours [case] Favours [control]
Study or Subgroup	Events	e Total	Events	Tota	Weight	M-H, Random, 95% (	3	M-H. Random, 95% Cl
Agrawal 2007 Chap 2012	86	184	88	569	16.4%	4.80 [3.32, 6.93		
Datta 2010	25	100	12	100	10.3%	2.44 [1.15, 5.20		
Gutiérrez -Amavizca 2013 Nomani 2016	7	110	24	125	3.8%	4.18 [0.85, 20.56 2.75 [1.56, 4.83		
Siddarth 2014	47	270	31	270	14.4%	1.62 [1.00, 2.65		
Yang 2004	57	220	67	485	15.9%	2.18 [1.47, 3.24		-
Total (95% CI)				2037	100.0%	2.32 [1.64, 3.28]		•
		1307						
Total events	336	1307	283					
Total events Heterogeneity: Tau <sup>a</sup> = 0.16; Test for overall effect: Z = 4	336 Chi <sup>2</sup> = 21	1307 .68, df =	283 7 (P =	0.003):	1° = 68%		0.01	0.1 1 10 100
Total events Heterogeneity: Tau <sup>2</sup> = 0.16; Test for overall effect: Z = 4 D	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case	1307 .68, df = .00001)	283 7 (P = ) Contro	i 0.003): I	1 <sup>2</sup> = 68%	Odds Ratio	0.01	0.1 1 10 100 Favours [case] Favours [control] Odds Ratio
Total events Heterogeneity: Tau <sup>2</sup> = 0.16; Test for overall effect: Z = 4 D Study or Subgroup E	336 Chi <sup>2</sup> = 21 77 (P < 0 Case	1307 .68, df = .00001)	283 7 (P = 1 Contro	0.003): I Total	1° = 68%	Odds Ratio M-H. Random, 95% C	0.01	0.1 1 10 100 Favours [case] Favours [control] Odds Ratio M-H. Random, 95% Cl
Total events Heterogeneity: Tau <sup>2</sup> = 0.16; Test for overall effect: Z = 4 D Study or Subgroup E Agrawal 2007 Akgul 2012	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case <u>vents T</u> 137 41	1307 .68, df = .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001)	283 = 7 (P = 1 Contro vents 266 16	0.003): I Total 1138 102	Veight 12.1% 7.3%	Odds Ratio <u>M-H. Random, 95% C</u> 1.94 [1.51, 2.50] 1.09 [0.58, 2.04]	0.01	0.1 1 10 100 Favours [case] Favours [control] Odds Ratio M-H. Random, 95% Cl
Total events Heterogeneity: Tau <sup>2</sup> = 0.16; Test for overall effect: 2 = 4 D Study or Subgroup E Agrawal 2007 Akgui 2012 deLima 2018 Makun 2012	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case <u>vents Tr</u> 137 41 70	1307 .68, df = .00001) .011 E .00001) .012 E .00001) .012 E .012	283 = 7 (P = 1 Contro vents 2 266 16 69 ee	0.003): I Total 1138 102 174 216	Weight 12.1% 7.3% 9.9% 9.7%	Odds Ratio M-H. Random, 95% C 1.94 [1.51, 2.50] 1.09 [0.58, 2.04] 0.81 [0.53, 1.23] 1.00 [0.55   541	0.01	0.1 10 100 Favours [case] Favours [control] Odds Ratio M-H. Random. 95% Cl
Total events Heterogeneity: Tau <sup>2</sup> = 0.16; Test for overall effect: 2 = 4 D Study or Subgroup E Agrawal 2007 Akgui 2012 deLima 2018 Makuc 2012 Nomani 2016	336 Chi <sup>2</sup> = 21 77 (P < 0 Case <u>vents Ta</u> 137 41 53 68	1307 .68, df - .00001) .00001, .0000000000	283 = 7 (P = 1 Contro vents 2 266 16 69 66 78	0.003): I Total 1138 102 174 216 274	Veight 12.1% 7.3% 9.9% 9.7% 10.4%	Odds Ratio M-H. Random. 95% C 1.94 (1.51, 2.50) 1.09 (0.58, 2.04) 0.81 (0.53, 1.23) 1.00 (0.65, 1.54) 0.86 (0.59, 1.26)	0.01	0.1 10 100 Favours [costrol] Odds Ratio M-H. Random, 95% Cl
Total events Heterogeneity: Tau? = 0.16; Test for overall effect: Z = 4 D Study or Subgroup E Agrawal 2007 Akgul 2012 deLima 2018 Makuc 2012 Nomani 2016 Relic 2013 Sinch 2019	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case <u>vents Tr</u> 137 41 53 68 124 158	1307 .68, df = .00001) .00001) .01001) .01001 .010001 .010000000000	283 7 (P = Contro vents 266 16 69 66 78 93 103	0.003): I Total 1138 102 174 216 274 274 274	Veight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6%	Odds Ratio M.H. Random, 95% C 1.94 (1.51, 2.50) 1.09 (0.58, 2.04) 0.81 (0.53, 1.23) 1.00 (0.65, 1.54) 0.86 (0.59, 1.26) 0.84 (0.61, 1.17) 1.36 (1.02, 1.81)	0.01	0.1 10 Favous (centrol) Odds Ratio M-H. Random, 95% Cl
Total events Test for overall effect: Z = 4 D Study or Subgroup E Agraval 2007 Akgul 2012 deLima 2018 Makuc 2012 Nomani 2016 Reijic 2013 Singh 2009 Suvakov 2013	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case <u>vents Tr</u> 137 41 53 68 124 158 171	1307 68, df - 00001) 011 E 368 244 202 174 266 410 546 398	283 7 (P = 1 266 16 69 66 78 93 103 150	0.003): 1 1 1 1 1 1 1 1 1 1 1 1 1	Veight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6% 11.7%	Odds Ratio <u>M-H, Random, 95% C</u> 1,94 (1.51, 2.60) 1,09 (0.58, 2.04) 0.81 (0.53, 1.23) 1,00 (0.65, 1.54) 0.86 (0.59, 1.26) 0.84 (0.61, 1.17) 1,36 (1.02, 1.81) 1,25 (0.94, 1.65)	0.01	0.1 10 100 Farours (cotrol) Farours (cotrol) 0646 Ratio M41. Random 39% Cl
Total events Heterogenety: Tau" = 0.16: Test for overall effect: Z = 4 D Study or Subgroup E Agraval 2007 Adgut 2012 deLima 2018 Maku: 2012 Maku: 2012 Nomani 2016 Singh 2009 Suvakov 2013 Tiwari 2009 Zaki 2015	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case <u>vents Tr</u> 137 53 68 124 158 124 158 171 78	1307 .68, df = .00001) .00001000 .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .00001) .000010 .0000000000	283 7 (P = 1 Contro vents 266 16 69 66 78 93 103 150 128 34	0.003): 1 1 1 1 1 1 1 1 1 1 1 1 1	Weight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6% 11.7% 11.2% 5.1%	Odds Ratio <u>M-H. Random, 95% C</u> 1.09 (1.54, 2.64) 0.81 (0.53, 1.23) 1.00 (0.65, 1.54) 0.86 (0.55, 1.26) 0.84 (0.65, 1.54) 0.86 (0.55, 1.26) 0.84 (0.65, 1.27) 1.36 (1.02, 1.81) 1.25 (0.94, 1.65) 0.63 (0.46, 0.87) 1.18 m de 2 part	0.01	0.1 10 100 Favours (control) Odds Ratio M-H. Random. 95% Cl
Total events Heterogenety: Tar2* = 0.16: Test for overall effect: 2 = 4 D Study or Subgroup E Aggraval 2007 Akgui 2012 det.ima 2018 Maku: 2012 Maku: 2012 Nomani 2016 Suvakov 2013 Singh 2009 Suvakov 2013 Timari 2006 Zall 2015	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case <u>vents Tr</u> 137 53 68 124 158 124 158 171 8	1307 .68, df = .00001) .00001000 .00001 .000001 .0000000000	283 = 7 (P = 1 Contro vents 266 16 69 66 78 93 103 150 128 34	0.003): Total 1 1138 102 174 216 274 274 446 398 448 174	Weight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6% 11.6% 11.2% 5.1%	Odds Ratio <u>M-H. Random. 9% C</u> 1.94 [1.51, 2.50] 1.09 [0.58, 2.04] 0.81 [0.53, 1.23] 1.00 [0.65, 1.54] 0.86 [0.55, 1.54] 0.84 [0.65, 1.517] 1.36 [1.02, 1.81] 1.25 [0.94, 1.65] 0.63 [0.46, 0.87] 1.18 [0.49, 2.81] 1.05 [0.52, 0.52] 1.05 [0.52, 0.52]	0.01	0.1 10 100 Favors (aze) Favors (control) 100 Odds Ratio M-H. Random. 95%, Cl
Total events	336 ChP = 21 .77 (P < 0 Case (vents Tr 137 41 .70 .53 .68 .124 .158 .124 .171 .8 .30 .908	1307 .68, df = .00001) .00001 .0000000000	283 7 (P = 1 266 16 69 66 78 93 103 150 128 34 1003	0.003): 1138 102 174 216 274 274 446 398 448 174 3644	Weight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6% 11.7% 11.2% 5.1% 100.0%	Odds Ratio <u>M-H. Random, 95% C</u> 1.94 [1.51, 2.80] 1.09 [0.58, 2.04] 0.05, 1.53 0.06 [0.56, 1.54] 0.86 [0.59, 1.26] 0.84 [0.61, 1.17] 1.25 [0.04, 1.61] 1.25 [0.04, 1.61] 1.25 [0.04, 1.61] 1.18 [0.49, 2.81] 1.05 [0.82, 1.34]	0.01	0.1 10 100 Farours (care) Farours (control) M-H. Rendom: 35% Cl
Total svents           Hedrogeneity: Tau" = 0.16:           Test for overall effect: 2 = 4           D           Study or Subaroup E           Agraval 2001           deLima 2018           Makuc 2012           odeLima 2018           Makuc 2012           Study or Subaroup E           Subarout 2012           Subarout 2013           Suvakov 2013           Twan 2006           Total (98% CI)           Total (98% CI)           Total (98% CI)	3366 : ChP = 21 .77 (P < 0 Case :vents Tr 137 : 41 : 53 : 68 : 124 : 68 : 124 : 78 : 8 : 8 : 908 : 908 : 908 : 908	1307 .68, df = .00001) .011 E .368 244 202 174 266 410 546 410 546 398 386 36 .30 .030	283 7 (P = 1 266 16 69 66 93 103 150 128 34 1003 aff = 9 (P	0.003): 1 1138 102 174 216 274 446 398 448 174 3644 < 0.00	P = 68% Weight 12.1% 9.9% 9.7% 10.4% 11.1% 11.6% 11.6% 11.6% 11.6% 11.6% 11.6% 11.6% 10.0% 0001; P =	Odds Ratio MH, Random, 25%, C 1.94 (15.1, 2.60) 0.81 (0.53, 1.23) 0.08 (10.56, 1.24) 0.86 (10.56, 1.26) 0.84 (0.56, 1.26) 0.84 (0.56, 1.26) 0.83 (0.64, 0.87) 1.36 (10.24, 1.81) 1.26 (0.44, 0.87) 1.18 (0.48, 2.81) 1.05 (0.82, 1.34] 78%	0.01	0.1 10 100 Farours (control) Odds Ratio MH. Random .95% Cl
Total events Test provents Test provents effect 2 = 4 D Study or Subarroup E Ageu 2012 deLima 2018 Makuc 2012 Makuc 2012 Makuc 2013 Suvakov 2013 Suvakov 2013 Tutal events Test provents effect 2 = 2 D D	336 : Chi <sup>2</sup> = 21 .77 (P < 0 Case <u>vents Tr</u> 137 : 41 : 53 : 124 - 158 : 171 : 78 : 8 30 908 11; Chi <sup>2</sup> = - 0.39 (P =	1307 68, df 4 600001) 00001 000000	283 = 7 (P = 1 Contro vents 2 266 16 69 66 78 93 103 150 128 34 1003 df = 9 (P	I Total 1 1138 102 174 274 274 274 274 446 398 448 174 3644 2< 0.00	<pre>I<sup>2</sup> = 68% Weight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6% 11.7% 11.2% 5.1% 100.0% 0001); I<sup>2</sup> =</pre>	Odds Ratio MH. Random, 55% C 1.94 (15.12,20) 0.05 (0.58,123) 0.06 (15.12,120) 0.06 (15.12) 0.06 (15.12) 0.06 (15.12) 0.06 (15.12) 0.06 (15.12) 0.05 (15.12) 0.05 (0.44, 155) 0.05 (0.44, 155) 0.05 (0.44, 155) 0.05 (0.42, 13.40) 1.05 (0.82, 13.40) 78%	0.01	0.1 10 100 Parcers (aae) Favours (control) M-H. Random. 95% Cl 
Total events           Heerogeneity, Tury – 0.16.           Test for ownall effect 2 = 4           D           Study or Subgroup, E           Agay 2012           Adau 2012           Adau 2012           Study or Subgroup, E           Agay 2012           Agay 2012           Stardy or Subgroup, E           Agay 2013           Singh 2009           Survakov 2013           Total events           Heterogeneity, Tury = 0.1           Total events           Heterogeneity, Tury = 0.2           Test for ownall effect - e           E	336 : Chi <sup>2</sup> = 21 .77 (P < 0 Case vents Tr 137 : 41 : 53 : 68 : 124 : 70 : 53 : 68 : 124 : 78 : 8 30 908 : 12, Chi <sup>2</sup> = - : 0.39 (P = Case : 24 : 25 : 24 : 25 :	1307 668, df + 600001) 0tal E 668 668 644 202 174 266 546 336 336 330 40.03, ( 0.70) 101 102 102 102 102 102 102 102	283 7 (P = ) 266 16 69 93 103 150 128 34 1003 if = 9 (P Contro	I Total 1 1138 102 174 276 274 274 274 274 446 398 448 174 3664 274 274	Veight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6% 5.1% 100.0% Wold:1; I <sup>2</sup> =	Odds Ratio MH. Random, 55% C 1.94 [15,12,60] 0.08 [0.05,123] 0.08 [0.08,126] 0.08 [0.08,126] 0.08 [0.08,126] 0.08 [0.08,126] 0.03 [0.04,087] 1.18 [0.48,281] 1.05 [0.82,134] 78% Odds Ratio	0.01	0.1 10 100 Farours (care) Ferours (control) Med. Rendom 25% C1 444 Rendom 25% C1 445 Rendom 25% C1 45% C1 45% Rendom 25% Rendom 25% C1 45%
Total events           Heterogeneity, Taur – 0.16.           Test for event effect. Z = 4           D           Study or Subgroup           Aguit 2012           deLima 2018           Mako: 2010           Mako: 2010           Subdy or 2012           deLima 2016           Helip: 2010           Subdy or 2013           Total (95% C1)           Study or coveral effect: Z = E           Study or Subgroup           Study or Subgroup           Agravel 2007	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case vents Tr 137 : 137 : 137 : 137 : 137 : 137 : 137 : 137 : 138 : 124 : 158 : 159 : 1	1307 .68, df + .000001) .011 E .022 .024 .022 .024	283 7 (P = Contro vents. 266 69 66 93 103 150 128 34 1003 if = 9 (P Contro vents. 59	0.003): 1 1138 102 174 216 274 274 274 274 274 3644 446 398 448 174 3644 274 274 274 274 274 274 274 2	I <sup>2</sup> = 68% Weight 12.1% 7.3% 9.7% 10.4% 11.1% 11.6% 11.7% 11.2% 5.1% 100.0% 100.0% 100.1); I <sup>2</sup> = Weight 15.7%	Odds Ratio 1.44 [L51,260] 1.96 [L53,264] 1.97 [L53,264] 1.97 [L53,264] 1.97 [L53,1264] 1.97 [L53,1264] 1.98 [L53,1264] 1.95 [L54,1374] 1.95 [L54,2164] 1.95 [L54,2164] 1.95 [L54,2164] 0.95 [L54,3744] 0.95 [L54,3744] 2.40 [L54,3744]	0.01	0.1 10 100 Farours (control) Odds Ratio MH. Random .95% Cl 0.1 10 Farours (cose) Farours (cose) Farours (cose) MH. Random .95% Cl
Total events           Test for event affect Z = 4           D           Study or Subarroup           Arguna 2016           Algu 2012           deLima 2018           Mako: 2018           Study or Subarroup           Total (#%5.0)           Total (#%3.0)           Subdy or Subgroup           E           Appu.2020 =	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case vents Tr 137 .53 .68 .124 .158 .158 .158 .158 .158 .158 .158 .158	1307 .68, df + .000001) .011 E .368 .244 .202 .227 .247 .266 .300 .330 .330 .011 E .184 .222 .191 .197	283 7 (P = Contro vents ' 2666 69 66 69 66 93 103 150 128 34 103 150 128 34 50 16 67 8 93 103 150 16 6 7 8 93 16 16 6 7 8 93 16 16 7 8 93 103 150 128 6 7 8 7 8 9 7 8 7 8 9 7 8 9 7 8 9 7 8 9 7 8 9 7 8 9 7 8 9 7 8 9 7 8 9 7 8 7 8 9 7 8 8 8 7 8 8 8 8 8 8 8 8 8 8 8 8 8	0.003): 1 1138 102 174 216 274 446 398 448 174 3644 < 0.00 1 569 51 569 51 87	<pre>I<sup>2</sup> = 68% Weight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6% 11.7% 15.1% 100.0% W001); I<sup>2</sup> = Weight 15.7% 4.7% 7.2%</pre>	Odds Ratio M-H, Random, <u>35%</u> , C 1.09 [0.58, 2.04] 0.09 [0.58, 2.04] 0.08 [0.59, 1.23] 1.00 [0.65, 1.54] 0.08 [0.59, 1.23] 1.32 [102, 1.13] 1.32 [102, 1.13] 1.32 [102, 1.13] 1.35 [102, 1.13] 1.35 [0.44, 0.87] 1.18 [0.48, 2.81] 1.05 [0.82, 1.34] 78% Odds Ratio <u>M-H, Random, 35%</u> , C 2.40 [1.54, 2.74] 0.63 [0.15, 4.26]	0.01	0.1 10 100 Finous (case) Ferous (control) 000 M-H. Random. 95% Cl 0.1 10 100 Farours (case) Ferours (control) Odds Ratio Odds Ratio Odds Ratio M-H. Random. 95% Cl
Total events Heterogeneity, Tar/ = 0.16, Tarat for varial effect 2 = 4 D Study or Subarroup E Aquiz 2012 Aquiz 2012 Aquiz 2012 Aquiz 2012 Aquiz 2012 Aquiz 2013 Simp 2020 Sixvatory 2013 Trivan 2006 Zaki 2015 Total events Heterogeneity, Tar/= 0.1 Express 2007 Aquiz 2017 Aquiz 2018 Aquiz 2017 Aquiz 2018 Aquiz 2017 Aquiz 2018 Aquiz	336 Chi <sup>2</sup> = 21 .77 (P < 0 Case vents T 137 53 124 158 171 78 3 908 908 1; Chi <sup>2</sup> = - 0.39 (P = Case vents T 4 4 4 4 6 4 4 5 3 908 4 1 7 1 1 1 1 1 1 1 1 1 1 1 1 1	1307 .68, df (- .00001) 	2832 7 (P = 1 Contro vents 16 69 66 678 93 103 128 34 1003 150 128 34 1003 159 (P = 1 Contro vents 150 150 150 150 150 150 150 150	0.003): 1 11138 102 174 216 274 274 274 274 274 446 398 448 174 3644 2<0.00 1 1 569 51 87 108	<pre>I<sup>2</sup> = 68% Weight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.6% 11.7% 15.1% 100.0% W001); I<sup>2</sup> = Weight 15.7% 4.7% 7.2% 9.3%</pre>	Odds Ratio MH. Bandom. <u>95%</u> C 1.94 (15.1, 260) 1.96 (15.8, 204) 0.81 (15.3, 123) 1.00 (15.6, 15.4) 0.84 (10.4), 1.77 1.86 (10.2, 181) 1.25 (10.4, 16.2) 0.25 (10.4, 16.2) 1.16 (0.48, 2.81) 1.16 (0.48, 2.81) 1.16 (0.48, 2.81) 1.16 (0.48, 2.81) 1.16 (0.48, 2.81) 1.16 (0.48, 2.81) 0.48 (0.47, 13.4) 0.49 (15.4, 13.4) 0.43 (15.1, 5.4) 0.43 (15.1, 5.4) 0.43 (15.1, 5.4)	0.01	0.1 10 100 Farcors (care) Farcous (control) M-U. Rendom 35% Cl 0.1 10 Farcors (care) Farcors (control) Odds Ratio Odds Ratio M-H. Random 35% Cl 
Total events           Test for event affects 2 = 4           D           Study or Subgroup           Aguit 2012           deLima 2018           Make: 2019           Make: 2019           Study or 2019           Study or 2019           Study or 2019           Make: 2019           Total (95% C1)           Agraval 2017           Agraval 2012           Aduinary 2018           Make: 2018           Make: 2018           Make: 2018           Make: 2012           Make: 2014           Make: 2014	3366 Chi <sup>2</sup> = 21 77 (P < 0) Case Vants Tr 137 137 137 137 137 137 137 137	1307 .68, df (- .00001) 	2832 = 7 (P = 1 Contro vents - 2266 16 69 93 103 150 128 34 1003 152 1003 159 2 7 7 13 11 10 12 13 159 13 14 159 159 159 159 159 159 159 159	0.003): 1 11138 102 174 276 274 274 274 274 274 446 398 448 174 3644 2<0.00 569 51 87 108 137 132	P = 68% Weight 12.1% 7.3% 9.9% 9.7% 10.4% 11.1% 11.5% 11.7% 11.2% 5.1% 100.0% 0001); P = Weight 15.7% 4.7% 7.2% 9.2% 9.2%	Odds Ratio 1.44 [5,12,60] 1.94 [1,5,1,2,60] 1.95 [0,5,8,2,04] 0.85 [0,5,1,124] 0.84 [0,61,1,17] 1.25 [0,44,1,65] 0.63 [0,46],2,61] 1.25 [0,44,1,65] 0.63 [0,46],2,61] 1.85 [0,42,1,34] 78% Odds Ratio MH.H.Randsm., 95%C MH.H.Rath, 3,24] 0.85 [0,23,1,26] 0.85 [0,23,2,26] 0.83 [0,23,2,26] 0.85 [0,23,2,26	0.01	0.1 10 100 Farours (care) Farours (control) 06d6 Ratio MH. Random 35% Cl 0.1 10 100 Farours (case) Farours (control) 06d6 Ratio MH. Random 95% Cl
Total events Heterogeneity: ray = 0.16. Tast for evenal effect 2 = 4 D Study: or Substroup E Aquit 2012 deLima 2018 Maku: 2012 Maku: 2012 Sunstav: 2013 Sunstav: 2013 Sunstav: 2013 Total (events Heterogeneity: Tayl= 0.1 Total (events Heterogeneity: Tayl= 0.2 Subdy: or Subgroup E Subdy: or Subgroup E Subgroup E Su	3366 : Chi'= 21 :77 (P < 0 Case (137 : 1 :137 : 1	1307 66, df 4 0,00001) 0tal E & 0,00001) 0tal X & 0,0001) 0tal X & 0,0001) 0,0001 0,0001 0,000	283 = 7 (P = 1 Contro 266 16 69 93 103 150 128 34 1003 152 26 Contro vents 103 152 34 152 103 152 103 152 103 152 152 152 152 152 152 152 152	0.003): 1 1 1 1 1 1 1 1 1 1 1 1 1	P = 68% Waight 12.1% 9.9% 10.4% 11.1% 11.6% 11.7% 11.6% 11.7% 10.0001; P = Weight 15.7% 10.0% 10.0% 12.2% 12.5%	Odds Ratio MH, Bandom, 35%, C 1.94 (1.63, 1.23) 1.09 [0.68, 2.04] 0.81 [0.53, 1.23] 0.82 [0.64, 1.54] 0.82 [0.64, 1.84] 0.83 [0.64, 0.87] 1.18 [0.46, 0.87] 1.18 [0.46, 0.87] 1.18 [0.46, 0.87] 1.18 [0.46, 0.87] 1.8 [0.42, 1.34] 78% Odds Ratio MH, Bandom, 35%, C 2.40 [1.54, 3.48] 0.43 [0.15, 3.48] 0.43 [0.15, 3.48] 0.43 [0.15, 3.48] 0.43 [0.13, 2.68] 0.43 [0.13, 2.68] 0.44 [0.13, 2.68] 0.4	0.01	0.1 10 100 Finours (care) Ferours (control) 0dds Ratio M-H. Random .95% Cl 0dds Ratio 0.1 10 100 Farours (case) Ferours (control) 0dds Ratio 0dds Ratio
Total events           Test for vanil effect 2 = 4           D           Study or Subgroup           Again 2012           dulma 2019           Mater 2013           Singly constraints           Survactor 2013           Singly constraints           Singly constraints           Singly constraints           Singly constraints           Singly constraints           Singly constraints           Test for overall effect 2 =           Singly constraints           Singly constraints           Mater 2016           Mater 2017           Mater 2018           Singly 2008           Singly 2001           Singly 2001	3366 : Chi'= 21 :77 (P < 0 Case (137 : 137 :	1307 66, df 4 000001) 0tal E 6 244 202 244 202 244 202 244 202 244 202 244 202 205 273 199 202	283 = 7 (P = 1 Contro 266 16 69 93 103 150 128 34 1003 152 20 Contro vents 10 27 7 13 11 18 11 33 17 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 15 10 128 13 13 15 10 13 15 10 128 13 13 15 10 13 15 13 13 13 13 13 13 13 13 13 13	i 0.003): i 1138 102 174 216 274 274 274 274 274 398 448 398 448 398 448 3644 i 264 569 51 87 108 137 223 199 924	P = 68% Waight 12.1% 9.9% 9.7% 9.7% 9.7% 10.4% 11.1% 11.1% 11.1% 11.1% 11.1% 11.2% 10.0001; P = Weight 15.7% 9.3% 10.2% 12.2% 12.2% 12.1% 15.1% 12.1% 15.2% 15.	Odds Ratio MH. Bandom, <u>95% C</u> 1,94 (15,1,260) 1,99 (15,8,204) 0,81 (10,3,123) 1,00 (10,65, 15,41) 0,86 (10,81, 127) 1,00 (10,65, 15,41) 0,84 (10,41, 117) 1,35 (10,44, 128) 1,16 (10,46, 281) 1,16 (10,46, 281) 1,16 (10,46, 281) 1,16 (10,46, 281) 1,16 (10,46, 281) 1,16 (10,46, 281) 1,16 (10,46, 281) 0,04 (10,13, 148) 0,04 (10,13, 148) 0,04 (10,13, 148) 0,04 (10,13, 148) 0,04 (10,13, 148) 0,04 (10,13, 148) 0,05 (10,27, 114) 1,16 (10,65, 2,268)	0.01	0.1 10 100 Farours (care) Farours (control) M-H. Rendom 55% Cl 
Total events Test events Test for event affect 2 = 4 D Study or Subgroup E Aguard 2012 deLima 2018 Makac 2012 Makac 2012 Makac 2012 Makac 2012 Makac 2012 Total (95% C1) Total (9	3366 : Chi <sup>+</sup> = 21 : Chi <sup>+</sup> = 1 : Chi	1307 568, df + 568, df + 568 244 202 202 277 266 538 244 202 202 277 410 538 40.03, ( 50.70) 548 412 203 546 546 546 546 546 546 546 546	2833 =7 (P = 1 Contro vents - 2266 69 66 69 66 93 103 150 128 34 150 128 34 150 128 34 150 128 34 150 150 150 150 150 150 150 150	0.003): 1 1 1 1 1 1 1 1 1 1 1 1 1	P* = 68% Weight: 7.3% 9.9% 9.7% 10.4% 11.7% 11.7% 11.7% 11.7% 11.7% 11.7% 11.7% 11.7% 11.7% 11.7% 10.0001); P = Weight: 15.7% 4.7% 9.3% 12.5% 12.5% 12.5% 12.5% 13.5% 12.5% 13.5% 1.4%	Odds Ratio MH, Random, 55% C 1.94 (15.7, 260) 0.57 (10.5, 12.0) 0.57 (10.5, 12.0) 0.58 (10.5, 12.0) 0.	0.01	ddd Ratio M-H. Random .95%. Cl Favours (case) Gate State M-H. Random .95%. Cl Hereitik (case) M-H. Random .95%. Cl Hereitik (case) M-H. Random .95%. Cl Hereitik (case) M-H. Random .95%. Cl
Total events Test events Test for wall effect 2 = 4 D Study or Subarroup E Adjust 2012 Adjust 2012 Adjust 2012 Adjust 2012 Adjust 2012 Adjust 2012 Singh 2008 Sixvatore 2013 Tiware 2009 Zaki 2015 Total (eVents Heterogeneity: Tayl <sup>®</sup> = 0.1 Total events Study or Subarroup E Aggust 2012 de Lima 2018 Adjust 2012 de Lima 2018 Adjust 2012 de Lima 2018 Adjust 2012 de Lima 2018 Adjust 2017 de Lima 2018 de Lima 20	3366 Chi <sup>2</sup> = 21 (2), 77 (P < 0) Case (vants Tr T 137 c) 137 c) 1	1307 568, df + 568, df + 568 244 202 202 277 266 538 244 202 202 277 266 538 244 202 202 202 202 202 202 202	2833 =7 (P = 1 Contro vents - 2266 69 66 69 66 93 103 150 128 34 150 128 34 150 128 34 150 128 34 150 150 150 128 34 150 150 150 150 150 150 150 150	0.003): 1 1 1 1 1 1 1 1 1 1 1 1 1	P* = 68% Weight: 7.3% 9.9% 9.7% 7.3% 9.7% 10.4% 11.6% 11.7% 11.7% 5.1% 100.0% 0001); P = Weight: 15.7% 7.2% 9.3% 12.5% 12.5% 12.5% 12.5% 12.5% 15.5% 15.5% 15.5% 0.3% 0.0	Odds Ratio MH, Bandom, 35% C 1.94 (1.53, 1.23) 1.09 [0.58, 2.04] 0.85 (0.54, 1.54] 0.85 (0.54, 1.54] 0.85 (0.54, 1.52) 0.83 [0.64, 0.87] 1.18 [0.46, 0.87] 1.18 [0.46, 0.87] 1.18 [0.46, 0.87] 1.18 [0.46, 0.87] 1.85 [0.84, 1.34] 78% Odds Ratio MH, Bandom, 35% C 2.40 [1.54, 3.74] 0.43 [0.15, 3.74] 0.53 [0.25, 1.14] 0.53 [0.25, 1.14] 0.55	0.01	0.1 10 Finous (case) Ferous (control) M-H. Random .95% Cl 
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Total events Test for event affects 2 = 4 D Study or Subgroup E Aquit 2012 dulma 2019 Makuz 2013 dulma 2019 Makuz 2013 Singh 2009 Suvakov 2013 Tixael 2009 Suvakov 2013 Ti	3366 ChP = 21,77 (P = 0 Case venta T, 70 137 : 137 : 137 : 137 : 137 : 137 : 137 : 137 : 138 : 1	1307 .66, df -( .60, d0001) .0111 E .000001) .0111 E .0011 .0111 E .0111 E	2833 = 7 (P = 1 Contro vents - 2266 66 69 93 103 1150 1157 115	I           I	IP = 68%           Weight           12.1%           7.3%           9.9%           9.7%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.2%           5.1%           100.0%           0001); IP =           02%           1.9%           12.1%           15.1%           102.5%           12.1%           12.5%           12.1%           12.1%           15.1%           100.0%           65); I* = 62           Keipht	Odds Ratio MH. Bandon, <u>55% C</u> 1.94 (15.1, 260) 1.96 [0.58, 2.04] 0.87 [0.58, 12.04] 0.88 [0.56], 1.62 0.84 [0.61, 1.77] 1.68 [0.42, 1.81] 1.25 [0.44, 2.81] 1.16 [0.48, 2.81] 1.16 [0.48, 2.81] 1.16 [0.48, 2.81] 1.16 [0.48, 2.81] 0.45 [0.42, 2.81] 0.45 [0.43, 2.81] 0.45 [0.44, 2.81] 0.45 [0.45, 2.81]	0.01	0.1 10 100 Farocons (cost) Forouns (control) M-H. Rendom 55% C1 0.1 100 Forouns (cost) Forouns (control) 0.1 100 Forouns (cost) Forouns (control) 0.1 100 Forouns (cost) Forouns (control) 0.1 100 Forouns (cost) Forouns (control) 0.1 100 0.1 100 Forouns (cost) Forouns (control) 0.1 100 Forouns (cost) Forouns
Total events Test events Test for event effect 2 = 4 D Study or Suberoup E Agua 2012 dullma 2018 Makuc 2012 dullma 2018 Makuc 2012 Makuc 2012 Makuc 2012 Total (95% C1) Total (95	3366 CnF = 21, 77 (P = 0 Case Venta: T, 77 (P = 0 Case Venta: T, 70 53 53 53 53 53 53 53 53 53 53 53 53 53	1307 1307 1307 166, df + 000001) 10tal E 368 36 36 30 40.03, cf 222 101 122 101 122 101 122 101 122 101 123 36 15 5 273 199 103 18 15 5 23.53, c 0.99) 0 1 2 23.53, c 0 9 3	2833 = 7 (P = 1 Contro vents - 2266 66 93 103 150 1128 34 1003 150 1128 34 103 150 128 34 103 150 128 34 113 103 150 1128 34 1128 117 1178 1178 1175 1178 1178 1177 1178 1178 1177 1178 1177 1178 1177 1178 1177 1178 1177 1178 1177 1178 1177 1178 1177 1178 1177 1178 1177 1178 1177 1178	I           I	IP = 68%           Weight           12.1%           7.3%           9.9%           9.7%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.1%           11.2%           5.1%           100.0%           15.7%           12.1%           12.5%           12.5%           12.5%           15.1%           11.4%           1.9%           11.4%           1.9%           11.5%	Odds Ratio MH, Bandom, <u>95%</u> , C 1.94 (15.8, 2.04) 0.91 (10.5, 1.23) 0.92 (10.5, 1.24) 0.82 (10.5, 1.24) 0.83 (10.5, 1.24) 0.85 (10.5, 1.24) 0.85 (10.5, 1.24) 1.35 (10.2, 1.34) 1.35 (10.2, 1.34) 78% Odds Ratio MH, Random, <u>19%</u> , C 2.40 (1.5, 1.74) 0.47 (10.3, 1.27) 0.47 (10.3, 1.27) 0.43 (10.4, 3.72) 1.60 (10.5, 1.24) 0.50 (10.3, 2.08) 0.50 (10.3, 1.72) 1.60 (10.5, 1.55) % Odds Ratio	0.01	0.1 10 100 Fisiours (case) Favours (control) Odds Ratio M-H. Random. 95% Cl Odds Ratio M-H. Random. 95% Cl 0.1 10 100 Favours (case) Favours (control) Odds Ratio M-H. Random. 95% Cl
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(caption on next page)

**Fig. 2.** Forest plot for overall analysis. (A) Analysis of the null genotype vs. the present genotype of *GSTM1*. (B) Analysis of the null genotype vs. the present genotype of *GSTM1*+*GSTT1*. (D) Analysis of the double null genotype vs. the present genotype of *GSTM1*+*GSTT1*. (D) Analysis of the *G* allele vs. the *G* + *A* allele in allelic comparison of GSTP1 rs1695. (E) Analysis of the *GG* genotype vs. the *GG* + *GA* + *AA* genotypes in the recessive model of GSTP1 rs1695. (F) Analysis of the *GG* + *GA* + *AA* genotypes in the dominant model of GSTP1 rs1695.

## 3.2. Meta-analysis of GST genes in overall CKD patients

In Fig. 2A–C, GSTM1 null (P = 0.0009), GSTT1 null (P = 0.0003), and double null (P < 0.00001) increased risk of CKD in total populations, but not in GSTP1 (in Fig. 2D–F).

# 3.3. Stratification analysis of studies by ethnicity

We performed a subanalysis on the Asian population (in Table 2) and other populations (in Table S6). The results showed significant associations in the total Asian population (P = 0.0001 for GSTM1 null, P = 0.001 for GSTT1 null, P < 0.00001 for double null, and P = 0.006 for GG allele, as shown in Table 2) and in the Southern Asian population (P = 0.03 for GSTM1 null, P = 0.02 for GSTT1 null, P = 0.008 for double null, and P = 0.002 for GG allele, as shown in Table 2) but not in the Eastern Asian and Western Asian populations. Surprisingly, no significant associations were found in the European population and USA population ( $P \ge 0.05$ , as shown in Table S6).

## 3.4. Risk of bias of the included studies

As shown in Table 3, GSTM1 null (P = 0.002), GSTT null (P = 0.001), and double null (P < 0.00001) were significantly associated with CKD risk in "low/moderate" studies but not in GSTP1. Furthermore, GSTT null (P = 0.03) and double null (P < 0.00001) were also related to the risk of CKD in "serious/critical" studies but not in GSTM1 null and GSTP1. This result indicated that the risk of bias might influence the overall results, and studies rated "low or moderate" had a higher quality for meta-analysis.

## 3.5. NOS star of selected studies

GSTM1 null (P = 0.005), GSTT null (P = 0.0003), and double null (P < 0.0001) were significantly associated with CKD risk in highquality studies assessed by the rewritten NOS in Table 4. Interestingly, the G allele (P = 0.04), GG allele (P = 0.0009), and GG + GA allele (P < 0.0001) were related to CKD risk in this approach, and these results were different from the results of studies rating "low/ moderate" bias. It was confirmed that GSTM1 and GSTT1 null genotypes were risk factors for CKD risk in a high-quality study assessed by two methods. However, the role of GSTP1 was ambiguous.

# Table 2

Stratification	analysis	of	studies	by	Asian 1	egion.
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Gene Symbols	Ethnicity	Location	No. of studies	Heterogeneity	Heterogeneity		OR	95%CI	Р
				Р	$I^2$				
GSTM1 null	Asian	All	16	0.0001	65 %	Random	1.48	1.21 - 1.81	0.0001
		Eastern Asian	5	0.41	0 %	Fixed	1.12	0.95-1.33	0.18
		Southern Asian	8	< 0.0001	79 %	Random	1.53	1.04 - 2.25	0.03
		Western Asian	3	0.004	82 %	Random	1.80	0.83-3.94	0.14
GSTT1 null	Asian	All	10	< 0.00001	82 %	Random	1.74	1.25-2.43	0.001
		Eastern Asian	2	Not Applicable	e				
		Southern Asian	5	< 0.00001	90 %	Random	2.05	1.11 - 3.79	0.02
		Western Asian	3	0.27	23 %	Fixed	1.33	0.99 - 1.78	0.06
double null	Asian	All	6	0.003	72 %	Random	2.43	1.65 - 3.57	< 0.00001
		Eastern Asian	2	Not Applicable	e				
		Southern Asian	3	0.002	84 %	Random	2.72	1.29-5.74	0.008
		Western Asian	1	Not Applicable	9				
G allele	Asian	All	4	< 0.00001	91 %	Random	1.11	0.67 - 1.83	0.70
		Eastern Asian	0	Not Applicable	e				
		Southern Asian	3	< 0.00001	93 %	Random	1.19	0.64-2.24	0.58
		Western Asian	1	Not Applicable	e				
GG	Asian	All	4	0.02	70 %	Random	1.57	1.14-2.16	0.006
		Eastern Asian	0	Not Applicable	9				
		Southern Asian	3	0.02	73 %	Random	1.72	1.23-2.43	0.002
		Western Asian	1	Not Applicable	e				
GG + GA	Asian	All	4	< 0.0001	88 %	Random	1.07	0.61 - 1.88	0.82
		Eastern Asian	0	Not Applicable	e				
		Southern Asian	3	< 0.00001	91 %	Random	1.15	0.56-2.36	0.70
		Western Asian	1	Not Applicable	9				

Not Applicable: 3 studies were needed to meta-analysis.

#### Table 3

Stratification analysis of risk of bias based on the ROBINS-I tool.

Gene Symbols	risk of bias	No. of studies	Heterogeneity	Heterogeneity		OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12 - 1.56	0.0009
	low/moderate	13	0.004	59 %	Random	1.35	1.11 - 1.65	0.002
	serious/critical	16	< 0.00001	79 %	Random	1.21	0.89-1.64	0.22
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	low/moderate	11	0.008	58 %	Random	1.50	1.17-1.91	0.001
	serious/critical	12	< 0.00001	82 %	Random	1.55	1.05 - 2.30	0.03
double null	All	8	0.003	68 %	Random	2.32	1.64 - 3.28	< 0.00001
	low/moderate	5	0.68	0 %	Fixed	1.83	1.45 - 2.31	< 0.00001
	serious/critical	3	0.27	24 %	Fixed	4.00	2.95-5.42	< 0.00001
G allele	All	10	< 0.00001	78 %	Random	1.05	0.82 - 1.34	0.70
	low/moderate	3	0.12	52 %	Random	1.15	0.88 - 1.51	0.30
	serious/critical	7	< 0.00001	83 %	Random	1.01	0.70-1.46	0.95
GG	All	10	0.005	62 %	Random	1.00	0.65 - 1.55	0.99
	low/moderate	3	0.18	41 %	Fixed	1.43	0.97 - 2.11	0.07
	serious/critical	7	0.003	69 %	Random	0.86	0.46 - 1.61	0.64
GG + GA	All	11	< 0.0001	73 %	Random	1.20	0.89-1.61	0.23
	low/moderate	4	0.28	22 %	Fixed	1.24	0.98 - 1.56	0.07
	serious/critical	7	< 0.0001	82 %	Random	1.23	0.77–1.96	0.38

## Table 4

Stratification analysis of study quality based on the reNOS tool.

Gene Symbols	NOS quality	No. of studies	Heterogeneity		Model	OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12-1.56	0.0009
	High quaility	14	0.001	61 %	Random	1.33	1.09-1.63	0.005
	Low quality	15	< 0.00001	80 %	Random	1.23	0.90 - 1.67	0.20
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	High quaility	12	< 0.00001	78 %	Random	1.84	1.33 - 2.56	0.0003
	Low quality	11	0.04	48 %	Random	1.22	0.97 - 1.55	0.09
double null	All	8	0.003	68 %	Random	2.32	1.64 - 3.28	< 0.00001
	High quaility	6	0.003	72 %	Random	2.44	1.58 - 3.78	< 0.0001
	Low quality	2	Not Applicable	e				
G allele	All	10	< 0.00001	78 %	Random	1.05	0.82 - 1.34	0.70
	High quaility	4	0.04	64 %	Random	1.40	1.02-1.93	0.04
	Low quality	6	0.07	52 %	Random	0.91	0.73-1.14	0.41
GG	All	10	0.005	62 %	Random	1.00	0.65 - 1.55	0.99
	High quaility	4	0.09	55 %	Random	1.84	1.28 - 2.63	0.0009
	Low quality	6	0.16	37 %	Fixed	0.92	0.67 - 1.25	0.59
GG + GA	All	11	< 0.0001	73 %	Random	1.20	0.89-1.61	0.23
	High quaility	5	0.12	45 %	Fixed	1.52	1.23-1.87	< 0.0001
	Low quality	6	0.003	73 %	Random	1.04	0.70 - 1.53	0.86

Not Applicable: 3 studies were needed to meta-analysis.

# 3.6. Subgroup analysis of deductions

Significant associations with CKD risk (P < 0.0001, and P < 0.0001 for double null) were found among studies with no deductions but not in GSTM1 null in Table 5. Not surprisingly, the GG allele (P = 0.001) and GG + GA allele (P = 0.01) were also related to CKD risk in a study with no deductions. GSTM1 null was associated with CKD risk in studies with deductions but not in GSTT1 null and GSTP1. These results were somewhat different from those in high-quality studies based on the reNOS scale.

## 3.7. Subgroup results of PCR methods

When stratified for multiplex PCR as shown in Table 6, GSTM1 (P < 0.0001) and GSTT1 (P = 0.008) null genotypes showed significant associations with CKD risk, but not in other PCR methods (P = 0.57 for GSTM1). These positive results should be carefully interpreted, as PCR methods are generally not considered a risk factor for quality, and they might not be representative. Subanalysis for PCR methods might be necessary due to judgement of genotypes with less arbitrariness.

## 3.8. Subgroup results of control selection

Control selection is important for case-control studies and might lead to different conclusions. We conducted this subanalysis to

#### Table 5

Subgroup analysis of deductions assessed by the rewritten NOS.

Gene Symbols	deductions	No. of studies	Heterogeneity	Heterogeneity		OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12-1.56	0.0009
	no deductions	17	0.0005	61 %	Random	1.22	1.00-1.49	0.05
	deductions	12	< 0.00001	75 %	Random	1.47	1.11-1.94	0.008
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	no deductions	13	< 0.00001	74 %	Random	1.96	1.44-2.67	< 0.0001
	deductions	10	0.19	28 %	Fixed	1.14	0.97 - 1.34	0.11
double null	All	8	0.003	68 %	Random	2.32	1.64-3.28	< 0.00001
	no deductions	6	0.003	72 %	Random	2.44	1.58 - 3.78	< 0.0001
	deductions	2	Not Applicable	2				
G allele	All	10	< 0.00001	78 %	Random	1.05	0.82 - 1.34	0.70
	no deductions	5	0.008	71 %	Random	1.27	0.90 - 1.80	0.17
	deductions	5	0.04	60 %	Random	0.90	0.70 - 1.14	0.38
GG	All	10	0.005	62 %	Random	1.00	0.65-1.55	0.99
	no deductions	5	0.11	46 %	Fixed	1.78	1.25-2.53	0.001
	deductions	5	0.10	49 %	Fixed	0.92	0.67 - 1.26	0.61
GG + GA	All	11	< 0.0001	73 %	Random	1.20	0.89-1.61	0.23
	no deductions	6	0.04	58 %	Random	1.61	1.12 - 2.31	0.01
	deductions	5	0.08	53 %	Random	0.89	0.66–1.19	0.42

Not Applicable: 3 studies were needed to meta-analysis.

# Table 6

# Subgroup results of PCR methods.

Gene Symbols	PCR method	No. of studies	Heterogeneity	Heterogeneity		OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12-1.56	0.0009
	Multiplex PCR	15	< 0.00001	72 %	Random	1.58	1.26 - 1.98	< 0.0001
	other PCR	14	0.006	55 %	Random	1.07	0.85 - 1.33	0.57
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	Multiplex PCR	14	0.0004	65 %	Random	1.38	1.09-1.76	0.008
	other PCR	9	< 0.00001	80 %	Random	1.75	1.13 - 2.71	0.01
double null	All	8	0.003	68 %	Random	2.32	1.64-3.28	< 0.00001
	Multiplex PCR	6	0.51	0 %	Fixed	2.04	1.63 - 2.56	< 0.00001
	other PCR	2	Not Applicable	2				

Not Applicable: 3 studies were needed to meta-analysis.

# Table 7

# Subgroup analysis of control selection.

Gene Symbols	Control	No. of studies	Heterogeneity		Model	OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12-1.56	0.0009
	healthy control	16	< 0.00001	75 %	Random	1.38	1.06 - 1.78	0.02
	selected control	16	0.0009	60 %	Random	1.36	1.10 - 1.68	0.004
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	healthy control	12	< 0.00001	83 %	Random	1.75	1.18 - 2.60	0.006
	selected control	13	0.07	40 %	Fixed	1.40	1.21 - 1.62	< 0.00001
GSTM1 + GSTT1 double null	All	8	0.003	68 %	Random	2.32	1.64 - 3.28	< 0.00001
	healthy control	5	0.03	63 %	Random	3.17	1.90-5.31	< 0.0001
	selected control	4	0.65	0 %	Fixed	1.78	1.38 - 2.30	< 0.0001
G allele	All	10	< 0.00001	78 %	Random	1.05	0.82 - 1.34	0.70
	healthy control	5	< 0.0001	84 %	Random	1.06	0.70 - 1.60	0.80
	selected control	5	0.007	71 %	Random	1.04	0.76 - 1.42	0.82
GG allele	All	10	0.005	62 %	Random	1.00	0.65 - 1.55	0.99
	healthy control	5	0.002	76 %	Random	0.91	0.41 - 2.02	0.82
	selected control	5	0.14	42 %	Fixed	1.19	0.85-1.66	0.31
GG + GA allele	All	11	< 0.0001	73 %	Random	1.20	0.89-1.61	0.23
	healthy control	5	0.0004	80 %	Random	1.28	0.76-2.16	0.35
	selected control	6	0.007	69 %	Random	1.14	0.79–1.65	0.48

reduce the potential arbitrariness. Three studies [18,40,44] had both healthy controls and selected controls. Data synthesis of these studies was recalculated. As shown in Table 7, the results showed significant associations in the healthy control population (P = 0.02 for GSTM1 null, P = 0.006 for GSTT1 null, P < 0.0001 for both null) and in the selected control population (P = 0.004 for GSTM1 null, P < 0.0001 for GSTT1 null, P < 0.0001 for both null) but not in GSTP1. These results were consistent with those of the total population, which indicated that control selection might have no effect on the final conclusions. The result might change our opinions that community control might be more representative than selected control in terms of these meta-results.

## 3.9. Data synthesis of case selection based on ESRD and non-ESRD

In the above results, patients were enrolled based on different stages of CKD. To reach accurate conclusions and verify the results of the cohort studies, the patients were divided into different groups based on the progression of CKD (ESRD and non–ESRD), etiology of CKD (DN and non–DN), allograft function (allograft dysfunction and stable graft function, SGF), and all–cause death (death and alive).

We performed this subanalysis to ascertain whether GST polymorphisms were associated with the progression of CKD. As shown in Table 8, GSTM1 deletion was associated with ESRD (P = 0.003) but not non–ESRD (P = 0.07). GSTT1 deletion was associated with ESRD risk (P = 0.02) and non–ESRD risk (P = 0.003). Double null also showed a significant association with ESRD risk (P < 0.0001) and non–ESRD risk (P = 0.001). Surprisingly, the G allele was a protective factor for the risk of non–ESRD, but not in GG and GG + GA. However, only 3 studies were included in this subgroup, and the results should be interpreted with caution.

#### 3.10. Data synthesis of case selection based on DN and non-DN

The results in Table 9 show that GSTM1 null was weakly associated with DN risk (P = 0.04) but not in non-DN (P = 0.06). GSTT1 null was associated with DN risk (P = 0.005) and non-DN risk (P = 0.005). Double null showed a significant association with non-DN risk (P = 0.0004). G (P = 0.02) allele also seemed to be a protective factor for DN risk, but not in the GG + GA allele. However, only 4 studies were included in this subgroup, and the result should be interpreted with caution.

## 3.11. Data synthesis of case selection based on DM-ESRD and DM-CKD

Subsequently, data synthesis of DM–ESRD and DM–CKD was conducted to confirm whether GST polymorphisms were associated with the progression of CKD in DM patients. When we reanalyzed the association among patients with DM in Table 10, the positive effects of GSTM1 deletion were missing in DM–ESRD (P = 0.17) and DM–CKD (P = 0.14). GSTT1 deletion showed a weak association with DM–ESRD (P = 0.04) but not with DM–CKD (P = 0.11). Double null was not suitable for analysis. Interestingly, the G (P = 0.04) allele showed a weakly diminished risk of DM–CKD, but not the GG genotype and GG + GA genotype.

## 3.12. Data synthesis of case selection based on DM-ESRD and non-DM ESRD

ESRD patients were divided into two groups (DM–ESRD and non–DM ESRD). The positive effects of GSTM1 deletion were also missing in DM–ESRD (P = 0.17) and non–DM ESRD (P = 0.28), as shown in Table 11. GSTT1 deletion showed a weak association with DM–ESRD (P = 0.04) but not with non–DM ESRD (P = 0.36). No significant associations were observed in double null and GSTP1 polymorphisms (all P > 0.05). These results revealed that GSTM1 and GSTT1 deletions were not associated with ESRD in non–DM

## Table 8

Subgroup	analysis	of ESRE	) and	non-ESRD
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Gene Symbols	ESRD vs. non-ESRD	No. of studies	Heterogeneity		Model	OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12-1.56	0.0009
	ESRD	17	< 0.00001	75 %	Random	1.38	1.12 - 1.70	0.003
	non-ESRD	14	0.0009	63 %	Random	1.31	0.98-1.74	0.07
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	ESRD	14	< 0.00001	81 %	Random	1.46	1.07-1.99	0.02
	non-ESRD	11	0.02	52 %	Random	1.52	1.15 - 2.01	0.003
double null	All	8	0.003	68 %	Random	2.32	1.64-3.28	< 0.00001
	ESRD	5	0.004	74 %	Random	2.7	1.68-4.33	< 0.0001
	non-ESRD	3	0.62	0 %	Fixed	1.75	1.25-2.46	0.001
G allele	All	10	< 0.00001	78 %	Random	1.05	0.82 - 1.34	0.70
	ESRD	7	0.0003	77 %	Random	1.14	0.87 - 1.48	0.35
	non-ESRD	3	0.16	46 %	Fixed	0.77	0.60-0.99	0.04
GG	All	10	0.005	62 %	Random	1.00	0.65 - 1.55	0.99
	ESRD	7	0.009	65 %	Random	1.17	0.72 - 1.90	0.52
	non-ESRD	3	0.94	0 %	Fixed	0.61	0.33 - 1.12	0.11
GG + GA	All	11	< 0.0001	73 %	Random	1.20	0.89-1.61	0.23
	ESRD	8	0.03	68 %	Random	1.3	0.95 - 1.78	0.10
	non-ESRD	4	0.01	73 %	Random	1.08	0.60-1.97	0.79

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#### Table 9

Subgroup analysis of DN and non-DN.

Gene Symbols	DN vs. non-DN	No. of studies	Heterogeneity		Model	OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12-1.56	0.0009
	DN	13	< 0.0001	73 %	Random	1.40	1.02 - 1.92	0.04
	non-DN	19	< 0.00001	71 %	Random	1.21	0.99-1.48	0.06
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	DN	10	0.002	66 %	Random	1.67	1.17 - 2.39	0.005
	non-DN	16	< 0.00001	79 %	Random	1.49	1.13-1.96	0.005
double null	All	8	0.003	68 %	Random	2.32	1.64 - 3.28	< 0.00001
	DN	2	Not Applicabl	e:				
	non-DN	8	0.0005	73 %	Random	2.09	1.39-3.15	0.0004
G allele	All	10	< 0.00001	78 %	Random	1.05	0.82 - 1.34	0.70
	DN	4	0.29	19 %	Fixed	0.78	0.63-0.96	0.02
	non-DN	6	0.0007	77 %	Random	1.20	0.90 - 1.58	0.21
GG	All	10	0.005	62 %	Random	1.00	0.65 - 1.55	0.99
	DN	4	0.97	0 %	Fixed	0.58	0.33 - 1.00	0.05
	non-DN	6	0.02	64 %	Random	1.29	0.80 - 2.10	0.30
GG + GA	All	11	< 0.0001	73 %	Random	1.20	0.89-1.61	0.23
	DN	5	0.02	67 %	Random	1.01	0.62 - 1.63	0.97
	non-DN	6	0.003	72 %	Random	1.35	0.95 - 1.90	0.09

Not Applicable: 3 studies were needed to meta-analysis.

## Table 10

Subgroup analysis of DM-ESRD and DM-CKD.

Gene Symbols	DM-ESRD vs. DM-CKD	No. of studies	Heterogeneity		Model	OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12 - 1.56	0.0009
	DM with ESRD	6	0.005	70 %	Random	1.31	0.89 - 1.92	0.17
	DM with CKD	8	0.0003	74 %	Random	1.47	0.88 - 2.45	0.14
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	DM with ESRD	4	0.03	66 %	Random	1.74	1.02 - 2.97	0.04
	DM with CKD	7	0.005	67 %	Random	1.51	0.92-2.48	0.11
double null	All	8	0.003	68 %	Random	2.32	1.64-3.28	< 0.00001
	DM with ESRD	1	Not Applicabl	e:				
	DM with CKD	1	Not Applicabl	e:				
G allele	All	10	< 0.00001	78 %	Random	1.05	0.82 - 1.34	0.70
	DM with ESRD	1	Not Applicabl	e:				
	DM with CKD	3	0.16	46 %	Fixed	0.77	0.60-0.99	0.04
GG	All	10	0.005	62 %	Random	1.00	0.65 - 1.55	0.99
	DM with ESRD	1	Not Applicable:					
	DM with CKD	3	0.94	0 %	Fixed	0.61	0.33 - 1.12	0.11
GG + GA	All	11	< 0.0001	73 %	Random	1.20	0.89-1.61	0.23
	DM with ESRD	2	Not Applicabl	e:				
	DM with CKD	4	0.01	73 %	Random	1.08	0.60–1.97	0.79

Not Applicable: 3 studies were needed to meta-analysis.

#### patients.

## 3.13. Data synthesis of case selection based on allograft function

Finally, allograft dysfunction (such as delayed graft function and rejection episodes) of renal transplant recipients (RTRs) was analyzed compared to that of stable graft function (SGF) controls or healthy controls. Two studies [7,25] were additionally included in this subgroup, as they had SGF controls. As shown in Table 12, the GSTM1 null showed a weak association with allograft dysfunction in studies that selected SGF patients as controls but not in normal controls and GSTT1 null patients. This result indicated that GSTM1 deletion might be a risk factor for allograft dysfunction in RTRs.

# 3.14. Data synthesis of case selection based on all-cause mortality

Considering that GSTM1 might be involved in allograft dysfunction, we tried to determine the association between GSTM1 null and all-cause mortality in RTRs. Only three studies [10,29,46] were included in this subgroup. Interestingly, the GSTM1 null genotype had a 2.43-fold increased risk for all-cause death, as shown in Table 13. This proved that GSTM1 might be a risk factor for all-cause mortality in RTRs. This conclusion should be carefully interpreted as only three studies were included, and anti-OS therapy might be

## Table 11

Subgroup analysis of DM-ESRD and non-DM ESRD.

Gene Symbols	DM-ESRD vs. non-DM ESRD	No. of studies	Heterogeneity		Model	OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All	29	< 0.00001	68 %	Random	1.32	1.12 - 1.56	0.0009
	DM-ESRD	6	0.005	70 %	Random	1.31	0.89 - 1.92	0.17
	non-DM ESRD	5	0.04	60 %	Random	1.17	0.88 - 1.57	0.28
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	DM-ESRD	4	0.03	66 %	Random	1.74	1.02 - 2.97	0.04
	non-DM ESRD	5	< 0.00001	93 %	Random	1.48	0.64-3.44	0.36
double null	All	8	0.003	68 %	Random	2.32	1.64 - 3.28	< 0.00001
	DM-ESRD	1	Not Applicable					
	non-DM ESRD	3	< 0.00001	95 %	Random	2.14	0.39-11.62	0.38
G allele	All	10	< 0.00001	78 %	Random	1.05	0.82 - 1.34	0.70
	DM-ESRD	1	Not Applicable					
	non-DM ESRD	2	Not Applicable					
GG	All	10	0.005	62 %	Random	1.00	0.65 - 1.55	0.99
	DM-ESRD	2	Not Applicable					
	non-DM ESRD	2	Not Applicable					
GG + GA	All	11	< 0.0001	73 %	Random	1.20	0.89-1.61	0.23
	DM-ESRD	2	Not Applicable					
	non-DM ESRD	2	Not Applicable					

Not Applicable: 3 studies were needed to meta-analysis.

## Table 12

Subgroup analysis of SGF control and normal control.

Gene Symbols	allograft dysfunction	No. of studies	Heterogeneity		Model	OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All SGF control	29 5	<0.00001 0.19	68 % 34 %	Random Fixed	1.32 1.31	1.12–1.56 1.01–1.70	0.0009 0.04
	normal control	3	0.04	69 %	Random	1.31	0.70-2.45	0.40
GSTT1 null	All	23	< 0.00001	74 %	Random	1.52	1.21 - 1.90	0.0003
	SGF control normal control	4 3	0.14 0.54	45 % 0 %	Fixed Fixed	0.91 1.04	0.65 - 1.28 0.68 - 1.60	0.61 0.84

meaningful for the treatment of these patients.

# 4. Discussion

We found significant associations between deletions of GSTM1 and GSTT1 and CKD risk in Southwest China but failed to find associations in GSTP1 rs1695. While the results of cohort studies were positive and consistent, the positions of GST genes were somewhat different in case-control studies. In the USA, Yan reported that deletions of GSTM1 and GSTT1 were not associated with ESRD [15]. Negative associations were also found in European populations [21,38], Eastern Asia [37], and Northern Africa [39]. The participants in these studies were mainly DM patients, which might be the reason why no associations were found. As shown in Table 10, the meta-results also proved that GSTM1 null was not associated with DM-CKD or DM-ESRD. These results suggested that GSTM1 deletion might behave differently in DM-CKD patients. Our case-control results showed no differences in the distributions of GST gene polymorphisms based on gender (data not shown). We reviewed other case-control studies listed in Table S1 to fully understand the associations and found that the results were conflicting.

Then, we conducted this meta-analysis on 30 studies and quantified positive associations between deletions of GSTM1 and GSTT1 and CKD risk but failed in GSTP1 rs1695 among three genetic models. We also found that the current evidence was influenced by ethnicity, study quality, and PCR method but not control selection. Given the extended concept of CKD patients, a subanalysis of disease types was executed, and the results remained positive. While these polymorphisms were not associated with DM–CKD, we considered that CKD patients could benefit from anti–OS therapy.

Table 13					
Subgroup analysis	of dead	patients	and	alive	patients.

Gene Symbols	Dead vs. alive	No. of studies	Heterogeneity		Model	OR	95%CI	Р
			Р	$I^2$				
GSTM1 null	All dead/alive	29 3	<0.00001 0.68	68 % 0 %	Random Fixed	1.32 2.43	1.12–1.56 1.71–3.44	0.0009 <0.00001

When restricted to studies with ethnicity, positive results were observed only in Southern Asia but not in Eastern Asia or other populations. This finding revealed that race might provide diversity and the Southern Asian population with GST gene polymorphisms might be more susceptible to CKD risk. Our case-control results were positive and different from the overall results in Eastern Asia. The different baseline characteristics of the population in Southwest China might explain this different finding. The present study provides a new perspective on the delicate position of GST genes in Chinese CKD patients.

To fully understand the possible influence of study quality, three methods were used for quality assessment. In the approach using the ROBINS–I tool, bias mainly came from the "bias due to confounding" domain and "bias due to selective reporting of the results" domain. Potential data bias and heterogeneity might be the reason for negative results in studies rated as "serious/critical" bias. In terms of the rewritten NOS scale, the selection of controls (such as community controls), comparability (such as age, sex, smoking, and BMI), and inconsistent data might be the other important factors for quality assessment. As shown in Table 4, significant associations were also found in GSTM1 null, GSTT1 null, double null and G allele of GSTP1. These results indicated that quality assessment was necessary for meta-analysis and that the synthetic results of high-quality studies were more robust.

To explore the potential arbitrariness, we also conducted subanalysis for PCR methods and control selection. Interestingly, PCR methodology might affect the conclusions, but not control selection. Since the multiplex PCR technique was not more accurate than other PCR methods, these results were somewhat impressive and hard to interpret. One possible reason was that the genotype determined by multiplex PCR might be more comparable due to less arbitrariness. Different methods have different abilities to identify SNPs or indels, and the genotypes should be rechecked with manual inspection [53]. Multiplex PCR had the same quality for the determination of genotypes and might have less arbitrariness in large amounts of data.

We also conducted another subanalysis. First, GSTM1 null was proven to be a risk factor for CKD progression, which was the same as in cohort studies [2,5,8,11]. Positive associations were also found in the GSTT1 null and double null groups. These results provide more awareness of the relationships between GST genes and CKD risk. Other factors, such as cruciferous vegetables, were also involved in CKD events and progression in a cohort study [9]. This finding provided a possible explanation for why the GSTM1 null behaved differently in the Southwest Chinese population compared to other Eastern Asian populations. In Southwest China, dietary factors, such as Houttuynia cordata, might account for this difference. In our case–control study, more than 63 % of patients had ESRD or were under hemodialysis. It also provided a new perspective on the delicate position of GSTT1 and GSTP1 in ESRD patients.

Second, the previous meta-analysis showed that GSTM1 and GSTT1 deletions were associated with T2DM [47,48]. However, conflicting results were observed between GSTM1/T1 deletions and DN [49–51]. Our results indicated that GSTM1 and GSTT1 null genotypes were risk factors for DN. Interestingly, the G allele was somewhat different, and the role of GSTP1 might be protective and ambiguous. However, its effects should be interpreted with caution due to the small sample size effect.

Third, our results showed that GSTM1 null was not associated with CKD progression in DM patients. The subanalysis produced interesting results compared to the positive associations in DN. GSTM1 null was reported to be associated with DM and DN risk, whereas it might not be a risk factor for CKD progression in DM patients. Considering that the positive effect of GSTM1 null was missing, a subanalysis for non–DM ESRD also showed that GSTM1 null was not associated with non-DM ESRD. One possible reason was that the etiology of CKD was different, and the baseline characteristics of DM–CKD patients were different from those of other disease types.

Fourth, allograft dysfunction and all-cause death of RTRs were subsequently analyzed to determine the potential role of GSTM1 deletion. It was shown that GSTM1 null, but not GSTT1 null, might be a risk factor for allograft dysfunction and all-cause death. Although GSTT1 antigens were reported to be responsible for the occurrence of antibody-mediated kidney graft rejection [52], GSTM1 null might be more important in terms of the meta-results. This result suggested that identification of the GSTM1 genotype might be useful and that anti-OS therapy should be promoted in RTRs or dialysis patients.

There were some limitations in this study. First, the survival rate was not analyzed in the case–control study. Second, other factors might have effects on the results of GSTM1 and GSTT1 deletions, such as baseline characteristics. However, this information was hard to collect. Third, an extended concept of CKD was used for data collection, which might introduce some selection bias. Subanalysis of disease types was performed to eliminate selection bias, and the results were still positive.

Thus, we found significant associations between deletions of GSTM1 and GSTT1 and CKD patients in Southwest China but failed in GSTP1 rs1695. The meta–analysis of 30 included studies also showed positive results, and the results were influenced by ethnicity, study quality, and PCR method but not control selection. Subanalysis of disease types remained positive. However, the relationship between GST genes and DM-CKD patients was different and should be further researched.

#### **Ethics statement**

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chengdu Medical College (CYFY-2022011). Written informed consent was obtained from all participants.

#### Author contribution statement

Jie Peng: Conceived and designed the experiments; Performed the experiments; Wrote the paper, Pei Ma; Xueqin Wu; Tianrong Yang; Yuting Hu: Performed the experiments. Ying Xu: Contributed reagents, materials, analysis tools or data. Shuang Li: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data. Hang Zhang: Contributed reagents, materials, analysis tools or data; Wrote the paper. Hongzhou Liu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

#### Data availability statement

Data will be made available on request.

#### Declaration of competing interest

The authors declared no potential conflicts of interest.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21183.

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