

Effects of genetic polymorphisms of glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) on the risk of diabetic nephropathy: a meta-analysis

Jan Orlewski¹, Ewa Orlewska²

¹ Chair and Department of Internal Diseases and Endocrinology, Medical University of Warsaw, Warsaw, Poland

² Department of Physiology, Pathophysiology and Infectious Microbiology, The Jan Kochanowski University, Kielce, Poland

KEY WORDS

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ABSTRACT

INTRODUCTION Glutathione S-transferases (GSTs) belong to a family of ubiquitous and multifunctional enzymes that protect the cells against oxidative stress.

OBJECTIVES The aim of the study was to evaluate the association between the polymorphisms of glutathione-S-transferase (GST) genes and diabetic nephropathy (DN).

PATIENTS AND METHODS PubMed, EMBASE, and Google Scholar databases were systematically searched to identify relevant studies. The odds ratio (OR) for the association was determined using a fixed or random effects model. Tests for heterogeneity of the results and sensitivity analyses were performed.

RESULTS A total of 9 publications (874 patients in the study group, 966 controls) were included. With the exception of 1 study, GSTT1 and GSTM1 genotypes were not assessed by methods that measure a gene copy number. A significantly increased risk of DN was found for the GSTM1(–) genotype (OR, 1.27; 95% CI, 1.02–1.58) and the combination of GSTT1(–)/GSTM1(–) (OR, 2.02; 95% CI, 1.22–3.36). We did not observe a correlation between DN and the GSTT1(–) genotype or the presence of Val alleles. In a subgroup analysis, an association between DN and the GSTM1(–) genotype was significant in Asians but not in Caucasians.

CONCLUSIONS Our results indicate that the GSTM1(–) genotype and the combination of GSTT1(–)/GSTM1(–) increase the risk of DN. The combination of the GST polymorphisms rather than individual polymorphism should be investigated. Genotyping allowing a trimodular determination of the GST copy number variations may better describe an association between the risk of disease and a given genotype.

INTRODUCTION Diabetic nephropathy (DN), one of the major microangiopathic chronic diabetic complications, is associated with an increased risk of major cardiovascular events and all-cause mortality.¹ DN is now the major cause of chronic kidney disease throughout the world and is the largest single cause of end-stage renal disease, accounting for nearly half of the patients entering dialysis each year.² The etiopathogenesis of DN is clearly multifactorial, including genetic and environmental factors. The most well-known factor is long-lasting hyperglycemia, which causes tissue damage through 5 major mechanisms: 1) increased flux of glucose and other sugars through

the polyol pathway; 2) increased intracellular formation of advanced glycation endproducts (AGEs); 3) increased expression of the receptor for AGEs and its activating ligands; 4) activation of protein kinase C isoforms; and 5) overactivity of the hexosamine pathway.³ Evidence indicates that all 5 mechanisms are activated by mitochondrial overproduction of reactive oxygen species (ROS).^{3,4} Oxidative stress occurs when the production of oxidants exceeds local antioxidant capacity; thus, genetic polymorphisms reducing the activity of antioxidant enzymes could increase a person's susceptibility to DN.⁵

Correspondence to:
Ewa Orlewska, MD, PhD, Zakład
Fizjologii, Patofizjologii i Mikrobiologii
Infekcyjnej, Uniwersytet Jana
Kochanowskiego, Al. IX Wieków
Kielce 19, 25-317 Kielce, Poland,
phone: +48 41 349 69 09,
fax: +48 41 349 69 16, e-mail:
eorl@ujk.edu.pl

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Glutathione-S transferases (GSTs; EC 2.5.1.18) play an important role in the body's defense against ROS—they inactivate the cyto- and genotoxic electrophiles (secondary metabolites of ROS) by catalyzing their conjugation with glutathione.⁶ GST enzymes are coded by at least 8 distinct loci: α (GSTA), μ (GSTM), θ (GSTT), π (GSTP), σ (GSTS), κ (GSTK), ω (GSTO), and τ (GSTZ). Three loci in particular, GSTM1, GSTT1, and GSTP1, have received most of the attention. The human μ -class GST is encoded by a 100-kb gene cluster ordered 5' GSTM4-GSTM2-GSTM1-GSTM5-GSTM3 3', located on chromosome 1p13.3. GSTM1 is one of the genes encoding the μ class of enzymes and 3 polymorphisms have been identified. One polymorphism is a deletion that results in a lack of functional gene product (GSTM1[–]). Individuals who are homozygous for this allele are unable to produce the GSTM1 protein.^{7,8} The other two, GSTM1*A and GSTM1*B, differ by a C519G substitution, resulting in asparagine (Asn) to Lys substitution at amino acid 173.⁷ Despite the limited number of substrate types used for comparison tests, no evidence of functional difference between GSTM1*A and GSTM1*B variants was found; thus, these alleles are typically categorized together as a single functional phenotype.⁷ The θ class *GSTT1* located on chromosome 22q11.2 is also polymorphic and presents 2 alleles, *GSTT1*1* active allele and the *GSTT1*0* null gene. *GSTT1*0* is a nonfunctional allele resulting from the deletion of the *GSTT1* gene. Persons with homozygous deletion of the GSTT1 locus (GSTT1 0/0) have no enzymatic functional activity of the respective enzyme.^{9,10} The glutathione S-transferase P1 (GSTP1) gene is located on chromosome 11q13. The human GSTP1 locus comprises 4 different alleles: GSTP1*A (wild-type Ile¹⁰⁵ → Ala¹¹⁴), GSTP1*B (Val¹⁰⁵ → Ala¹¹⁴), GSTP1*C (Val¹⁰⁵ → Val¹¹⁴), and GSTP1*D (Ile¹⁰⁵ → Val¹¹⁴). GST enzyme activity is significantly lower among individuals with 105Val allele owing to a polymorphism at nucleotide 313 in the GSTP1 gene (A-to-G transition at nucleotide 313, causing a change of isoleucine [Ile] to valine [Val] at codon 105).¹¹

In the last few years, some investigations have been done on the associations of DN with the genetic polymorphism of GSTs. However, these studies reported inconsistent results, possibly owing to a small effect of the polymorphism on the risk of DN, relatively small sample size, ethnic background, or differences in the clinical status of patients.

Meta-analysis is a widely used method to augment statistical power and to draw a more convincing conclusion through the pooling of data from individual association studies.¹² Therefore, we performed a meta-analysis of the published studies to clarify inconsistency in study results and to establish a comprehensive picture of the relationship between the deletion polymorphisms in GSTM1 and GSTT1 as well as a single nucleotide polymorphism in GSTP1 (rs1695) genes and DN.

PATIENTS AND METHODS Literature search and data extraction Papers published before the end of January 2015 were identified through a search of Pubmed, Embase, Cochrane Library, and Google Scholar, using different possible variations and combinations of the following terms “glutathione S-transferases” or “GST” and “polymorphism” and “diabetic nephropathy” without restriction in language and time. The reference list of each relevant publication was also examined to identify additional studies appropriate for inclusion in the meta-analysis. Studies that met all of the following criteria were considered eligible: 1) case-control study, 2) investigation of the association between GSTM1/T1/P1 polymorphisms and DN, and 3) providing the information on genotype frequencies of GSTM1/T1/P1 polymorphism in both cases and controls. For each study, the following information was extracted independently by 2 investigators: first author's surname, year of publication, country/region, ethnicity, gender, genotyping method, clinical characteristics, confirmation of diagnosis, sample size of cases and controls, genotype and allele frequencies of cases and controls, Hardy-Weinberg equilibrium (HWE). The results were compared and disagreements were discussed and resolved with consensus. Where essential information was not presented in articles, every effort was made to contact the authors.

Statistical analysis The main meta-analysis for each polymorphism compared DN (considered as cases) vs diabetes patients without DN (considered as controls). The association between GSTM1, T1, and P1 (Ile105Val) polymorphisms and risk of DN was expressed as odds ratio (OR) and 95% confidence interval (CI). Statistical heterogeneity across studies included in the meta-analysis was assessed by Cochran Q statistic (a significant Q-statistic [$P < 0.10$] indicated heterogeneity across the studies). An I^2 statistic was used to evaluate whether inconsistencies among studies were attributed to heterogeneity rather than chance. The following suggested cut-off points were used: $I^2 = 0\%$ – 25% , no heterogeneity; $I^2 = 25\%$ – 50% , moderate heterogeneity; $I^2 = 50\%$ – 75% , large heterogeneity; $I^2 = 75\%$ – 100% , extreme heterogeneity.¹³ If heterogeneity existed, the random effects model was adopted to calculate the overall OR value.¹⁴ Otherwise, the fixed effects model was used. In addition, sources of heterogeneity were investigated by stratified meta-analyses based on the type of diabetes, ethnicity, and sample size (the total number of patients, <150 or ≥ 100), existence of diabetic retinopathy, hemoglobin A_{1c} (HbA_{1c}) level, and duration of diabetes. To look for bias, we used 3 tools: 1) the funnel plot, 2) Begg and Mazumdar test based on the rank correlation between the observed effect sizes and observed standard errors, and 3) Egger linear regression test at a P level of significance of less than 0.10.¹⁵ To assess the stability of the result, sensitivity analyses were performed, each

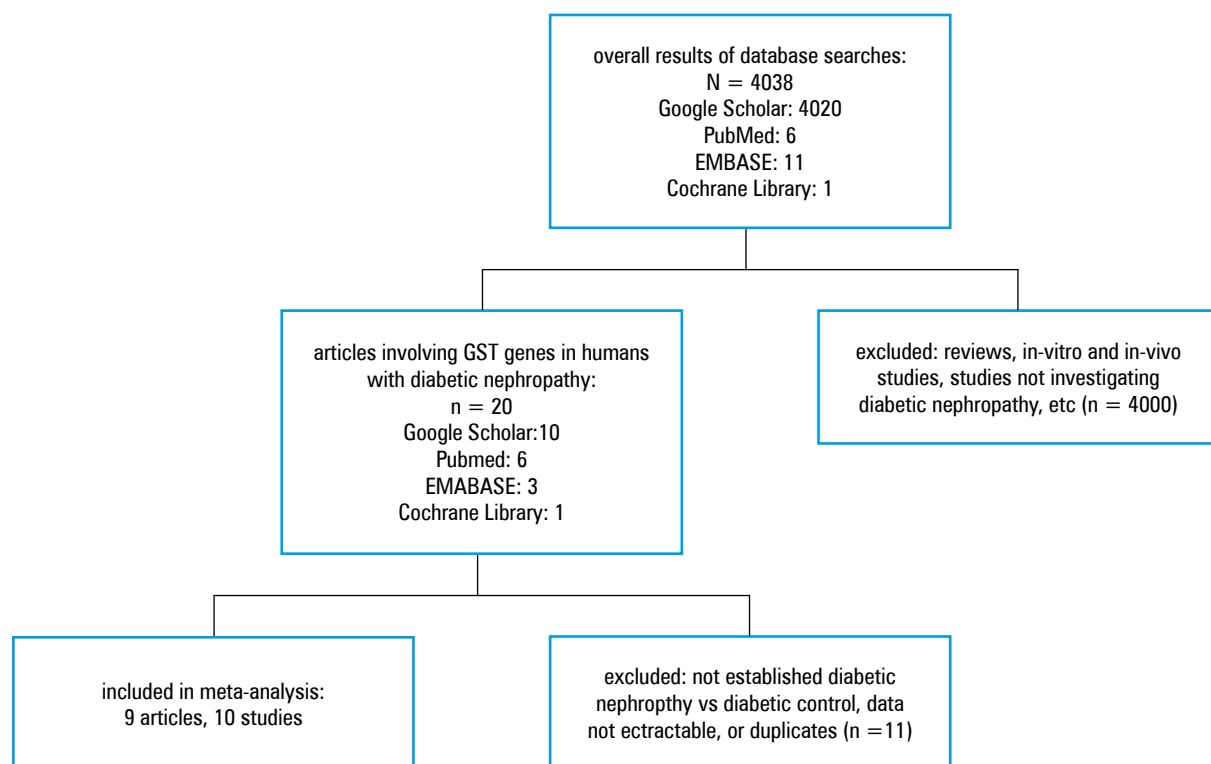


FIGURE 1 Flowchart of study selection

study in turn was removed from the total, and the remaining were reanalyzed. All the analyses were carried out using StatsDirect version 2.8.0. All *P* values were 2-sided at a *P* value of 0.05, except where otherwise specified.

RESULTS Characteristics of studies The initial literature search yielded 4038 references (PubMed, 6; EMBASE, 11; Cochrane Library, 1; Google Scholar, 4020). Of these, 4011 were excluded because they did not meet the criteria or were overlapping references (FIGURE 1). Finally, a total of 9 publications (10 studies) were included, involving 874 cases (125 Caucasian, 731 Asians, and 18 Egyptians) and 966 controls (197 Caucasians, 760 Asians, and 9 Egyptians).^{16–24} Sample sizes ranged between 27²² and 361.¹⁸ Type 1 diabetes was reported for 124 patients, type 2 diabetes—for 1716. The detailed characteristics of the studies included in this meta-analysis are shown in TABLE 1. There were 6 studies with 481 DN cases and 558 controls concerning the GSTT1 polymorphism,^{17,18,20–22,24} 8 studies with 678 DN cases and 742 controls concerning the GSTM1 polymorphism,^{16–18,20–24} and 5 studies (4 publications) with 475 DN cases and 525 controls concerning the GSTP1 polymorphism.^{18,19,21,22} The frequencies of homozygous deletion of both GSTM1 and GSTT1 genes were reported only in 2 studies.^{17,20} The GSTP1 polymorphism was found to occur in frequencies consistent with HWE in the control populations of the vast majority of the published studies evaluating this genotype. HWE was not assessed for the GSTM1 and GSTT1 variants because heterozygous individuals could not be distinguished from the homozygous wild type. In 9 studies, the genotyping approach did not allow

for detecting heterozygous carriers of GSTM1 or GSTT1 deletion; hence, the GSTM1-0 or GSTT1-0 genotype group included only patients homozygous for GSTM1 or GSTT1 deletion. The GSTM1-1 or GSTT1-1 genotype group included homozygous and heterozygous carriers of the functional allele. Blind genotyping was not reported in any study.

In the majority of the studies, patients in case and control groups were well matched with regard to age, body mass index, diabetes duration, and HbA_{1c}. Only in 2 studies, HbA_{1c} level was significantly lower in the DN groups,^{17,18} and in 2 studies duration of diabetes was significantly shorter in the DN groups.^{21,19}

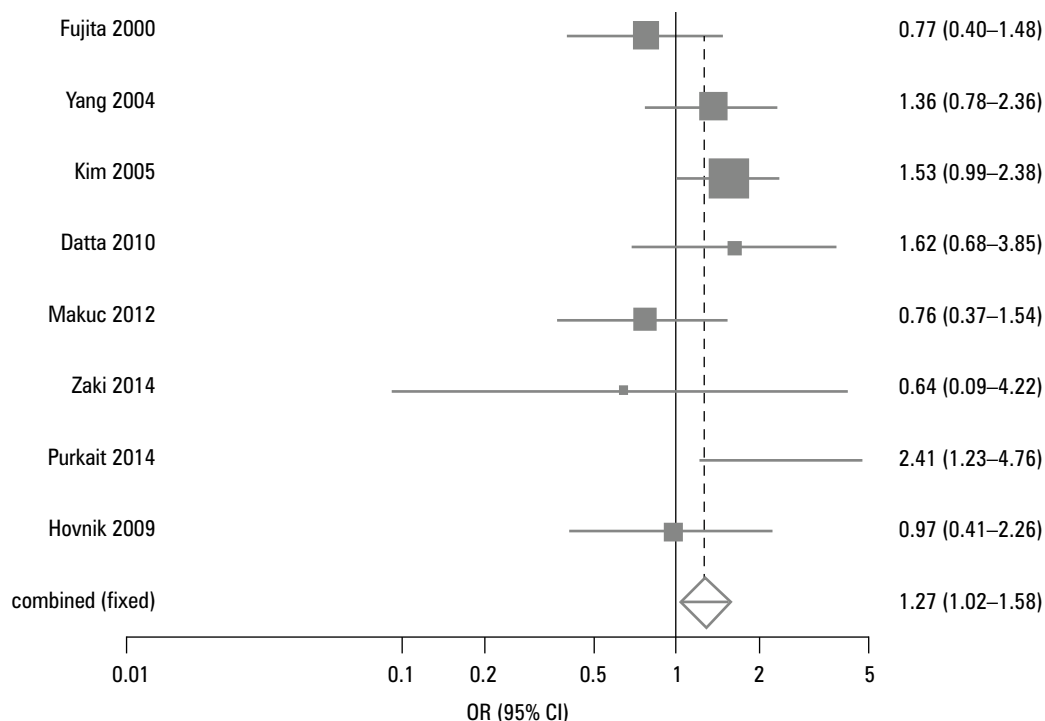
Meta-analysis results GSTM1 For DN risk and the null genotype of GSTM1, our meta-analysis gave a statistically significant overall OR of 1.27 (95% CI, 1.02–1.58; *P* = 0.03) with statistically nonsignificant between-study heterogeneity ($P_{\text{heterogeneity}} = 0.13$, $I^2 = 37.8\%$; FIGURE 2). This analysis is based on pooling of data from a number of different ethnic populations and both type 1 and type 2 diabetes. Subgroup analyses on ethnicity indicated that the association between the GSTM1(–) genotype and risk of DN was significant in Asians but not in Caucasians (OR, 1.43; 95% CI, 1.12–1.83; *P* = 0.005; $I^2 = 41.6\%$; $P_{\text{heterogeneity}} = 0.14$ and OR, 0.84; 95% CI, 0.50–1.39; *P* = 0.58; $P_{\text{heterogeneity}} = 0.646$; respectively). When only type 2 diabetes patients were analyzed, no significant association was found between the GSTM1(–) genotype and DN (OR, 1.27; 95% CI, 0.92–1.76; *P* = 0.15; $I^2 = 44.1\%$; $P_{\text{heterogeneity}} = 0.097$). In the stratified analysis by sample size, no significant associations were found in large studies (OR, 1.26; 95% CI, 0.85–1.86; *P* = 0.24; $I^2 = 58.7\%$; $P_{\text{heterogeneity}}$

TABLE 1 Characteristics of studies included in the meta-analysis

First author	Country	Ethnicity	Case/control	HbA _{1c}	Retinopathy	Duration of diabetes	Method of genotyping	Genes	HWE	Results
type 2 diabetes										
Fujita ¹⁶	Japan	Asian	105/69	7.8 ±1.7 case; 7.6 ±2 control	100%, both groups	no data	PCR	GSTM1	NA	NS
Yang ¹⁷	Taiwan	Asian	119/111	7.08 ±2.19 case; 7.86 ±1.85 control	no data	no data	multiplex PCR	GSTT1, GSTM1, both null, both positive	NA	S for GSTT1, S for both null
Kim ¹⁸	Korea	Asian	177/184	7.2 ±1.5 case; 7.7 ±1.2 control	100%, both groups	18.0 ± 8.0 cases; 20.0 ± 5.0 control	PCR	GSTT1, GSTM1, GSTP1	NA	S for GSTM1, NS for GSTP1 and GSTT1
Tiwari ¹⁹	India	Asian (SI: Dravidian)	106/149	7.3 ±1.4 case; 7.2 ±1.4 control	100% case/30% control	13.97 ± 6.5 cases; 15.45 ± 9.9 control	RFLP	GSTT1, GSTM1, GSTP1	yes	S for GSTP1, NS for GSTT1 and GSTM1 (not shown)
		Asian (NI: Indo-European)	90/75	10.4 ±7.7 case; 7.3 ±1.0 control	100% case/64.4% control	9.62 ± 6.8 cases; 15.39 ± 8.2 control	RFLP	GSTT1, GSTM1 (NS, not shown), GSTP1	yes	NS for GSTP1, NS for GSTT1 and GSTM1 (not shown)
Datta ²⁰	India	Asian	50/50	no data	100% case	no data	multiplex PCR	GSTT1, GSTM1, both null, both positive, GSTT1+/GSTM1-, GSTT1-/GSTM1+	NA	S for GSTT1 (with GSTM1+ and GSTM1-)
Makuc ²¹	Slovenia	Caucasian	88/109	8.12 ±1.50 cases; 7.47 ±1.15 control	37.5% case/23.9% control	12.2 ± 8.1 cases; 15.0 ± 4.9 control	PCR, RFLP real-time PCR	GSTT1, GSTM1, GSTP1	yes	NS
Zaki ²²	Egypt	Egyptian	18/9	8.52 ±2.25	present, no detailed data	10.5 ± 5.49	PCR for GSTT1 and GSTM1; RFLP for GSTP1	GSTT1, GSTM1, GSTP1	yes, for GSTP1	NS
Purkait ²³	India	Asian	84/123	no data	no data	no data	PCR	GSTM1	NA	S for GSTM1
type 1 diabetes										
Hovnik ²⁴	Slovenia	Caucasian	37	8.2 ±1.0 cases; 8.1 ±1.1 control	0/0	19.3 ± 5.8 cases; 17.9 ± 5.6 control	multiplex PCR	GSTM1, GSTT1	yes, GSTM1	NS

Abbreviations: GSTM1, glutathione S-transferase M1; GSTP1, glutathione S-transferase P1; GSTT1, glutathione S-transferase T1; HbA_{1c}, hemoglobin A_{1c}; NA, not applicable; NS, nonsignificant; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; S, significant

FIGURE 2 Meta-analysis of GSTM1(–) genotype associated with diabetic nephropathy. Each box represents the odds ratio point estimate, and its area is proportional to the weight of the study. The diamond represents the overall summary estimate, with confidence interval represented by its width. Abbreviations: CI, confidence interval; OR, odds ratio



pooled OR = 1.273126 (95% CI = 1.023188–1.584118); $\chi^2 = 4.470448$; $P = 0.0345$;
Cochran Q = 11.261752 (df = 7); $P = 0.1276$; moment-based estimate of between-study variance = 0.064889;
 $I^2 = 37.8\%$ (95% CI = 0%–71.3%)

TABLE 2 Meta-analysis of the association of the GSTM1(–) polymorphism with diabetic nephropathy; results of sensitivity analysis: exclusion of individual studies and results of meta-analysis

Study excluded	Statistical method	OR (95% CI)	P value	I^2
Fujita ¹⁹	fixed	1.37 (1.09–1.74)	0.009	27.1%
Yang ²⁰	random	1.21 (0.85–1.72)	0.3	30.3%
Kim ²¹	fixed	1.18 (0.92–1.53)	0.21	41.4%
Datta ²³	random	1.2 (0.86–1.66)	0.28	44.9%
Makuc ²⁴	fixed	1.36 (1.08–1.71)	0.01	30.9%
Zaki ²⁵	fixed	1.29 (1.03–1.61)	0.03	43.1%
Purkait ²⁶	fixed	1.17 (0.92–1.47)	0.22	11.3%
Hovnik ²⁷	random	1.27 (0.91–1.76)	0.15	44.1%

Abbreviations: see **FIGURE 2**

= 0.05). By considering the presence of diabetic retinopathy in both DN and control groups, the OR was 1.28 (95% CI, 0.94–1.76; $P = 0.14$; $I^2 = 46.2\%$; $P_{\text{heterogeneity}} = 0.16$). Sensitivity analyses showed that 5 studies of 8 included in the meta-analysis^{17,18,20,23,24} influenced the pooled OR qualitatively, suggesting that the results of this meta-analysis are not stable (**TABLE 2**).

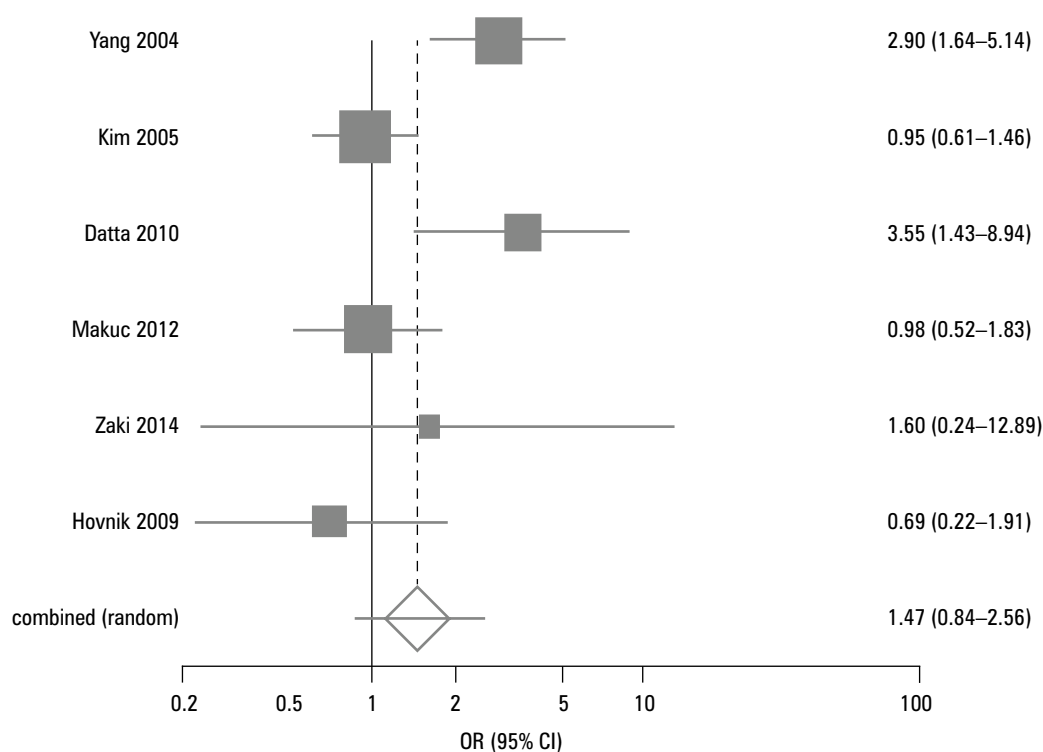
GSTT1 The meta-analysis showed a statistically nonsignificant association between the GSTT1(–) genotype and DN. The overall OR was 1.47 (95% CI, 0.84–2.56, $P = 0.18$) with significant between-study heterogeneity ($P_{\text{heterogeneity}} = 0.002$; $I^2 = 73.6\%$) (**FIGURE 3**). No significant association

was found between the GSTT1(–) genotype and DN in stratified analyses according to ethnicity, type 2 diabetes, sample size, and presence of diabetic retinopathy in both DN and control groups. The OR was 2.05 (95% CI, 0.85–4.96; $P = 0.11$; $I^2 = 86.2\%$; $P_{\text{heterogeneity}} = 0.0007$) in Asians; 0.88 (96% CI, 0.54–1.45; $P = 0.72$; $P_{\text{heterogeneity}} = 0.54$) in Caucasians; 1.67 (95% CI, 0.91–3.07; $P = 0.09$; $P_{\text{heterogeneity}} = 0.002$, $I^2 = 76.2\%$) in type 2 diabetes patients; 1.38 (95% CI, 0.68–2.81; $P = 0.38$; $P_{\text{heterogeneity}} = 0.003$, $I^2 = 83.1\%$) in big studies; and 1.74 (95% CI, 0.48–6.34; $P = 0.4$; $P_{\text{heterogeneity}} = 0.005$) in population with diabetic retinopathy in both DN and control groups. In a sensitivity analysis, the exclusion of individual studies did not affect these results.

GSTP1 There were no significant associations between the presence of the Val allele (Ile/Val genotype, Val/Val genotype and Val / Val and Ile / Val genotypes combined) and DN in the overall and subgroup analyses (**TABLE 3**). In the sensitivity analysis, the exclusion of individual studies did not affect these results.

Gene–gene interaction The data on both null genotype of GSTs among cases and controls were available in 2 studies,^{17,20} which included 169 cases and 161 controls. The interaction between GSTM1(–) and GSTT1(–), for which an OR of 2.02 (95% CI, 1.22–3.36; $P = 0.009$; $P_{\text{heterogeneity}} = 0.88$) for DN appeared in comparison with individuals with the positive genotypes.

FIGURE 3 Meta-analysis of GSTT1 null genotype associated with DN. Each box represents odds ratio point estimate, and its area is proportional to the weight of the study. The diamond represents the overall summary estimate, with confidence interval represented by its width. Abbreviations: see



pooled OR = 1.469066 (95% CI = 0.84256–2.561425); $\chi^2 = 1.838751$ ($df = 1$); $P = 0.1751$;
Cochran Q = 18.968903 ($df = 5$); $P = 0.0019$; moment-based estimate of between-study variance = 0.321453
 $I^2 = 73.6\%$ (95% CI = 16.5%–86.6%)

TABLE 3 Meta-analysis of the association between GSTP1 genotypes and diabetic nephropathy

Genotypes	No. of studies	Statistical method	OR (95% CI)	P value	I^2
total population					
Ile/Ile	5	random	1.16 (0.71–1.91)	0.55	66.4%
Val/Ile	5	random	0.93 (0.54–1.60)	0.80	70.1%
Val/Val	4	fixed	0.77 (0.44–1.34)	0.43	0.00%
combined (Val/Ile & Val/Val)	5	random	0.87 (0.53–1.43)	0.58	66.9%
Asian population					
Ile/Ile	3	random	1.46 (0.86–2.45)	0.16	65.8%
Val/Ile	3	random	0.7 (0.42–1.16)	0.17	61.2%
Val/Val	3	fixed	0.9 (0.46–1.78)	0.90	3.8%
combined (Val/Ile & Val/Val)	3	random	0.69 (0.41–1.18)	0.18	67.5%

Abbreviations: see **FIGURE 2**

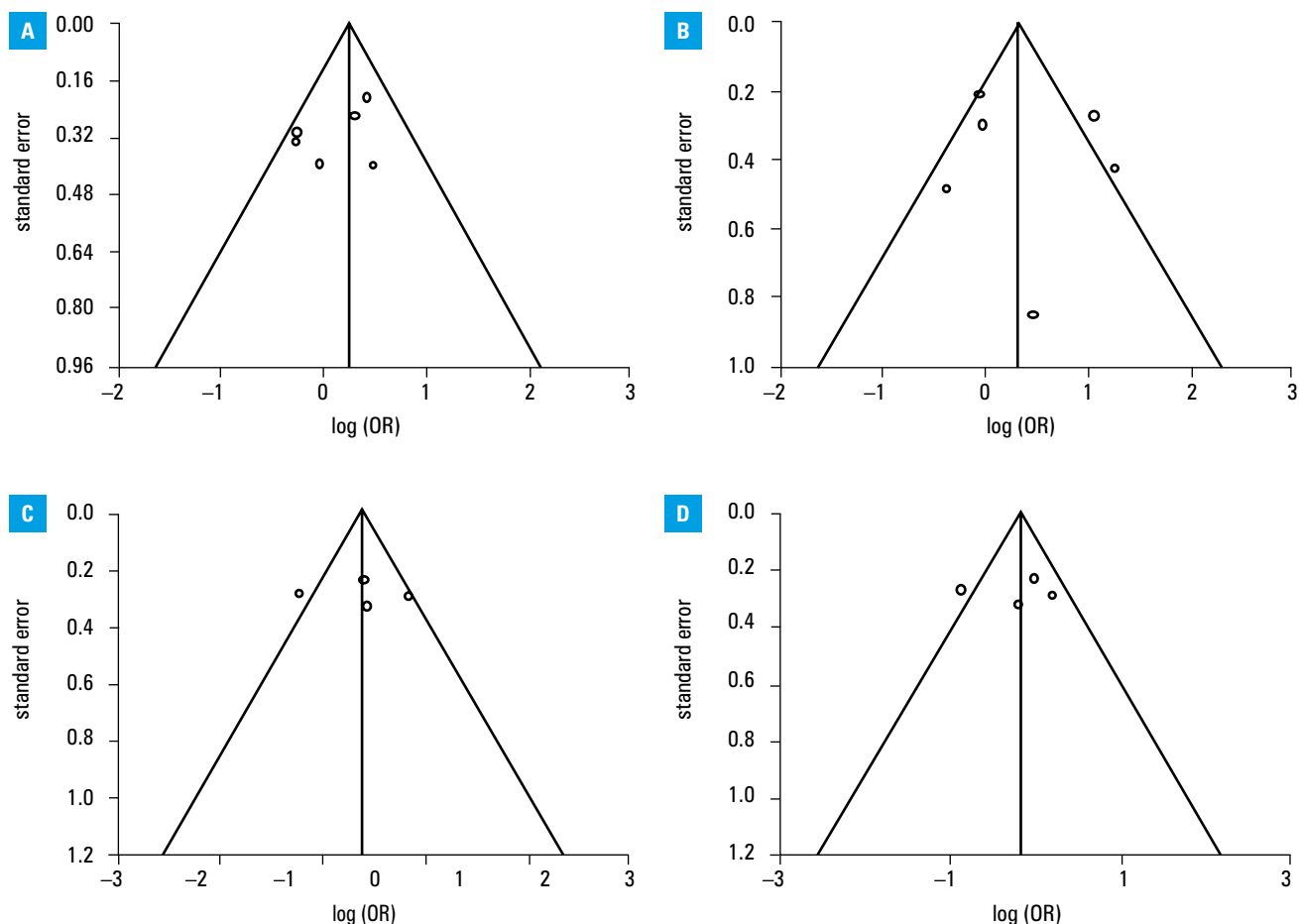
Potential publication bias Funnel plots and Egger test were generated to evaluate potential publication bias for GSTM1 (**FIGURE 4A**), GSTT1 (**FIGURE 4B**), and GSTP1 (**FIGURE 4CD**). The shape of the funnel plots was symmetrical for these polymorphisms. The statistical results still did not show publication bias (Egger test, $P = 0.38$ for GSTM1, $P = 0.73$ for GSTT1, $P = 0.41$ for GSTP1 Ile/Val, and 0.42 for GSTP1 Ile/Val and Val/Val combined).

DISCUSSION The present study provides the most comprehensive assessment so far of the

relationship between susceptibility to DN and GST polymorphisms. Meta-analyses of several individual genetic variants in relation to DN have been performed previously,^{25–27} but this is the first complete overview of the association between GST polymorphism and DN. An accurate estimation of this association provided by a meta-analysis could provide a better insight into the underlying pathogenetic mechanisms of DN. As GSTM1(–), GSTT1(–), and GSTP1 Val allele are associated with a lower activity of the corresponding enzymes, it is plausible to speculate that an inadequate GST function may predispose to the development of DN in the presence of increased oxidative stress.³

Overall, our results suggest that individuals with the GSTM1(–) or GSTM1(–)/GSTT1(–) genotype have a significantly increased risk of DN compared with individuals who do not possess these allelic variants, whereas the GSTT1 polymorphism (null vs nondeleted) and GSTP1 polymorphism (possession of the Val allele) seems to be unrelated to DN risk. A significantly increased risk of DN for individuals with combined deletion mutation in GSTM1 and GSTT1 genes in comparison with individuals with positive genotypes suggests that combinations of certain genotypes may be more discriminating as risk factors than a single locus genotype.

However, our results should be interpreted with caution. Sensitivity analyses showed that the results of the meta-analysis of the association between the GSTM1(–) genotype and DN



A) Egger: bias = -1.42 (95% CI = -5.08 to 2.25); $P = 0.38$; B) Egger: bias = 0.86 (95% CI = -5.62 to 7.33); $P = 0.73$
C) Egger: bias = 2.2 (95% CI = -5.21 to 9.61); $P = 0.41$; D) Egger: bias = 2.05 (95% CI = -4.96 to 9.05); $P = 0.42$

FIGURE 4 Funnel plot of association between GSTM1 (A), GSTT1 (B), GSTP1 Ile/Val (C), and GSTP1 Va/Val (D) polymorphism and DN. Formal statistical criteria by the Egger test were also performed to investigate the symmetry of the funnel plot.

Abbreviations: see

FIGURE 2

are not stable. No significant association has been found between the GSTM1(–) genotype and DN in stratified analyses according to the type of diabetes, sample size, presence of diabetic retinopathy, mean HbA_{1c} level, and diabetes duration in both DN and control groups. Considering Caucasian and Asian studies separately, the association between the GSTM1(–) status and DN reaches a significant level among Asians, but not in Caucasians. The prevalence of GSTM1 homozygous null individuals in Caucasian and Asian populations is about 50%.²⁸ It should be noted that in 2 studies included in the meta-analysis, 1 Caucasian and 1 Asian,^{21,23} the prevalence of GSTM1(–) individuals was 2-fold lower in the control group. It should be stressed that for convenience and for hypothesis testing, we decided to perform the frequency analyses starting with conventional definitions of ethnicity and being aware that, for example, the Asian population can be heterogeneous. However, the numbers of subjects for each of these countries was quite small, making any conclusions regarding heterogeneity within the Asian group premature. In general, ethnic identification is a difficult task, especially in situations where considerable admixture has been known to occur, and misclassification of individuals of mixed ancestry is very likely. Furthermore, defining ethnicity is probably not a biologically

plausible way to divide the human population in terms of genetic differences.²⁹

A meta-analysis of the role of the GSTM1(–)/GSTT1(–) genotype in the development of DN was based on the results of only 2 Asian studies that reported gene–gene interaction.^{17,20} Furthermore, only these 2 studies showed a significant association between homozygous deletion of GSTT1 and the risk of DN.^{17,20} Therefore, further research is necessary to fully explore the possible interaction between combinations of certain genotypes and DN risk.

The strength of our meta-analysis was based on the accumulation of published data, providing more information to detect significant differences. We have searched in different databases for reports and included also non-English-language studies to minimize selection bias. Heterogeneity between studies was assessed by statistical analysis. To explore potential clinical heterogeneity, we decided a priori to perform several subgroup meta-analyses according to ethnicity, diabetes type, sample size, presence of diabetic retinopathy, mean HbA_{1c} level, and diabetes duration in both DN and control groups.

Despite the fact that we made an accurate and comprehensive analysis, our study has several limitations. Regarding the quality of the included studies, blind genotyping was not reported

in any study, the selection of cases and controls was usually not reported to be done in a consecutive or random fashion, and the differences in patients' characteristics account for a large part of the observed heterogeneity. Owing to the lack of detailed data, not all studies can be included in stratified subgroup analyses. We could not analyze environment-gene interactions, as not enough information was available to do so. There are limitations in the interpretation regarding GSTM1 and GSTT1 genotypes common to other studies. With the exception of 1 study,²⁰ both genotypes were assessed by "yes-no" polymerase chain reaction (PCR) methods that do not measure gene copy number and therefore cannot distinguish between heterozygous (+/0) and homozygous (+/+ or even ++/+) non-null genotypes. GSTT1 or GSTM1 copy number variations are correlated with altered enzyme activity, and the analysis in a dose-dependent manner would best describe any disease outcome association.³⁰

Despite these remarks, some interesting conclusions have emerged. Our results indicate that both GSTM1(-) genotype and the combination of GSTT1(-)/GSTM1(-) genotypes increase the risk of DN. Therefore, these polymorphic variants could be considered genetic markers for the risk of this disease. Considering a worldwide increase in diabetes, the recognition of the genes/genetic variants as having a significant association with risk of DN may have clinical importance in the future: it will provide new therapeutic targets, help identify patients with an increased risk of DN, and lead to the development of opportunities for disease prevention and improved measures of personalized medicine.

Our study sought to synthesize evidence regarding the association between GST polymorphisms and DN risk but also provided us with an overview of the past research and an opportunity to identify gaps and areas for future research. Firstly, considering the possible additive/modulating effect of different GST genotypes, the combination of the GST polymorphisms rather than individual polymorphism should be investigated. Secondly, a clearer picture of the interaction between different polymorphisms and environmental factors on DN should be adequately addressed by large and well-designed epidemiological studies. Thirdly, true genotyping, which identifies the null allele and can distinguish homozygous wild-type +/+ from heterozygous +/- individuals, is important because of the gene dosage effect associated with having 2, 1, or no alleles. New methods using either long-range PCR or real-time PCR are available to definitively identify +/+, +/-, and -/- genotypes.

All but 1 study included in our meta-analysis have used old methodology and, not surprisingly, obtained confusing data, which resulted in inconsistent or contradictory publications on the association of the GSTM1 and GSTT1 genotypes with DN. The investigation of associations between the GST polymorphism and DN risk will

not advance unless investigators use state-of-the-art genotyping allowing a trimodular determination of the GST copy number variations.^{31,32}

Recent developments in genotyping technology and increased information on the human genome have facilitated genome-wide association studies (GWAS) for investigating novel disease susceptibility across the entire human genome. GWAS of DN have been conducted in several populations. However, most of the identified risk loci could not be replicated by independent studies with a few exceptions including those in *ELMO1*, *FRMD3*, *CARS*, *APOL3-MYH9*, and 13q33 between *MYO16* and *IRS2* genes.³³⁻³⁸ Functional studies of these genes revealed the involvement of cytoskeleton reorganization, phagocytosis of apoptotic cells, fibroblast migration, insulin signaling, and epithelial clonal expansion in the pathogenesis of DN. The failure of GWAS in DN may be explained by small sample sizes, different phenotype definitions between studies, population-specific associations, and strong influence of environmental factors. It should be stressed, however, that the comparison of chromosomal regions demonstrated by GWAS as potentially associated with DN with the positions of oxidative stress-related genes that have been tested in candidate gene studies shows that many of them are positional candidates, for example, 22q, 11p, 7q.⁵

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Wpływ polimorfizmów genetycznych S-transferaz glutationowych (GSTM1, GSTT1, GSTP1) na ryzyko nefropatii cukrzycowej – metaanaliza

Jan Orlewski¹, Ewa Orlewska²

¹ Katedra i Klinika Chorób Wewnętrznych i Endokrynologii, Warszawski Uniwersytet Medyczny, Warszawa

² Zakład Fizjologii, Patofizjologii i Mikrobiologii Infekcyjnej, Uniwersytet Jana Kochanowskiego, Kielce

SŁOWA KLUCZOWE

nefropatia
cukrzycowa, GSTM1,
GSTT1, GSTP1,
S-transferazy
glutationowe

STRESZCZENIE

WPROWADZENIE S-transferazy glutationowe (GST) należą do rodziny wszechobecnych i wielozadaniowych enzymów, które chronią komórki przed produktami stresu oksydacyjnego.

CELE Celem badania była ocena związku między polimorfizmami genów GST a nefropatią cukrzycową (*diabetic nephropathy* – DN).

PACJENCI I METODY Przeprowadzono systematyczny przegląd literatury w bazach PubMed, EMBASE i Google Scholar. Do obliczenia skumulowanego ilorazu szans (*odds ratio* – OR) użyto modelu efektu stałego lub zmiennego. Przeprowadzono testy heterogeniczności wyników oraz analizy wrażliwości.

WYNIKI Do analizy włączono 9 publikacji (874 pacjentów w grupie eksperymentalnej, 966 pacjentów w grupie kontrolnej). Poza jednym badaniem genotypy GSTT1 oraz GSTM1 nie były oceniane metodami określającymi liczbę kopii genów. Istotny wzrost ryzyka DN zaobserwowano dla genotypów GSTM1(–) (OR = 1,27; 95% CI: 1,02–1,58) oraz GSTT1(–)/GSTM1(–) (OR = 2,02 95% CI: 1,22–3,36). Nie zaobserwowano związku między DN a genotypem GSTT1(–) lub występowaniem allelu Val. W analizie podgrup zaobserwowano istotny związek między DN a genotypem GSTM1(–) u rasy azjatyckiej i brak tego związku u rasy kaukaskiej.

WNIOSKI Wyniki wskazują, że genotypy GSTM1(–) oraz GSTT1(–)/GSTM1(–) zwiększają ryzyko DN. Badania powinny raczej uwzględniać kombinacje występowania polimorfizmów genetycznych GST, a nie polimorfizm pojedynczego genu. Ilościowe oznaczanie kopii genów GST może lepiej oceniać związek pomiędzy ryzykiem zachorowania a określonym genotypem.

Adres do korespondencji:

dr hab. n. med. Ewa Orlewska, Zakład
Fizjologii, Patofizjologii i Mikrobiologii
Infekcyjnej, Uniwersytet Jana
Kochanowskiego, Al. IX Wieków Kielce
19, 25-317 Kielce, tel.: 41 349 69 09,
fax: 41 349 69 16, e-mail:
eorl@ujk.edu.pl

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