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Association of manganese superoxide dismutase Ala16Val gene polymorphism with diabetic retinopathy risk in type 2 diabetes: A systematic review and meta-analysis

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ABSTRACT

Background: Diabetic retinopathy (DR) is renowned as a prominent cause of visual impairment worldwide. The association between manganese superoxide dismutase (MnSOD) gene, Ala16Val (rs4880), and DR susceptibility in people with type 2 diabetes mellitus (T2DM) remains contentious.

Objective: This meta-analysis aims to evaluate risk of DR in T2DM patients with MnSOD Ala16Val polymorphism.

Methods: A literature search was conducted using MEDLINE, Scopus, Web of Science, ScienceDirect, EMBASE, and grey literature to identify potential studies assessing the link between MnSOD polymorphism and DR risk among T2DM patients. The data was further analyzed in fixed/random effect models using RevMan 5.3 under five genetic models.

Results: Six studies comprising 2,132 subjects from four distinct ethnicities were included. The present study revealed that MnSOD gene polymorphism was associated with a significantly increasing DR risk in T2DM patients under the co-dominant model (VV vs. AA) (OR 1.87 [1.42, 2.46], p<0.0001) and dominant model (VV+AV vs. AA) (OR 1.85 [1.02, 3.33], p=0.0400).

Conclusions: T2DM individuals with rs4880 VV alleles are more susceptible to DR development, making them as a potential marker for heightened DR susceptibility in T2DM patients, laying the foundation for a gene panel to assess their susceptibility to develop DR.

Keywords: genetic polymorphism, superoxide dismutase, diabetic retinopathy, type 2 diabetes mellitus

INTRODUCTION

Diabetic retinopathy (DR), recognized as the earliest and most prevalent complication arising from diabetes, represents a leading cause of vision loss among the working-age population globally [1-3]. Remarkably, nearly one-third of diabetic patients above the age of 40 will develop DR, with approximately one in six facing severe vision impairments such as diabetic macular edema [4]. Clinically characterized by progressive microvascular alterations, DR pathogenesis involves occluded capillaries, neovascularization, breakdown of the blood-retinal barrier (BRB), altered retinal permeability, and macular edema [5]. Multiple factors contribute to the development of DR, including persistent hyperglycemia, uncontrolled lipid levels, hypertension, and genetic influences [3, 6].

In recent years, research has highlighted biochemical mechanisms and genetic factors as promoters of DR, mainly through oxidative stress mechanisms [7, 8]. In diabetic patients, increased oxidative stress resulted from increased polyol and hexosamine pathway activity, hyperactivation of protein kinase C isoforms, and the accumulation of advanced glycation end products [9]. These pathways collectively trigger a cascade of biochemical alterations, ultimately causing excessive reactive oxygen species (ROS) production. This hyperglycemia-induced oxidative stress is recognized as a contributor to endothelial cell dysfunction and apoptosis and accelerated loss of retinal capillary cells [10, 11]. Consequently, these vascular changes are linked to the development of DR.

Manganese superoxide dismutase (MnSOD), the first-line antioxidant, is a scavenger enzyme to combat excessive free radicals. The overproduction of ROS and suppression of its elimination will lead to a pathological cascade of diabetes and

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its complications, including retinopathy diabetes (DR) [12]. The MnSOD enzyme catalyzes the dismutation of superoxide radicals into H_2O_2, thus eliminating free radicals from the cell [13]. Previous studies demonstrated that overexpression of MnSOD abrogates retinal mitochondria dysfunction by protecting retinal endothelial cells from oxidative damage, thereby preventing DR [14]. Previous animal models also support the idea that overexpression of MnSOD in diabetic mice prevents the development of retinopathy [15]. As fundamental as its antioxidant role, any structural or functional alterations of MnSOD, including polymorphisms, could have crucial consequences for its functions [9].

Ala16Val (rs4880), the encoding gene of human MnSOD, is located on chromosome 6q25.2. By this far, Ala16Val is the best-studied single-nucleotide polymorphism (SNP) in MnSOD that has been revealed to be functionally pertinent. Previous studies have shown that a single nucleotide polymorphism in the MnSOD enzyme could accelerate the manifestation of diabetic complications, including nephropathy, coronary artery diseases, and acute myocardial infarction [8,16]. This condition occurs due to post-translational covalent modifications in SOD [17]. It is then postulated that the Ala16Val genetic polymorphism may contribute to individual variations in DR susceptibility.

However, previous studies yielded controversial results on the association between Ala16Val polymorphism and DR, specifically the allele linked with its development. Due to the lack of consistent results among prior studies, the present study is crucial in determining the association between the polymorphism of Ala16Val and DR susceptibility in type 2 diabetes mellitus (T2DM) patients statistically. This study is the first systematic review and meta-analysis to analyze the association of Ala16Val polymorphism with DR among individuals with T2DM.

METHODS

The present meta-analysis was based on the preferred reporting items for systematic reviews and meta-analysis (PRISMA) 2020 protocol. This study has been registered in PROSPERO under the registration number CRD42023384576. The complete PRISMA 2020 checklist for systematic reviews and meta-analysis for this study is accessible in **Table A1** in **Appendix A**.

Database Searching

The present study gathered previously published case-control or cohort studies investigating the association between MnSOD gene polymorphism Ala16Val and the risk of DR in T2DM individuals. The literature was searched using electronic databases from MEDLINE, Scopus, Web of Science, ScienceDirect, EMBASE, and grey literature by using MeSH terms: “genetic polymorphism,” “superoxide dismutase,” and “diabetic retinopathy”. The search was conducted in May 2022. PRISMA reporting was then concluded with the assistance of COVIDENCE. The complete searching strategy is provided in **Table B1** in **Appendix B**.

Inclusion & Exclusion Criteria

Patients & samples

The eligibility criteria for patient inclusion are past individuals diagnosed with T2DM as per American Diabetes Association guideline 2021, along with DR through clinical evaluation by ophthalmologists through fundoscopy or fluorescein angiography. Samples included in the analysis were obtained via standardized molecular techniques such as PCR-RFLP, sequencing, or TaqMan genotyping to confirm the presence of MnSOD Ala16Val polymorphism. The framework of inclusion criteria is available in **Table B2** in **Appendix B**.

Studies

All included studies must meet the following criteria:

(1) a case-control or cohort study,

(2) investigated the association between MnSOD Ala16Val genetic polymorphism and the risk of DR among T2DM individuals,

(3) reported the risk as odds ratio (OR) with 95% confidence interval (CI) or provided sufficient data to extract OR with 95% CI data,

(4) investigated only human subjects, and

(5) in English.

No specific ethnicities were addressed in this meta-analysis. The articles with unextractable data (unmeasurable data, full-text not available, unstandardized reporting) and duplicated studies were excluded from the record.

Study Selection

Literature search and retrieval were performed by two authors independently. Any disagreements were resolved through discussion, and the final decision was established upon approval of all authors. A systematic reporting of database searching, and study selection is provided in **Figure 1**.

Data Extraction

To minimize bias, two reviewers investigated the potential articles independently. The following data were extracted from each study: primary author, publication year, ethnicity, number of case/control, genotyping method, genotype distribution in case and control, and Newcastle-Ottawa scale (NOS).

Quality Assessment

NOS was used to examine the quality of included studies, ensuring the production of a high-quality meta-analysis. The NOS assessment evaluated articles from three major domains: selection of participants, comparability between groups, and ascertainment of exposure or outcome. Ratings were ranging from zero-nine, categorizing studies as poor quality (zero-two), fair quality (three-five), and good or high quality (six-nine) [18].

Data Analysis & Statistical Methods

The association strength between MnSOD Ala16Val polymorphism was estimated using OR and 95% CI. The present study investigates five genetic models, including allele (A vs. V), dominant (VV+AV vs. AA), recessive (VV vs. AA+AV), co-dominant major vs. minor homozygote (VV vs. AA), and co-dominant heterozygote vs. major homozygote (AV vs. AA).

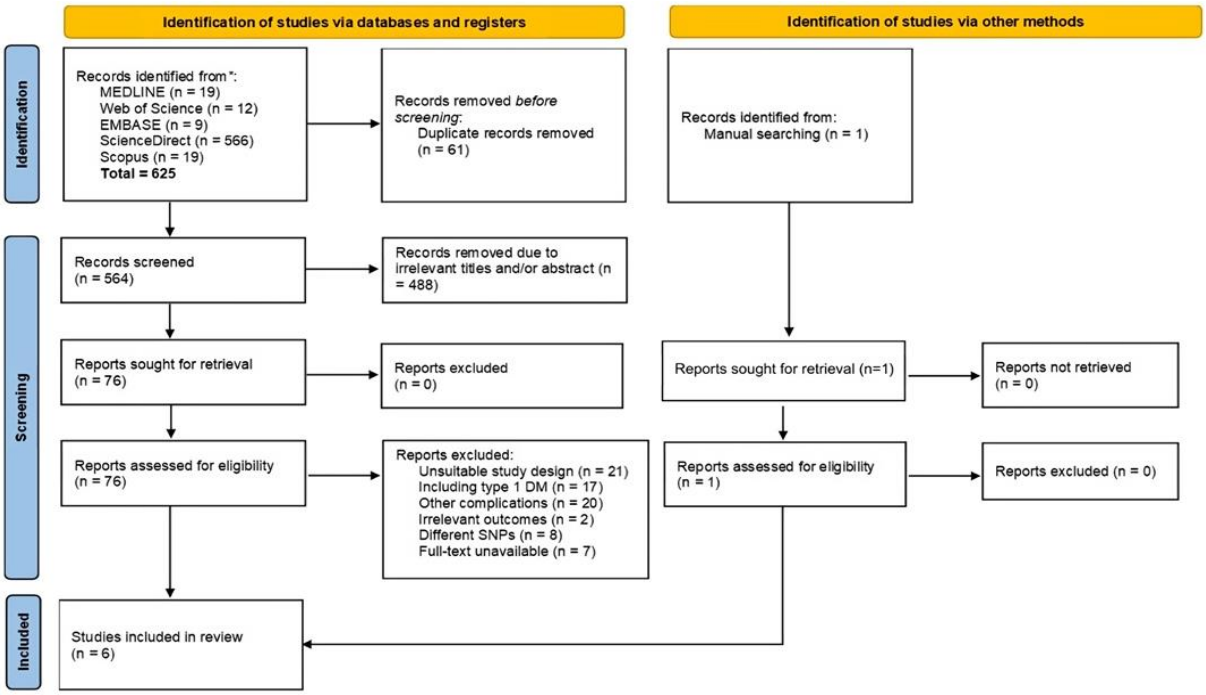

Figure 1. PRISMA diagram of included studies (Source: Authors' own elaboration)

Table 1. Characteristics & genotype distribution frequency of included studies

No	R	Ethnicity	Age		Case/ control	Genotyping method	Genotype distribution						Chi-square for HWE	HWE p-value	NOS score
							DR (+)			DR (-)					
			Case	Control			VV	AV	AA	VV	AV	AA			
1	[2]	Egyptian	60.10±8.80	57.80±9.20	53/47	TaqMan	23	22	8	10	25	12	0.5041	0.4780	8
2	[20]	Iranian	52.50±22.50	52.50±22.50	140/140	PCR-RFLP	32	100	8	56	54	30	5.1900	0.0220	9
3	[21]	Slovenian	66.00±9.50	66.90±11.50	283/143	PCR-RFLP	80	140	63	23	69	51	0.1390	0.9050	8
4	[22]	North Indian	55.92±8.90	55.84±12.20	446/312	TaqMan	127	218	101	58	194	60	5.8900	0.0150	9
5	[23]	South Korean	53.40± 13.30	52.10±13.20	130/174	PCR-RFLP	105	23	2	138	34	2	0.0990	0.7610	7
6	[24]	Chinese	N/A	N/A	125/198	PCR-RFLP	99	8	18	93	10	36	174.2200	<0.0100	8

Note. R: Reference; HWE: Hardy Weinberg equilibrium

The heterogeneity assumption was examined using Chi-square-based Q-test and I2 statistics. The pool estimated ORs were determined using either a fixed or random effects model. The random effects model was applied in the case of between-study heterogeneity (I2>50% and p for heterogeneity <0.0500). Otherwise, the fixed effect model was utilized to generate pooled OR [19].

Sensitivity analysis was done to analyze the influence of data from each study on pooled ORs by eliminating a single study at a time. Based on the available data, a subgroup analysis was performed on ethnicities. All statistical tests were carried out using Review Manager 5.4 software (The Cochrane Collaboration, UK), with two-sided test p-values, and p<0.0500 was considered statistically significant.

Publication Bias & Risk of Bias

Publication bias was assessed using a funnel plot and Egger's test. The publication bias was indicated when the data in a funnel plot were distributed disproportionately. In contrast, the absence of publication bias was suggested when the data were distributed approximately symmetrically. Egger's test was calculated using STATA version 17 and significant Egger's test imply a significant publication bias and small study effects.

RESULTS

Study Selection

A total of 625 studies were initially obtained from five databases (MEDLINE, Web of Sciences, EMBASE, ScienceDirect, and Scopus) and manually from previous reviews. Among them, 61 duplicate records were removed automatically before screening. During the screening process, 488 articles with irrelevant titles/abstracts were excluded, leaving 76 potential ones for further identification. A total of 71 studies were excluded due to unsuitable study design (review/case report/letters to the editor), including other type(s) of diabetes or complication(s), different SNP, irrelevant outcome(s), or unavailable full-text. Six studies fulfilled the criteria then assessed using NOS as attached in **Table B3** in **Appendix B**. The detail of study flow diagram (PRISMA) can be seen in **Figure 1**.

Eventually, six studies recruited 2,132 participants (1,177 cases and 951 controls) were included. The papers that were retrieved were published from 2006 to 2015. Each included study has a sample size ranging from 280 to 758 participants. Of six studies, two were from East Asia (China and South Korea), two were from West Asia (Egypt and Iran), one from South Asia (North India), and one was from Slovenia [20-24]. Allele and genotype distribution frequencies from each study are concluded in **Table 1**.

Table 2. Pooled risk estimate under five different genetic model analyses

SNP MnSOD Ala16Val	n	OR (95% CI)	p-value (Z test)	I_2 for heterogeneity	p-value for heterogeneity	FE/RE
V vs. A	4	1.29 [0.99, 1.68]	0.0600	63	0.0400	RE
VV+AV vs. AA	5	1.85 [1.02, 3.33]	0.0400**	81	0.0004	RE
VV vs. AA	5	1.87 [1.42, 2.46]	<0.0001**	28	0.2200	FE
VV vs. AA+AV	6	1.63 [0.71, 3.76]	0.3800	88	<0.0001	RE
AV vs. AA	6	1.21 [0.73, 1.98]	0.4600	80	0.0001	RE

Note. FE: Fixed effects & RE: Random effects

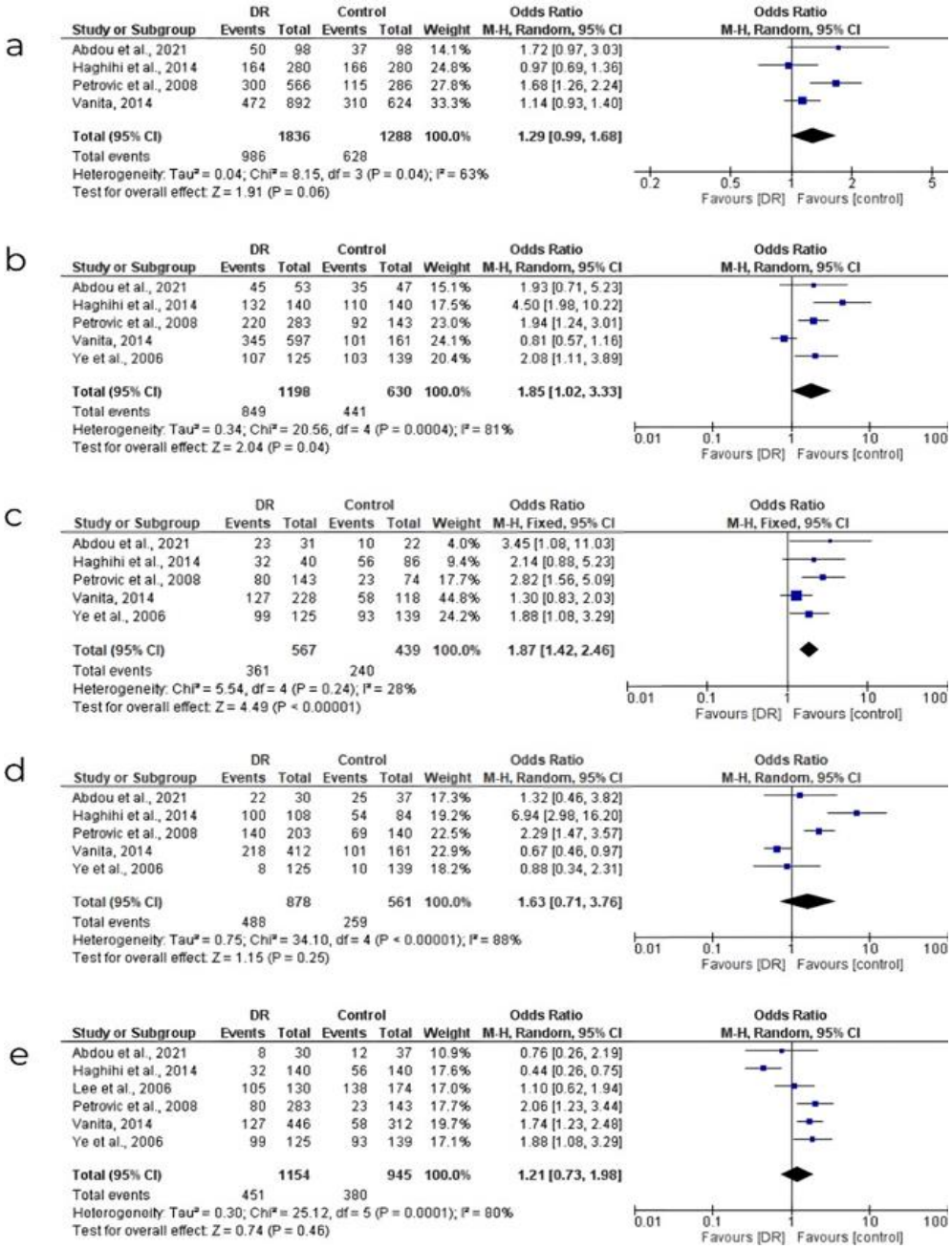

Figure 2. Forest plot analysis under (a) allele model (A vs. V); (b) dominant model (VV+AV vs. AA); (c) co-dominant model (AA vs. VV); (d) heterozygote vs. major homozygote (VV vs. AA+AV); & (e) recessive model (AA vs. AV) (Source: Authors' own elaboration)

The other five studies showed the raw data for each genotype distribution frequency in three different genotypes (AA, AV, and VV). As seen in **Table 1**, the study in [23] did not provide a specific frequency for either AV or AA but showed only the frequency of those two combined (AV+AA). This reporting discrepancy resulted in varying study numbers (n) on the pooled risk estimate, as shown in **Table 2**.

Association Between MnSOD Ala16Val Polymorphism with DR Risk in T2DM Patients

The present study determines the strength of association between MnSOD Ala16Val polymorphism with retinopathy diabetes risk in T2DM patients using pooled OR and 95% CI. Five genetic model analyses were conducted on the raw data in the included studies. **Figure 2** shows forest plot analysis.

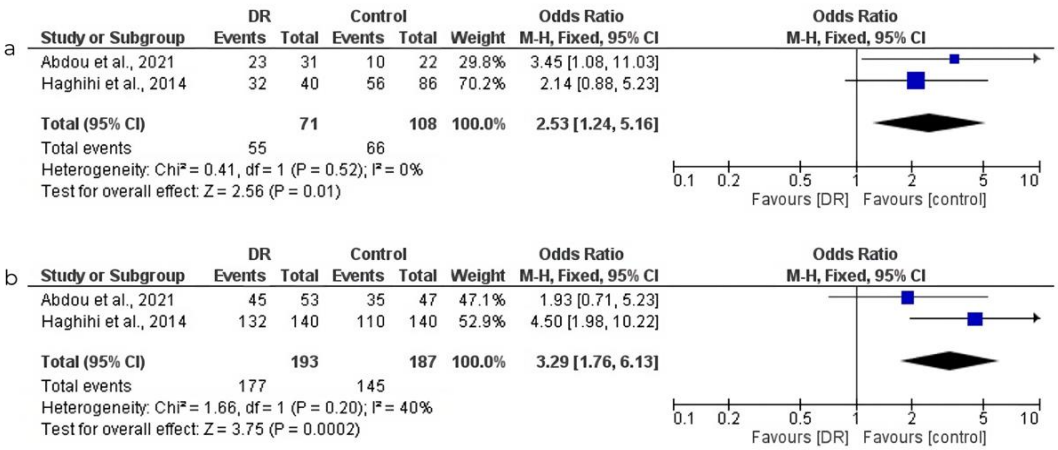

Figure 3. Subgroup analysis: Association of MnSOD Ala16Val to DR susceptibility among T2DM patients in West Asia under (a) co-dominant model (VV vs. AA); & (b) dominant model (VV+AV v. AA) (Source: Authors' own elaboration)

The present meta-analysis found that MnSOD Ala16Val genetic polymorphism was significantly associated with DR risk in T2DM patients under the co-dominant model (OR=1.87 [1.42, 2.46], p<0.0001) (part c in **Figure 2**) and dominant model (OR=1.85 [1.02, 3.33] P=0.0800, p=0.0400) (part b in **Figure 2**) but were not significant under the following genetic models: allele (OR=1.29 [0.99, 1.68], p=0.0600) (part a in **Figure 2**), heterozygote vs. major homozygote (OR=11.63 [0.71, 3.76], p=0.3800) (part d in **Figure 2**), and the recessive model (OR=11.21 [0.73, 1.98], p=0.4600) (part e in **Figure 2**). The random effects model was applied to all five genetic models. The pooled risk estimate using five different models can be seen in **Table 2**.

Subgroup Analysis

We performed a subgroup analysis to assess the association of MnSOD Ala16Val to DR susceptibility among West Asians with T2DM. It is found that the pooled ORs was even stronger among the West Asians patients under the co-dominant model (VV vs. AA) OR=2.53 [1.24, 5.16], p=0.0100 and dominant model (VV+AV vs. AA) OR=3.29 [1.76, 6.13], p=0.0002 (part a in **Figure 3** and part b in **Figure 3**).

Sensitivity Analysis

There were no significant differences in pooled ORs when one study was excluded at a time, indicating that no single study altered the statistical significance of the overall findings.

Risk of Publication Bias

The funnel plot analysis suggested no obvious evidence of publication bias in the co-dominant model, as the plot displayed approximate symmetrical (**Figure 4**). This finding was further confirmed by Egger's test analysis, which revealed insignificant result for co-dominant model analysis (Z=1.12, p=0.2600), indicating no small sample size affecting the overall effect size in this study. The summary of the risk of bias graph can be seen in **Figure 5**.

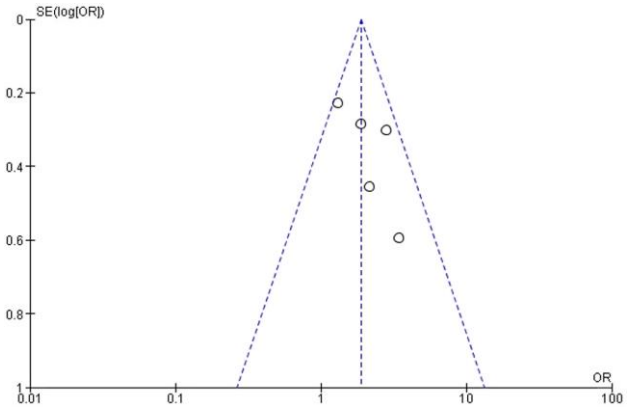

Figure 4. Funnel plot of meta-analysis for association between MnSOD Ala16Val gene polymorphism & DR susceptibility in T2DM (AA vs. VV) (Source: Authors' own elaboration)

DISCUSSION

The present meta-analysis of case-control studies demonstrated that MnSOD Ala16Val genetic polymorphism was significantly associated with the escalating risk of retinopathy diabetes in T2DM patients under the co-dominant model (VV vs. AA). Other genetic model analyses found no significant association between DR risk and MnSOD Ala16Val polymorphism despite DR incidence linked to the higher frequency of the V allele. The escalating importance of DR has prompted the investigation of genetic risk factor research for DR. Even previously researched, the association between MnSOD Ala16Val polymorphism and DR risk in type 2 diabetes patients remains contentious. This result prompted further comprehensive research, specifically for T2DM patients, as it contributes to more than 90% of diabetes cases [2, 3].

Manganese Superoxide Dismutase Ala16Val Gene Polymorphism & Free Radicals

MnSOD is a metalloenzyme that serves as the first line of defense against ROS. SOD enzyme detoxifies superoxide radicals and catalyzes their transition into H_2O_2. The MnSOD gene exists on chromosome 6 (6q25.3) and encodes human MnSOD, recognized as SOD2 [25]. MnSOD is vital in maintaining antioxidant balance in living cells as the first-line antioxidant agent inside mitochondria. Any structural or functional alteration in MnSOD, including polymorphism in its encoding gene, may unfavorably diminish its scavenging role in oxidative stress [9, 12].

As the only enzyme inside mitochondria, the impact of polymorphism in MnSOD encoding gene on oxidative stress modulation and subsequent disease prevention has piqued

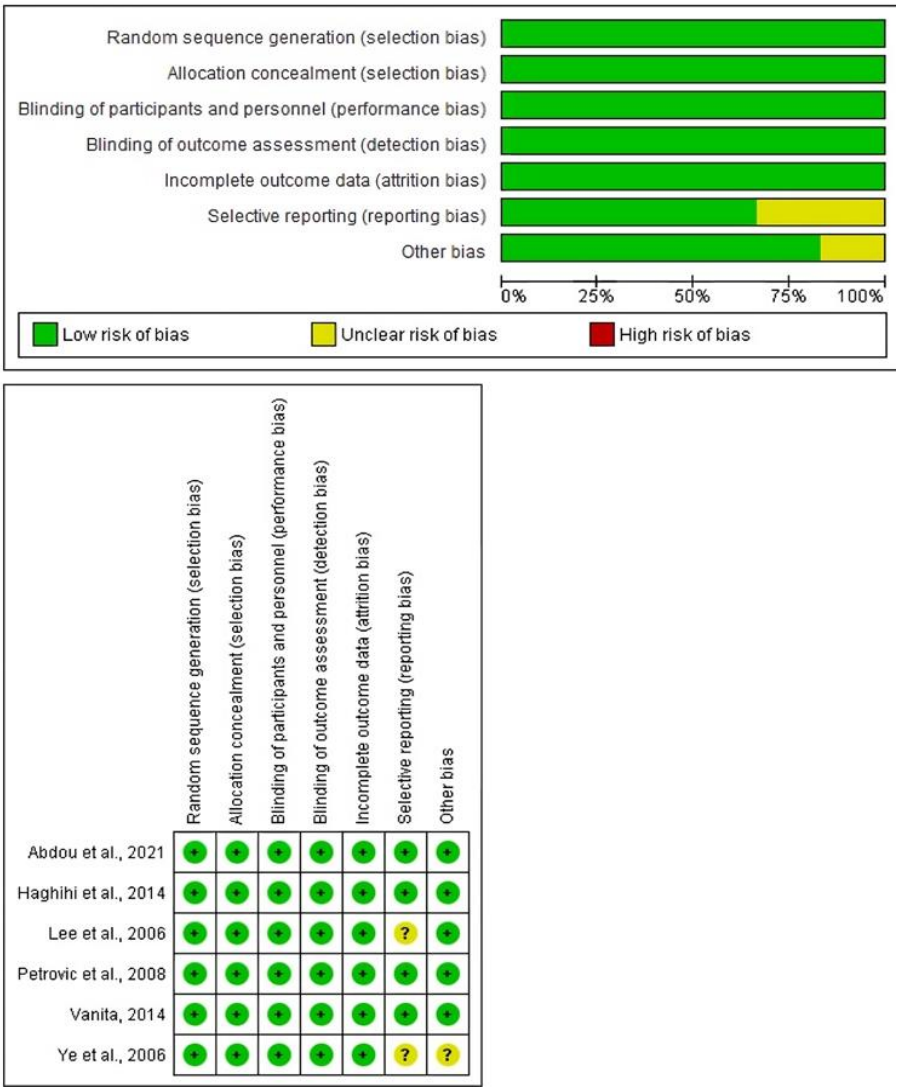

Figure 5. Risk of bias for included studies (Source: Authors' own elaboration)

growing interest [12]. It is revealed that the polymorphism in exon 2 of the SOD2 gene A16V (C/T) (rs4880) gene is considered functional as it alters the structural configuration in the mitochondrial targeting domain, eventually resulting in diminished antioxidant capability and limited post-transcriptional enzymatic transport [26]. It was previously explained the immense role of MnSOD polymorphism at the free radical level [27]. Substituting C to T (GCT to GTT) in MnSOD polymorphism alters the translation from alanine (Ala) to valine (Val) [27]. mRNA will be easily degraded, forming a β-sheet secondary structure rather than the expected α-helix. The newly formed configuration then will be poorly recognized by the receptor, resulting in mistargeting and leading to reduced SOD transport efficacy in complex I mitochondria. The processes then finally lead to 30-40% less MnSOD activity, causing less scavenging activity and more oxidative stress in mitochondria [26, 28, 29].

Previous findings revealed that MTS-Ala precursor MnSOD (A) can be optimally transported across both mitochondrial membranes quickly to reach the matrix, while MTS-Val precursor MnSOD (B) is instead partially anchored to the mitochondrial inner membrane. This finding concluded that the Ala precursor in Mn-SOD produces an active, matrixial MnSOD homotetramer, 30-40% more active than Val-MnSOD precursor. β-sheet secondary structure will eventually diminish MnSOD activity and escalate oxidative stress. This polymorphism plays a critical pathophysiological mechanism in development of retinopathy and other diabetic complications as it worsens hyperglycemia condition [27, 29].

Impaired Antioxidant Response & Retinopathy Diabetes

Retina, a tissue rich in polyunsaturated fatty acids, is constantly exposed to visible light and consumes more oxygen, making it particularly susceptible to oxidative stress damage [11, 21]. The increasing level of oxidative stress could particularly contribute to the significant mitochondrial fragmentation in retinal endothelial cells. This phenomenon can be explained as oxidative stress could initiate a pro-apoptosis cascade through several mechanisms, including epigenetic modification, mitochondrial dysfunction, and neurodegeneration [9, 30].

Hyperglycemia-induced epigenetic modification in retinopathy diabetes is closely linked to abnormal transcription in antioxidant genes. Alterations in transcription factors, histone modification (methylation and acetylation), or hypermethylation of mtDNA result in limited defense against free radicals and abnormally elevated levels of pro-inflammatory mediators. Eventually, overexpression of pro-inflammatory proteins results in vascular lesions, inferring its

crucial role as a contributor to diabetic vascular complications, including DR [31-34].

The epigenetic modifications in mitochondrial DNA (mtDNA) have been demonstrated as a latent influence to induce base mismatch in the mitochondrial system [35]. Damaged mtDNA will then cause impaired transcription and protein synthesis, resulting in abnormal function of the electron transport chain system, especially in complex I. The dysfunction of mitochondria promotes cytochrome C leakage to initiate subsequent pro-apoptosis caspase cascade [36, 37]. The retinal vascular cell apoptosis will then result in acellular capillaries and pericyte loss, the hallmark of retinopathy diabetes itself [30].

Pericyte cells will go through abnormal apoptosis, causing altered permeability in the membrane, hence causing acellular capillaries. Various growth factor pathways will be altered, resulting in structural and functional changes in the retina. Excessive ROS levels will destroy BRB by elevating the VEGF level, altering vascular permeability. In addition, elevated VEGF levels will cause neovascularization in the retina. However, the newly established vessels are vulnerable and quickly go through a breakdown, causing outpouching and microaneurysms. These processes then will lead to retinopathy diabetes, especially when excessive oxidative stress remains uncontrolled [9].

To our knowledge, this is the first meta-analysis that investigated the association between MnSOD Ala16Val genetic polymorphism and DR risk in T2DM patients. The identification of a significant association of MnSOD Ala16Val polymorphism, specifically in the co-dominant model (AA vs. VV), provides a potentially useful marker for increased DR susceptibility in T2DM patients, paving the way for the development of a targeted gene panel for assessing individual predisposition. The inclusion of a small number of studies, however, may have impacted the statistical power of our meta-analysis. Further studies involving larger sample sizes across diverse ethnic groups is necessary to enhance understanding of the association between MnSOD Ala16Val polymorphism and DR susceptibility among T2DM patients.

CONCLUSIONS

This meta-analysis revealed a significant association between the MnSOD Ala16Val genetic polymorphism and an elevated risk of DR in individuals with T2DM, specifically under the co-dominant (VV vs. AA) and dominant model (VV+VA vs. AA). Therefore, the MnSOD Ala16Val genetic polymorphism emerges as a potential marker for heightened DR susceptibility in T2DM patients, laying the foundation for a gene panel to assess their tendency to develop DR.

Author contributions: ASDN, GIP, AP, & CDKW: draft manuscript; **ASDN, GIP, & AP:** methodology, literature search, study screening, data acquisition, & risk of bias assessment; **ASDN & AP:** project conceptualization; **ASDN:** formal statistical analysis & result interpretation; **GIP, AP, & CDKW:** extensive research on topic & critical revisions; & **CDKW:** project supervision. All authors have agreed with the results and conclusions.

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Ethical statement: The authors stated that the study does not require any ethical approval as it only extract secondary data that is accessible online in medical metadata.

Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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APPENDIX A

Table A1. PRISMA 2020 checklist for systematic reviews & meta-analysis

Section & topic	Item	Checklist item	RPN
Title			
Title	1	Identify the report as a systematic review, meta-analysis or both.	0
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	1-3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	1 & 3
Methods			
Protocol & registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	3
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	3-4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3 & SM
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	SM
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4 & **Figure 1**
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	SM
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4, 5, & **Figure 5**
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	1, 4, 5, & **Table 2**
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	4 & 5
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6 & **Figure 5**
Additional analysis	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5-7 & **Figure 3**
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5 & **Figure 1**
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	4, **Table 1**, & SM
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (item 12).	**Figure 5**
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	**Table 1** & **Figure 2**
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals & measures of consistency.	6, **Figure 2**, & SM
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (item 15).	6 & **Figure 4**
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [item 16]).	5-7 & **Figure 3**
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8-10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of results in context of other evidence, & implications for future research.	10
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	11

Note. RPN: Reported on page number & SM: Supplementary material

APPENDIX B

Table B1. Search strategies

Database	Search strategy	First hit results
MEDLINE via PubMed	#1 “polymorphism, genetic” “[MeSH Terms]” OR “polymorphism, single nucleotide [MeSH Terms]” #2 ““diabetic retinopathy”[MeSH Terms]” #3 ““superoxide dismutase”[MeSH Terms]” #4 **#1 AND #2 AND #3**	19
Scopus	#1 TITLE-ABS-KEY (“polymorphism, genetic”) OR TITLE-ABS-KEY (“polymorphism, single nucleotide”) #2 TITLE-ABS-KEY (“diabetic retinopathy”) #3 TITLE-ABS-KEY (“superoxide dismutase”) #4 **#1 AND #2 AND #3**	19
Web of Science	#1 TS=(polymorphism, genetic) OR TS=(polymorphism, single nucleotide) #2 TS=(diabetic retinopathy) #3 TS=(superoxide dismutase) #4 **#1 AND #2 AND #3**	12
ScienceDirect	(polymorphism, genetic OR polymorphism, single nucleotide) AND (diabetic retinopathy) AND (superoxide dismutase)	566
EMBASE	#1 *Polymorphism, Genetic/ or polymorphism, single nucleotide/ #2 *Superoxide Dismutase/ #3 *Diabetic Retinopathy/	9

Table B2. PICO framework

Components of PICO	Definition
Population	Adults aged 18 or older diagnosed with T2DM according to American Diabetes Association 2021, along with diabetic retinopathy as diagnosed by ophthalmologists through standardized test.
Index test	Manganese superoxide dismutase (MnSOD) Ala16Val genetic polymorphism.
Comparator	Control (T2DM with no DR).
Outcome	DR susceptibility (odds ratio, 95% confidence interval).

Table B3. Quality assessment for observational cohorts using NOS tool

Included studies	Selection (0-4 points)				Comparability (0-2 points)		Outcome (0-3 points)			TP
	REC	SNEC	AE	DOIW	AIR	AOR	AO	FL	LFR	
[2]	1	1	1	1	1	1	1	1	1	9
[20]	1	1	1	1	1	1	1	1	1	9
[21]	1	0	1	1	1	1	1	1	1	8
[22]	1	1	1	1	1	1	1	1	1	9
[23]	1	1	1	1	1	0	1	1	1	8
[24]	1	1	1	1	1	0	0	1	1	7

Note. REC: Representativeness of exposed cohort; SNEC: Selection of non-exposed cohort; AE: Ascertainment of exposure; DOIW: Demonstration that outcome of interest was not present at start of study; AIR: Adjust for the important risk factors; AOR: Adjust for other risk factors; AO: Assessment of outcome; FL: Follow-up length; LFR: Loss to follow-up rate; & TP: Total points