Journal of Research in Applied and Basic Medical Sciences

ORIGINAL RESEARCH ARTICLE

Identification of CTLA-4 +49 A/G (Rs231775) Polymorphism in Diabetic Retinopathy and its Allelic Association with Cardiovascular Disease Risk Factors

Praneeth Putti[1], Karnati Rohith[2], Vijaya bhaskar Potuganti[3], M Lakshmi Surya Prabha[4], Juhi Aggarwal[5], Nasrin[6], Meraj Mohiuddin[7], Shabana Shireen[8], Babulal S[9], Swarna deepak Kuragayala[10], Mahaboob Vali Shaik[11]

Published: 15 November 2025

Abstract

Background The Study investigates the association between the CTLA-4 +49 A/G (rs231775) polymorphism and cardiovascular disease risk factors in patients with diabetic retinopathy.

Methods Fifty cases with diabetic retinopathy were compared to fifty healthy controls. Blood samples were subjected to ARMS-PCR with primers specific to the CTLA-4 +49 A/G (rs231775) gene.

Results Hypertension was identified as a co-factor for diabetic retinopathy, with an odds ratio of 1.32 (95% CI: 0.45-4.85). Furthermore, the A allele was more frequently observed in patients with a history of myocardial infarction than the G allele, with counts of 25 versus 10 and 11 versus 4 (p = 0.455). The A allele was also more prevalent in the hyperlipidemia group than the G allele, with frequencies of 34 versus 1 and 9 versus 6 (p = 0.255). Hyperlipidemia was recognized as a co-factor for diabetic retinopathy, with an odds ratio of 2.25 (95% CI: 0.42-12.65). The A allele showed an odds ratio of 3.85 (95% CI: 1.95-7.99), indicating an increased risk of developing diabetic retinopathy. The AG genotype was significantly more common in the diabetic retinopathy group compared to the control group (31 versus 11) (p = 0.003), and this genotype was associated with a higher risk for diabetic retinopathy (odds ratio: 12.2, 95% CI: 4.56-41.85). The AA genotype was more frequently observed in the diabetic retinopathy group than the control group (15 versus 22) (p = 0.312) (odds ratio: 3.2, 95% CI: 0.598-7).

Conclusion The AG genotype and the A allele were more prevalent in patients with diabetic retinopathy, suggesting that they may be risk factors. The AA genotype, the AG genotype, and the G allele exhibited significant associations with diabetic retinopathy.

Keywords Allele, Diabetic retinopathy, Hyperlipidemia, Hypertension, Obesity, Polymorphism

✉ Mahaboob Vali Shaik
research.nmc1@gmail.com

1. Department of Ophthalmology, Mallareddy Institute of Medical Sciences, GHMC Quthbullapur, Jeedimetla, Hyderabad, Telangana, India
2. Associate Professor, General Medicine, Madha Medical College and Research Institute, Kovur, Chennai, Tamil Nadu, India
3. Department of Anatomy, MNR Medical College and Hospital, Sangareddy, Hyderabad, India
4. Addl DME, Director (Research & Development), Dr NTR University of Health Sciences, Gunadala, Vijayawada, Andhra Pradesh, India
5. Department of Biochemistry, Santosh Medical College and Hospital, Santosh Deemed to be University, Ghaziabad, U.P., India
6. Senior General Ophthalmologist & Cornea specialist, Director of Medical Administration & Training, Sankar Foundation Eye Hospital, NaiduThota, Visakhapatnam, Andhra Pradesh, India
7. General Medicine, Nimra Institute of Medical Sciences, Vijayawada, A.P, India
8. Department of Biochemistry, Nimra Institute of Medical Sciences, Vijayawada, A.P, India
9. Department of Pulmonology, Nimra Institute of Medical Sciences, Vijayawada, India
10. Department of Critical Care Medicine, Apollo Health City, Jubilee Hills, Hyderabad, Telangana. India
11. Department of Research, Nimra Institute of Medical Sciences, Vijayawada, AP, India

Received: 25 July 2025/ Revised: 7 September 2025/ Accepted: 1 November 2025

1 Introduction

Diabetes mellitus is one of the leading global health issues, with India being the second most affected country after China. In 2019, it was projected that 77 million people in India had diabetes, with the number expected to rise to over 134 million by 2045.[1] Common eye problems related to diabetes include diabetic retinopathy, neovascular glaucoma, cataracts, strabismus, ptosis, oculomotor nerve palsy, and hordeolum.[2] Often, assessments of the ocular surface are neglected, with too much focus placed on retinopathy.[3]

Diabetic retinopathy (DR) is a microvascular complication of diabetes mellitus and a major cause of visual impairment and blindness globally. Non–vision–threatening stages of DR include mild and moderate non-proliferative DR (NPDR). Vision-threatening DR includes severe NPDR, proliferative DR (PDR), and diabetic macular edema (DME), which occurs at any DR stage. DR results from prolonged high blood sugar, damaging retinal blood vessels and causing leakage, ischemia, and new blood vessel growth. The International Diabetes Federation estimates over 537 million people worldwide have diabetes, with projections rising to 643 million by 2030, and about 34.6% of these individuals are affected by DR.[4] Diagnosis often requires fundus photography and Optical Coherence Tomography (OCT) to detect macular edema and other signs. Strict glycemic control can reduce the progression of DR by up to 76%. Treatment options include laser photocoagulation to seal leaking vessels and reduce neovascularization, as well as intravitreal injections of anti-VEGF agents, such as ranibizumab and aflibercept, to treat macular edema.[5] For advanced cases with vitreous hemorrhage or retinal detachment, pars plana vitrectomy is the standard surgical treatment.

Numerous studies have identified various genetic variations associated with an increased risk of type 2 diabetes mellitus (T2DM), providing important insights into the underlying pathophysiological mechanisms. A particularly significant genomic region is the CTLA-4 gene, which encodes the cytotoxic T-lymphocyte-associated protein. This gene plays a crucial role in regulating inhibitory signals that counteract T cell activation, thereby influencing immune homeostasis.[6] Located on chromosome 2q33, the CTLA-4 gene contains recurring single-nucleotide polymorphisms (SNPs) that have been studied for their potential associations. The CTLA-4 +49 A/G (rs231775) SNP has garnered considerable attention.[7] However, the impact of the CTLA-4 +49 A/G gene polymorphism on individuals with DR remains largely unexplored. Additionally, investigations into the association between these alleles and various cardiovascular disease risk factors have not yet been conducted. Gaining a better understanding of how CTLA-4 gene variants influence the risk of developing DR could aid in creating more effective prevention and management strategies.

2 Methods

Fifty patients diagnosed with DR were compared with fifty healthy individuals. The inclusion criteria included patients in both the case and control groups. In the case group (DR, n = 50), patients had to confirm a diagnosis of diabetes mellitus and be defined as having sight-threatening DR, including severe NPDR, PDR, or DME. Retinopathy status was confirmed through standardized ophthalmic evaluations, such as dilated slit-lamp biomicroscopy, multi-field fundus photography, or OCT. In the control group (diabetic patients without retinopathy), controls were required to have a confirmed diagnosis of DM (either type 1 or type 2, matching the case group) but without any signs of DR. The most stringent and valuable control groups consisted of "diabetes-resistant" individuals—patients with a long duration of diabetes (e.g., 10 to 15 years or more) yet no evidence of DR. This helped control the strong confounding effect of disease duration. Retinopathy status in controls was confirmed using the same standardized ophthalmic evaluations as the case group. Controls were defined as having no DR or, at most, minimal NPDR. Additionally, controls were matched to cases by age, gender, and ethnic/racial background to minimize the influence of population stratification on the results.

Exclusion criteria were applied to minimize confounding factors that could independently influence the development of retinopathy and interfere with genetic findings. Patients with severe comorbidities, such as advanced heart failure, liver disease, or malignancy, were excluded. Individuals with inflammatory diseases or those taking anti-inflammatory medications or antioxidant supplements were also excluded, as these factors can independently affect retinal health. Exclusion of other diabetic complications, such as severe nephropathy or neuropathy, was sometimes considered to isolate genetic factors specific to DR.

Other pre-existing or co-existing eye conditions not related to diabetes—such as age-related macular degeneration (AMD), glaucoma, or uveitis—were a basis for exclusion to prevent misinterpreting other retinal damage as DR. Patients with significant media opacities, including cataracts that interfere with high-quality fundus photography or accurate retinopathy grading, were also excluded.

Procedure

The research specifically targeted those with DR, intentionally omitting any other eye conditions or disorders. The study employed the ARMS-PCR method, using Tetra-ARMS-PCR primers (Table 1).

Table 1 The Teträ-ARMS-PCR primers for CTLA-4 +49 A/G

Primer	Sequence (5'–3')	Purpose	Amplicon size (bp)
Forward Inner (A-allele)	CACAAGGCTCAGCTGAACCTGGATG	Amplifies the A-allele-specific product	237
Reverse Inner (G-allele)	ACAGGAGAGTGCAGGGCCAGGTCCTAGT	Amplifies the G-allele-specific product	216
Forward Outer	TCTATTCAAGTGCCTTCTGTGTGTGCA	Produces the non-allele-specific control band	409
Reverse Outer	GCCAAGCCAGATTGGAGTTTTACCTT	Produces the non-allele-specific control band	409

Gel electrophoresis results for each genotype:
A/A Homozygote: 2 bands at 409 bp (control) & 237 bp (A-allele).
G/G Homozygote: 2 bands at 409 bp (control) & 216 bp (G-allele).
A/G Heterozygote: 3 bands at 409 bp (control), 237 bp (A-allele), & 216 bp (G-allele)

ARMS-PCR

Genomic DNA was extracted using a Blood DNA Extraction Kit. The concentration and purity of the extracted genomic DNA from blood samples were evaluated with a NanoDrop spectrophotometer (THERMO, USA).

The T-ARMS-PCR technique was employed to genotype the CTLA-4 +49 A/G (rs231775) gene polymorphism in both patient and healthy control groups. The T-ARMS-PCR master mix was prepared using the Taq G2 Green Master Mixture kit, with two reactions performed for each sample. The standard T-ARMS-PCR reaction mixture included 5 µL of DNA template, 1 µL of Forward Inner Primer (10 pmol), 1 µL of Reverse Inner Primer (10 pmol), 1 µL of Forward Primer (10 pmol), 1 µL of Reverse Primer (10 pmol), 12.5 µL of G2 Green Master Mix, and 3.5 µL of Nuclease-Free Water. The PCR master mix was transported to the centrifuge and spun at 3000 rpm for 3 minutes before being placed in a PCR Thermocycler (BioRad, USA). Cycling conditions: 10 min at 95°C followed by 35 cycles of 94°C for 30 seconds, annealing for 30 seconds, 72°C for 30 seconds, and a final cycle of 72°C for 7 minutes.

For statistical analysis, continuous variables were expressed as mean ± standard deviation. The associations of the odds ratio (OR) and 95% confidence interval (CI) were examined using the chi-square test. Statistical analysis was performed using SPSS software for Windows, version 25.0.

3 Results

The patient and control groups are statistically comparable in terms of age and gender, which is crucial for the validity of this case-control study. Hypertension was significantly more common in the DR group, with a prevalence of 76.0%, compared to just 8.0% in the control group (p = 0.001). The prevalence of obesity was notably higher in the DR group at 60%, while the control group had a mere 2.0% (p = 0.002). A significant difference was observed in the history of myocardial infarction, with 72% of the DR cases reporting such a history, compared to only 10% in the control group (p = 0.002). Moreover, hyperlipidemia was found to be significantly more prevalent in the DR cases, with an occurrence of 86%, in contrast to just 2% in the control group (p = 0.003) (Table 2).

Table 2 Demographics and patients with DR in association with cardiovascular disease risk factors

Characteristics	Patients	Control	P-value*
Age (years)	52.24 ± 14.9	49.55 ± 13.6	0.45
Range	21-75 yrs	22-72 yrs	
20-30	6 (12.00 %)	8 (16.00 %)	
31-45	6 (12.00 %)	8 (16.00 %)	
66-70	12 (24.00 %)	9 (18.00%)	0.59
46-65	17 (34.00 %)	17 (34.00%)	
> 70	9 (18.00 %)	8 (16.00 %)	
Sex			
Male, n (%)	23 (46.00 %)	27 (54.00 %)	0.725
Female, n (%)	27 (54.00 %)	23 (46.00 %)	
Hypertension			
Yes, n (%)	38 (76.00 %)	4 (8 %)	0.001
No, n (%)	12 (24.00 %)	46 (92 %)	
Obesity			
Yes, n (%)	30 (60 %)	2 (4 %)	0.002
No, n (%)	20 (40 %)	48 (96 %)	
History of myocardial infarction			
Yes, n (%)	36 (72 %)	5 (10 %)	0.002
No, n (%)	14 (28 %)	45 (90 %)	
Hyperlipidemia			
Yes, n (%)	43 (86.00 %)	1 (2.00 %)	0.003
No, n (%)	7 (14.00 %)	49 (98.00 %)	

*chi-square test

Recognition of CTLA-4 +49 A/G (rs231775) Polymorphism

The CTLA-4 +49 A/G (rs231775) polymorphism was examined using the ARMS-PCR method. Three different genotypes were detected at this locus: AG, AA, and GG. The wild-type homozygous genotype (AA) showed amplification exclusively of the A allele, while the mutant homozygous genotype (GG) amplified only the G allele. The heterozygous genotype (AG) exhibited amplification of both G and A alleles, each corresponding to a distinct product size. The AG genotype was significantly more common in DR cases than in the control group (31 vs. 11), with a p-value of 0.003. Additionally, the AG genotype was linked to a heightened risk of DR, with an odds ratio of 12.2 (95% CI: 4.56-41.85). Conversely, the homozygous genotype AA was more frequently observed in the DR group than in the control group (15 vs. 22), although this difference was not statistically significant (p = 0.312) (Table 3).

Allele A was more common in the DR group than in the control group, with frequencies of 77 and 46, respectively. This difference was statistically significant (p = 0.0045). Additionally, genotype A was identified as a risk factor for DR, with an OR of 3.85 (95% CI: 1.95-7.99) (Table 4).

The relationship between the CTLA-4 +49 A/G (rs231775) polymorphism and the risk of developing DR in obese individuals was investigated. Allele A was more common among hypertensive individuals than allele G (27 vs. 8 and 12 vs. 4), although this difference did not reach statistical significance (p = 0.65). As a result, allele A was suggested as a potential risk factor for DR, with an OR of 1.32 (95% CI: 0.45-4.85). In the obese group, allele A also showed a higher frequency than allele G (23 vs. 13 and 8 vs. 7). Still, this finding was not statistically significant (p = 0.399), resulting in an OR of 1.55 (95% CI: 0.39-4.66) for DR. Furthermore, allele A was more frequently found in individuals with a history of myocardial infarction compared to allele G (25 vs. 10 and 11 vs. 4), yet this difference was again not statistically significant (p = 0.455), indicating an OR of 1.399 (95% CI: 0.298-4.366) for DR. Additionally, allele A was significantly more prevalent in the hyperlipidemia group compared to allele G (34 vs. 1 and 9 vs. 6). However, this difference was also non-significant (p = 0.255). Therefore, allele A was associated with an increased risk for DR, as reflected by an OR of 2.25 (95% CI: 0.42-12.65) (Table 5).

The G allele in patients with diabetic retinopathy shows significant correlation with Hypertension, Hyperlipidemia, History of myocardial infarction, and Obesity risk factors. Similarly, AA and AG genotypes show significant correlations with risk factors such as hypertension, Hyperlipidemia, History of myocardial infarction, and obesity. Neither genotype nor allele has a significant correlation with the Age factor (Table 6).

4 Discussion

This study included a diverse age range of patients diagnosed with DR. The results regarding traditional risk factors were consistent with previous research conducted in various populations, including those in Europe.[8] Age and gender are acknowledged as major risk factors for diabetes, with older individuals facing a higher risk for T2DM due to increased insulin resistance (IR) and changes in pancreatic function that occur with aging.[9,10]

In this research, the prevalence and management of hypertension among patients with confirmed T2DM exceeded international standards. However, there is potential for improved hypertension management in patients who have both hypertension and DR through targeted interventions.[11]

This study highlights the importance of management strategies for individuals affected by DR. Our findings support earlier research linking longer diabetes duration

Table 3 CTLA-4 +49 A/G (rs231775) poly genotype in DR cases and the control group

CTLA-4 +49 A/G (rs231775)	Cases, n = 50	Control, n = 50	P1	P2	OR	95% CI
AA	15	22	0.0002	0.003	12.2	4.56-41.85
AG	31	11		0.312	3.2	0.598 – 7.98
GG	4	17		Reference	Reference	Reference

Important relations of the stately OR and 95% CI were evaluated using chi-square.

Table 4 CTLA-4 +49 A/G (rs231775) poly allele occurrence in DR cases & control group

CTLA-4+49 A/G (rs231775)	Patients n = 100	Control n = 100	P-value	OR	95%CI
A	77	46	0.0045	3.85	1.95- 7.99
G	23	54		0.2308	0.145- 0.524

Table 5 Association of alleles of CTLA-4 +49 A/G (rs231775) by cardiovascular risk factors

	Hypertension				
	Yes, n = 38	No, n = 12	OR	95% CI	P-value
Allele A, n (%)	27 (71.05%)	8 (66.67%)			0.65
Allele G, n (%)	11 (28.94%)	4 (33.33%)	1.32	0.45-4.85	
	Obesity				
	Yes, n = 30	No, n = 20	OR	95% CI	
Allele A, n (%)	22 (73.33%)	13 (65%)	1.55	0.39-4.66	0.399
Allele G, n (%)	8 (26.67%)	7 (35%)			
	History of myocardial infarction				
	Yes, n = 36	No, n = 14	OR	95% CI	
Allele A, n (%)	25 (69.45%)	10 (71.42%)			0.455
Allele G, n (%)	11 (30.56%)	4 (28.58%)	1.399	0.298-4.366	
	Hyperlipidemia				
	Yes, n = 43	No, n = 7	OR	95% CI	
Allele A, n (%)	34 (79.07%)	1 (14.28%)			0.255
Allele G, n (%)	9 (20.93)	6 (85.72%)	2.33	0.42-12.65	

Table 6 Correlation of CVD Risk Factors with DR

		Age	**Hypertension**	**Hyperlipidemia**	**History of myocardial infarction**	**Obesity**
Allele A	R-value	0.005	0.088	0.083	0.065	0.005
	P-value	0.960	0.409	0.439	0.545	0.960
	n	90	90	90	90	90
Allele G	R-value	0.088	0.439**	0.684**	0.759**	0.439**
	P-value	0.409	0.000	0.000	0.000	0.000
	n	90	90	90	90	90
AA	R-value	0.083	-0.684**	-0.439**	0.914**	-0.439**
	P-value	0.439	0.000	0.000	0.000	0.000
	n	90	90	90	90	90
AG	R-value	0.065	0.76**	0.914**	0.463**	-0.463**
	P-value	0.545	0.000	0.000	0.000	0.000
	n	90	90	90	90	90

**Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Pearson correlation = R-value

with hypertension. The relationship between DR, obesity, and IR is complex and interdependent, emphasizing the need to understand this connection.[12,13]

Our findings align with previous studies regarding the effect of the CTLA-4 +49 A/G polymorphism on the development of DR. Dong et al. analyzed 16 studies involving 3,713 diabetic patients and 3,862 controls, revealing that individuals with the AG genotype had a higher risk of developing T2DM, with an OR of 1.28 and a 95% CI of 1.05-1.56. No significant association was found between the AA genotype and the risk of T2DM.[14] Additionally, Zhou et al. conducted a meta-analysis that confirmed the association between the AG genotype and an increased risk of T2DM, while the AA genotype showed no significant correlation. The slight vulnerability associated with the AG genotype may stem from its potential influence on the expression and function of the CTLA-4 gene, which plays a key role in immune

regulation and inflammation.[15]

The CTLA-4 gene is essential for controlling T-cell activation, and its polymorphisms have been associated with various autoimmune and inflammatory diseases, including T2DM. Allelic analysis indicated a significantly higher frequency of the A allele in individuals with DR compared to the control group, with the A allele showing an OR of 3.85 (95% CI: 1.95-7.99), suggesting its association with an elevated risk of developing DR.[16-18]

In a separate study by Chang et al., which included 17 studies with a total of 5,244 T2DM patients and 5,608 healthy controls, the A allele was associated with an increased risk of T2DM, yielding an OR of 1.40 and a 95% CI of 1.20-1.63.[19]

The risk associated with the A allele may relate to its effects on the presence and function of the CTLA-4 gene. Further investigation is necessary to elucidate the intricate relationships between this genetic variation and both genetic and environmental factors in the progression of DR. Longitudinal studies are often considered crucial for evaluating the prognostic significance of this genetic variant in the development of T2DM and for exploring its potential implications for early interventions and personalized management strategies.[20]

The association between this polymorphism and obesity has not been extensively studied. Some evidence suggests it may affect lipid metabolism and increase cardiovascular risk. Previous research on the CTLA-4 CT60 G/A polymorphism and its health effects has produced mixed results. Research on lipid metabolism also suggests a potential association with cardiovascular risk, but the exact mechanisms remain to be investigated. This study could not compare its results due to a lack of prior research involving DR patients, highlighting the need for more studies to clarify the relationship between DR and cardiovascular risk factors. Additionally, the findings are limited by a small sample size.

5 Conclusion

The CTLA-4 +49 A/G (rs231775) polymorphism indicated that the AG genotype and A allele were more common among DR patients, suggesting these genetic factors may increase the risk of DR. However, although no statistically significant association was observed between CTLA-4 polymorphisms and obesity, the CTLA-4 +49 A allele was associated with an increased risk of hypertension, obesity, myocardial infarction, and hyperlipidemia in the DR patient group.

Declarations

Acknowledgments
Not applicable.

Artificial Intelligence Disclosure
No AI or any other related sources were used.

Authors' Contributions
Mahaboob Vali and Skandha Harshita contributed to the study theme, experimental design, data collection, and article writing. Munni Shaik, John Basha, Babulal, Nissi Molli, Swarna Deepak, Jayaram G, and Vijaya Shekar were involved in data analysis, article writing, and critical review of the manuscript.

Availability of Data and Materials
Data related to this article are available from the corresponding author upon request.

Conflict of Interest
There is no conflict of interest to be declared.

Consent for Publication
Not applicable.

Ethical Considerations
The study was approved by the Institutional Ethics Committee under the Code of Ethics IEC/NIMS-VJA/2023/RC.02.

Funding
No funding was received for this study.

References

1. Pradeepa R, Mohan V. Epidemiology of type 2 diabetes in India. Indian J Ophthalmol. 2021;69(11):2932-8. doi:10.4103/ijo.IJO_1627_21
2. Sayin N, Kara N, Pekel G. Ocular complications of diabetes mellitus. World J Diabetes. 2015;6(1):92-108. doi:10.4239/wjd.v6.i1.92
3. Wang S, Jia Y, Li T, Wang A, Gao L, Yang C, et al. Dry eye disease is more prevalent in children with diabetes than in those without diabetes. Curr Eye Res. 2019;44(12):1299-305. doi:10.1080/02713683.2019.1641827
4. Lundeen EA, Burke-Conte Z, Rein DB, Wittenborn JS, Saaddine J, Lee AY, et al. Prevalence of diabetic retinopathy in the US in 2021. JAMA Ophthalmol. 2023;141(8):747-54. doi: 10.1001/jamaophthalmol.2023.2289
5. Wallsh JO, Gallemore RP. Anti-VEGF-resistant retinal diseases: a review of the latest treatment options. Cells. 2021;10(5):1049. doi: 10.3390/cells10051049

6. Fuchsberger C, Flannick J, Teslovich TM, Mahajan A, Agarwala V, Gaulton KJ, et al. The genetic architecture of type 2 diabetes. Nature. 2016;536(7614):41-7. doi: 10.1038/nature18642

7. Mirsharif ES, Rostamian A, Salehi M, Askari N, Ghazanfari T. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)+ 49A> G (rs231775) gene polymorphism is not associated with COVID-19 severity and mortality in an Iranian population. Heliyon. 2024;10(1). doi: 10.1016/j.heliyon.2023.e23308

8. Lindarto D, Darmadi D. Association Between CTLA4 Level and Obesity, Dyslipidemia, and Type 2 Diabetes Mellitus. Med Arch. 2025;79(2):117. doi: 10.5455/medarh.2025.79.117-121

9. Russo G, Manicardi V, Rossi M, Orsi E, Solini A. Sex-and gender-differences in chronic long-term complications of type 1 and type 2 diabetes mellitus in Italy. Nutr Metab and Cardiovasc Dis. 2022;32(10):2297-309. doi:10.1016/j.numecd.2022.08.011

10. Zheng W, Chu J, Ren J, Dong J, Bambrick H, Wang N, et al. Age-and gender-specific differences in the seasonal distribution of diabetes mortality in Shandong, China: a spatial analysis. Int J Environ Res Public Health. 2022;19(24):17024. doi: 10.3390/ijerph192417024

11. Alwadeai KS, Alhammad SA. Prevalence of type 2 diabetes mellitus and related factors among the general adult population in Saudi Arabia between 2016–2022: A systematic review and meta-analysis of the cross-sectional studies. Medicine (Baltimore). 2023;102(24):e34021. doi: 10.1097/MD.0000000000034021

12. Kirkman MS, Briscoe VJ, Clark N, Florez H, Haas LB, Halter JB, et al. Diabetes in older adults. Diabetes Care. 2012;35(12):2650. doi: 10.2337/dc12-1801

13. Berraho M, El Achhab Y, Benslimane A, Rhazi KE, Chikri M, Nejjari C. Hypertension and type 2 diabetes: a cross-sectional study in Morocco (EPIDIAM Study). Pan Afr Med J. 2012;11(1).

14. Dong, Y., Zhou, J., Zhu, Y., Luo, L., He, T., Hu, H., ... & Yu, M. (2018). Association between sleep duration and HbA1c level: a systematic review and meta-analysis. Sleep medicine reviews, 48, 66-75.

15. Zhou Z. Jin P, Xiang B, Huang G, The association of cytotoxic T-lymphocyte antigen-4 + 49A/G and CT60 polymorphisms with type 1 diabetes and latent autoimmune diabetes in Chinese adults. J Endocrinol Invest. 2015;38:149–54.

16. Yanagawa T, Taniyama M, Enomoto S, Gomi K, Maruyama H, Ban Y, et al. CTLA4 gene polymorphism confers susceptibility to Graves' disease in Japanese. Thyroid. 1997;7(6):843-6. doi: 10.1089/thy.1997.7.843

17. Paterson AM, Lovitch SB, Sage PT, Juneja VR, Lee Y, Trombley JD, et al. Deletion of CTLA-4 on regulatory T cells during adulthood leads to resistance to autoimmunity. J Exp Med. 2015;212(10):1603-21. doi: 10.1084/jem.20141030

18. Ren D, He L, Pang X. Association of CLTA-4 gene polymorphisms with diabetes mellitus: a study based on the Han population of Northern China. Diabetes Metab Syndr Obes: Targets and Therapy. 2022:2705-12. doi:10.2147/DMSO.S374451

19. Chang Y-C, Liu P-H, Yu Y-H, Kuo S-S, Chang T-J, Jiang Y-D, et al. Validation of type 2 diabetes risk variants identified by genome-wide association studies in the Han Chinese population: a replication study and meta-analysis. PloS One. 2014;9(4):e95045. doi:10.1371/journal.pone.0095045

20. Veitch S, Njock M-S, Chandy M, Siraj MA, Chi L, Mak H, et al. MiR-30 promotes fatty acid beta-oxidation and endothelial cell dysfunction and is a circulating biomarker of coronary microvascular dysfunction in pre-clinical models of diabetes. Cardiovasc Diabetol. 2022;21(1):31. doi: 10.1186/s12933-022-01458-z