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The association of *KEAP1* and *NFE2L2* polymorphisms with glycemic control and late complications in patients with type 2 diabetes

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ABSTRACT

To investigate the association of *KEAP1* rs1048290, rs9676881 and *NFE2L2* rs6706649, rs6721961, rs35652124 polymorphisms with glycemic control and development of late complications in patients with type 2 diabetes mellitus (T2DM), a total of 316 T2DM patients were included in the retrospective genetic association study. Genotyping was performed using competitive allele-specific PCR. Data on HbA1c levels as a measure of glycemic control, and information on late complications, including ischemic heart disease, retinopathy, and nephropathy, was obtained from the medical records. Logistic regression analysis was used to assess the association between selected genetic polymorphisms and patients outcomes. Significant associations were observed between *KEAP1* rs9676881 ($p < 0.001$) and *NFE2L2* rs6721961 ($p = 0.006$) polymorphisms and elevated HbA1c levels. Additionally, *NFE2L2* rs35652124 polymorphism was linked to a nominally higher risk of late complications, including ischemic heart disease ($p = 0.036$), retinopathy ($p = 0.032$), and nephropathy ($p = 0.026$). Results indicate that polymorphisms in the *KEAP1* and *NFE2L2* genes may influence glycemic control and the development of late complications in T2DM patients. These findings provide valuable insights into the genetic factors underlying T2DM progression and its complications in European populations, highlighting the potential role of genetic markers in optimizing personalized treatment strategies.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex metabolic disease whose increasing global prevalence presents a significant public health challenge (International Diabetes Federation. IDF Diabetes Atlas Tenth edition 202Accessed 29 August 2024). Inflammation and oxidative stress play key roles in the pathogenesis of T2DM, with hyperglycemia inducing oxidative damage that significantly contributes to the progression of late microvascular complications, including nephropathy, retinopathy, and neuropathy (Wang et al., 2021), as well as macrovascular complications such as ischemic heart disease (Kibel et al., 2020). At the molecular level, the transcription factor NRF2 (Nuclear factor erythroid 2-related factor 2), encoded by the *NFE2L2* gene, is one of the primary regulators of the cellular response to oxidative stress (Baumel-Alterzon et al., 2021). The stability of NRF2 is regulated by the protein KEAP1 (Kelch-like ECH-associated protein 1), which acts as a sensor of oxidative stress and inhibits NRF2 under normal conditions by promoting its degradation (He and Sun, 2021).

The NRF2-KEAP1 pathway is essential for regulating the body's antioxidant defense mechanisms and protecting cells from oxidative damage. On the other hand, dysregulation of the NRF2-KEAP1 pathway can lead to increased inflammation and oxidative stress, contributing to the development of insulin resistance and disturbances in glucose metabolism (DeFronzo et al., 2014). In this context, NRF2 and KEAP1 together may play a protective role in preventing the development of T2DM and its late complications (Behl et al., 2021). Genome-wide

Abbreviations: T2DM, Type 2 diabetes mellitus; NRF2, Nuclear factor erythroid 2-related factor 2; KEAP1, Kelch-like ECH-associated protein 1; SNPs, Single nucleotide polymorphisms; SGLT-2, Sodium-glucose cotransporter 2; GLP-1, Glucagon-like peptide-1; MAF, Minor allele frequency; KASP, Competitive allele-specific PCR; HWE, Hardy-Weinberg equilibrium; ORs, Odds ratios; CIs, Confidence intervals; BMI, Body mass index.

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association studies have already identified several genetic variants that are associated with NRF2 expression and may play a crucial role in the pathogenic mechanisms associated with the development of T2DM (Shin et al., 2019). Epidemiological and genetic association studies in recent years have shown that polymorphisms in the promoter region of the *NFE2L2* gene are associated with diseases caused by oxidative stress, as they influence the regulation of gene expression and, consequently, the ability of cells to respond to oxidative damage (Teena et al., 2020; Wang et al., 2021; Jiménez-Osorio et al., 2016). Among the most commonly studied single nucleotide polymorphisms (SNPs) in the *NFE2L2* gene in this context are rs6721961, rs6706649, and rs35652124 (Teena et al., 2020). Moreover, genetic and epigenetic alterations within the NRF2-KEAP1 pathway can significantly impact the clinical progression of T2DM, affecting not only disease development but also the individual response to therapeutic treatment (Buse et al., 2019). Understanding the relationship between specific genetic polymorphisms and control of T2DM, particularly in relation to therapeutic outcomes, is therefore essential for advancing personalized medicine in diabetes care.

Beyond well-known treatments like metformin and sulfonylureas, which have long played a central role in T2DM management, in recent years, newer antihyperglycemic medications, such as sodium-glucose cotransporter 2 (SGLT-2) inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists, have been introduced into clinical practice (DeFronzo et al., 2014; Buse et al., 2019). These medications not only differ in their mechanisms of action and efficacy in glycemic control but also in their ability to protect against the development of late complications in T2DM (Susilawati et al., 2023). Importantly, oxidative stress may play a role in the effects of these medications, as it is a common factor influencing the pathophysiology of T2DM and its complications. For instance, metformin has been found to attenuate T2DM-induced oxidative stress by enhancing total antioxidant capacity and reducing total oxidant status through its ability to inhibit glucose autoxidation and suppress mitochondrial reactive oxygen species production (May Hassan Abdul-Hadi, 2020). Additionally, studies have shown that SGLT-2 inhibitors and GLP-1 receptor agonists provide therapeutic benefits beyond glycemic control by enhancing mitochondrial function, reducing oxidative stress, and alleviating inflammation (Luna-Marco et al., 2024; Winiarska et al., 2021).

The aim of this study was to investigate the impact of selected SNPs in the *NFE2L2* and *KEAP1* genes, which regulate antioxidant pathways, on the management of T2DM and the development of late complications in patients treated with different types of antihyperglycemic agents.

2. Materials and methods

2.1. Study Design

This retrospective genetic association study integrated two independent cohorts of patients with T2DM. One cohort comprised patients from a historical dataset collected between 2011 and 2012, while the second cohort included patients treated with newer class of antihyperglycemic agents between 2018 and 2022. Both cohorts were subjected to identical protocols for data collection, genotyping and analysis. Given the methodological consistency, data from both cohorts were combined and analysed as a single study population.

2.2. Study participants

A total of 316 clinically well-defined T2DM patients were included in the study. The study cohort included patients receiving treatment with a variety of antihyperglycemic agents, including both conventional and newer therapeutic options, as well as combination treatments. Patients were recruited from General Hospital Trbovlje, Health Center Kočevje, and University Medical Centre Ljubljana, Slovenia. Inclusion criteria were (1) confirmed diagnosis of T2DM, (2) age between 18 and 75 years, and (3) signed informed consent. Patients with other types of diabetes (e.g., type 1, gestational, pancreatogenic), drug-induced diabetes, or conditions potentially affecting study compliance, such as mental or cognitive impairment, substance abuse, and a history of cancer in the last five years, were not included in the study.

2.3. DNA isolation and genotyping

DNA samples were obtained from peripheral venous blood or buccal swabs. DNA isolation was performed according to the instructions of the manufacturer using E.Z.N.A.® SQ II Blood DNA Kit (Omega Bio-tek, Inc., USA) or the QIAamp DNA Mini Kit (Qiagen, Germany).

NFE2L2 and *KEAP1* polymorphisms were considered for the analysis based on their high minor allele frequencies (MAFs) and established functional relevance, particularly their involvement in the oxidative stress regulation and antioxidant response pathways. *In silico* predicted function selected polymorphisms was evaluated using SNP Function Prediction and FORGEdb (Breeze et al., 2024; Xu and Taylor, 2009). Only SNPs with MAF above 0.05 were included in the analysis. The selected *NFE2L2* rs6706649, rs6721961 and rs35652124, as well as *KEAP1* rs1048290 and rs9676881 polymorphisms were genotyped using competitive allele-specific PCR (KASP) assays according to the manufacturer's instructions (LGC Biosearch Technologies, UK). To ensure the accuracy and reproducibility of the genotyping, control samples with known genotypes were included on each PCR plate, and approximately 20% of the samples were independently re-genotyped.

2.4. Statistical analysis

Median and interquartile ranges (25th to 75th percentiles) were used to describe continuous variables, while frequency distributions were used to describe the distribution of categorical variables. The MAF was calculated for each polymorphism, and Hardy-Weinberg equilibrium (HWE) was assessed using the chi-squared test. Analyses were conducted using additive and dominant genetic models. Normality of continuous variables was assessed with the Shapiro-Wilk test; as they were not normally distributed, we used non-parametric tests to evaluate the association of SNPs with continuous variables, including the Mann-Whitney test for two independent groups and the Kruskal-Wallis test with *post hoc* Bonferroni correction for comparisons across more than two groups.

Logistic regression was employed to assess the association of polymorphisms with late complications, calculating odds ratios (ORs) and 95% confidence intervals (CIs) through univariate analyses. For multivariate logistic regression, key clinical covariates were selected using stepwise forward conditional selection from the following clinical variables: sex, age, smoking status, T2DM duration, body mass index (BMI), blood pressure, HbA1c, dyslipidemia, statin treatment, hypertension, and lipid profile measurements. In cases where no subjects were present in a particular group, Fisher's exact test was used for analysis.

All statistical tests were two-sided. To decrease the chance of false-positive findings due to the investigation of five polymorphisms, Bonferroni correction was used to account for multiple comparisons. The level of statistical significance was set at 0.010 and p-values between 0.010 and 0.050 were considered as nominally significant. Analyses were conducted using IBM SPSS Statistics version 27.0 (IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Patients' characteristics

A total of 192 male and 124 female patients with T2DM were included in the study. The median age of participants was 66 (60–72) years, with a median duration of diabetes of 14 (7–20) years. The majority of participants were overweight or obese, as reflected by a median body mass index (BMI) of 30 kg/m^2. The median HbA1c level was 7.5%,

indicating suboptimal long-term glycemic control. Demographic, clinical characteristics of patients are summarized in Table 1. A higher proportion of patients had microvascular complications (23.1%) compared to macrovascular complications (18.0%).

3.2. Genotype frequencies

The genotype distribution for the investigated *KEAP1* and *NFE2L2* polymorphisms, including the frequency of the minor allele and the adherence to HWE, is shown in Table 2. Deviations from HWE were observed for *KEAP1* rs9676881 ($p = 0.004$), *NFE2L2* rs6706649 ($p = 0.004$), and *NFE2L2* rs6721961 ($p < 0.001$) polymorphisms. MAFs in our study were comparable to MAF in the European population reported in dbSNP (Phan et al., 2025) for all SNPs except rs9676881 ($p = 0.06$). However, *KEAP1* and *NFE2L2* polymorphisms were previously associated with T2DM risk (Teena et al., 2020; Wang et al., 2021; Jiménez-Osorio et al., 2016; Fan et al., 2022; Khalili et al., 2022) that could contribute to the observed differences between the genotype distribution in our study group and the frequencies reported in European population. Based on this, we included all SNPs in further analyses. *In silico* predicted function of the investigated polymorphisms is presented in Supplementary Table 1.

Table 1
Patient's characteristics (N = 316).

Variable	Category/unit	N (%) / median (25 %-75 %)
Sex	Men, N (%)	192 (60.8)
	Women, N (%)	124 (39.2)
Age	Years, median (25 %-75 %)	66 (60–72)
Duration of T2DM	Years, median (25 %-75 %)	14 (7–20)
Smoking	No, N (%)	209 (66.1)
	Yes, N (%)	107 (33.9)
Body mass	kg, median (25 %-75 %)	87 (77–102)
BMI	kg/m^2, median (25 %-75 %)	30 (28–34) [1]
Systolic blood pressure	mmHg, median (25 %-75 %)	140 (130–150)
Diastolic blood pressure	mmHg, median (25 %-75 %)	80 (74.3–85)
HbA1c	%, median (25 %-75 %)	7.5 (6.6–8.4)
Total cholesterol	mmol/l, median (25 %-75 %)	4.2 (3.5–4.9) [3]
HDL cholesterol	mmol/l, median (25 %-75 %)	1.1 (0.9–1.4) [5]
LDL cholesterol	mmol/l, median (25 %-75 %)	2.3 (1.8–3.0) [4]
TAG	mmol/l, median (25 %-75 %)	1.7 (1.2–2.5) [3]
Urea	mmol/l, median (25 %-75 %)	6.2 (4.9–7.9) [4]
Creatinine	μmol/l, median (25 %-75 %)	78 (68–95.5) [3]
eGFR	$ml/min/1.73\ m^2$, median (25 %-75 %)	78 (64–90) [3]
Arterial hypertension	No, N (%)	44 (14.8) [19]
	Yes, N (%)	253 (85.2)
Diagnosed dyslipidaemia	No, N (%)	90 (28.5)
	Yes, N (%)	226 (71.5)
Statin treatment	No, N (%)	94 (30.0) [3]
	Yes, N (%)	219 (70.0)
Type of complication		
Macrovascular complications	No, N (%)	259 (82.0)
	Yes, N (%)	57 (18.0)
Peripheral artery occlusive disease	No, N (%)	304 (96.2)
	Yes, N (%)	12 (3.8)
Ischemic heart disease	No, N (%)	285 (90.2)
	Yes, N (%)	31 (9.8)
Microvascular complications	No, N (%)	243 (76.9)
	Yes, N (%)	73 (23.1)
Neuropathy	No, N (%)	295 (93.7)
	Yes, N (%)	20 (6.3)
Retinopathy	No, N (%)	269 (85.1)
	Yes, N (%)	47 (14.9)
Nephropathy	No, N (%)	287 (90.8)
	Yes, N (%)	29 (9.2)

The number of missing data is given in [] brackets. Categorical variables are presented as N (%), continuous variables as median, and interquartile range (25th and 75th percentile). N – number of patients, T2DM – type 2 diabetes mellitus, BMI – body mass index, HDL – high-density lipoprotein. HDL – high-density lipoprotein, LDL – low-density lipoprotein, TAG – triacylglycerides, eGFR – estimated glomerular filtration rate.

3.3. Association of polymorphisms with glycemic control of T2DM

The associations of *KEAP1* and *NFE2L2* polymorphisms with HbA1c concentration is presented in Table 3. For *KEAP1* rs9676881, a statistically significant difference in HbA1c levels was observed among different genotypes ($p < 0.001$) as the AA genotype carriers had significantly higher HbA1c levels compared to carriers of the GG ($p_{adj} = 0.029$) and GA genotype ($p_{adj} < 0.001$) (Fig. 1A).

A statistically significant difference in HbA1c levels was also observed for the *NFE2L2* gene polymorphism rs6721961 ($p = 0.006$) as TT genotype carriers exhibited markedly higher HbA1c levels compared to the carriers of the GG ($p_{adj} = 0.048$) or GT ($p_{adj} = 0.002$) genotypes (Fig. 1B).

3.4. Association of polymorphisms with late complications of T2DM

The association of *KEAP1* and *NFE2L2* polymorphisms with macrovascular and microvascular complications of T2DM is presented in Supplementary Tables 2 and 3. Carriers of two polymorphic *NFE2L2* rs35652124 alleles were slightly more likely to experience microvascular complications, but the association did not reach statistical significance in univariable (OR = 2.41 (1.00–5.84), p = 0.050) or multivariable analysis (OR = 2.19 (0.83–5.73), p = 0.111). No associations with late complications were observed for the other *NFE2L2* or *KEAP1* polymorphisms.

When evaluating the association of investigated polymorphisms with individual late complications, *NFE2L2* rs35652124 was associated with ischemic heart disease, retinopathy and nephropathy (Table 4). Carriers of two polymorphic *NFE2L2* rs35652124 alleles were more likely to experience ischemic heart disease (OR = 2.98 (1.08–8.24), p = 0.036), but the difference was not significant after adjustment for the duration of T2DM (p = 0.053). Similarly, carriers of two polymorphic *NFE2L2* rs35652124 alleles had nominally higher risk for retinopathy (OR = 2.87 (1.09–7.53), p = 0.032) and nephropathy (OR = 3.52 (1.16–10.66), p = 0.026), but the difference was not significant in multivariable analysis (p = 0.141 and p = 0.054, respectively). No associations with individual complications were observed for the other investigated polymorphisms (data not shown).

4. Discussion

Oxidative stress is a key factor in the development of late complications in T2DM, and *KEAP1* and *NFE2L2* genes encode transcription factors that regulate oxidative stress-related pathways. In this retrospective genetic association study, we examined the impact of five *KEAP1* and *NFE2L2* polymorphisms on glycemic control in T2DM patients. Our findings suggest that *KEAP1* rs9676881 and *NFE2L2* rs6721961 are associated with increased HbA1c, indicating poorer glycemic control, while *NFE2L2* rs35652124 is nominally associated with an increased risk of late complications.

We observed a statistically significant association of *KEAP1* rs9676881 with HbA1c levels. Individuals with the AA genotype had higher HbA1c levels compared to those with the GG or GA genotypes. These findings suggest that the presence of two polymorphic A alleles leads to worse glycemic control. Previous studies have highlighted the

Table 2
Frequency distribution of the investigated polymorphisms.

Gene	SNP	SNP description	Genotype	N (%)	MAF (%)	P_{HWE}	MAF in the European population*
KEAP1	rs1048290 [14]	p.Leu471=	GG	100 (33.1)	40.9	0.190	37.1
			GC	157 (52.0)			
			CC	45 (14.9)			
	rs9676881 [3]	c.*548G > A	GG	106 (33.9)	45.4	**0.004**	37.9
			GA	130 (41.5)			
			AA	77 (24.6)			
NFE2L2	rs35652124 [3[	c.-769 T > C	TT	139 (44.4)	32.3	0.235	32.7
			TC	146 (46.6.)			
			CC	28 (8.9)			
	rs6706649 [2]	c.-767C > T	CC	248 (79.0)	12.1	**0.004**	11.8
			CT	56 (17.8)			
			TT	10 (3.2)			
	rs6721961 [10]	c.-733 T > G	GG	231 (75.5)	14.9	**<0.001**	11.6
			GT	59 (19.3)			
			TT	16 (5.2)			

[] – number of patients in whom we were unable to determine the genotype. KEAP1 – Kelch like ECH associated protein 1 gene, NFE2L2 – NFE2 like bZIP transcription factor 2 gene, Leu – leucine, A – adenine, G – guanine, C – cytosine, T – thymine, MAF – minor allele frequency, P_{HWE} – p-value of the $\chi 2$ test for HWE, *ALFA project, dbSNP (Phan et al., 2025).

Table 3
Association of the studied polymorphisms with HbA1c concentration.

Gene	SNP	Genotype	HbA1c (%) Median (25 %-75 %)	P
KEAP1	rs1048290	GG	7.6 (6.5–8.5)	$P_{add} = 0.761$
		GC	7.4 (6.7–8.3)	
		CC	7.6 (6.6–8.7)	
		GC + CC	7.4 (6.7–8.4)	$P_{dom} = 0.777$
	rs9676881	GG	7.7 (6.6–8.5)	**$P_{add} < 0.001$**
		GA	7.1 (6.6–8.0)	Pairwise comparisons: AA vs GG P = 0.029 AA vs GA P < 0.001
		AA	8.2 (7.1–9.2)	
		GA + AA	7.4 (6.7–8.4)	$P_{dom} = 0.945$
NFE2L2	rs35652124	TT	7.4 (6.6–8.3)	$P_{add} = 0.179$
		TC	7.6 (6.6–8.4)	
		CC	8.1 (7.2–9.1)	
		TC + CC	7.7 (6.7–8.5)	$P_{dom} = 0.207$
	rs6706649	CC	7.5 (6.6–8.4)	$P_{add} = 0.863$
		CT	7.7 (6.6–8.4)	
		TT	7.2 (6.7–8.3)	
		CT + TT	7.7 (6.7–8.4)	$P_{dom} = 0.794$
	rs6721961	GG	7.5 (6.7–8.4)	**$P_{add} = 0.006$**
		GT	7.1 (6.6–7.9)	Pairwise comparisons: TT vs GG P = 0.048 TT vs GT P = 0.002
		TT	8.6 (7.5–9.5)	
		GT + TT	7.4 (6.6–8.3)	$P_{dom} = 0.583$

For the additive model (P_{add}), Kruskal-Wallis test was used for statistical analysis, and for the dominant model (P_{dom}), Mann-Whitney test. HbA1c – haemoglobin A1c, also glycated haemoglobin, A – adenine, G – guanine, C – cytosine, T – thymine.

important role of KEAP1 in regulating oxidative stress and inflammatory processes, which can influence the development and progression of T2DM. *KEAP1* rs9676881 is located in the 3′ untranslated region, near transcription factor binding and enhancer sites, and may be associated with varying levels of *KEAP1* gene expression, potentially influencing the body's ability to regulate oxidative stress (Soto et al., 2023). However, the exact impact of this polymorphism on *KEAP1* expression remains incompletely understood. Given the lack of comprehensive studies directly examining the association between the rs9676881 and HbA1c levels, further studies are required to validate our findings (Matana et al., 2020).

NFE2L2 rs6721961 was also statistically significantly associated with HbA1c levels. Individuals with the TT genotype had higher HbA1c levels compared to those with the GG or GT genotypes. Researchers have suggested that rs6721961 is associated with impaired β-cell function and enhanced insulin resistance, contributing to the progression of T2DM (Wang et al., 2015). The polymorphic rs6721961 T allele was associated with lower promoter activity and lower tissue NRF2 expression (Hartikainen et al., 2012; Marzec et al., 2007; Khadir et al., 2022). This can influence the balance between oxidative stress and antioxidant processes, increasing oxidative stress, potentially contributing to impaired glycemic control and higher HbA1c levels (He et al., 2020; Mhaibes and Ali, 2024). Despite an extensive review of the literature, no previous studies investigated the link between the rs6721961 and HbA1c levels. Therefore, our findings provide an important basis for further research, as genetic variability in the NRF2-KEAP1 pathway could significantly influence glycemic control in patients with T2DM.

The role of genetic polymorphisms in T2DM is an area of increasing research interest, particularly due to their potential association with the development of late complications of the disease. Among all the investigated polymorphisms, only polymorphic *NFE2L2* rs35652124 C allele was associated with an increased risk for specific late complications (ischemic heart disease, retinopathy, and nephropathy) in our study. However, the association was not significant after adjustment for clinical parameters, suggesting this polymorphism is not an independent prediction factor. The rs35652124 polymorphism, also located in the *NFE2L2* promoter region, may affect the binding of NRF2 to antioxidant response elements, therefore influencing the expression of its target genes (Teena et al., 2020; Marzec et al., 2007). It has been associated with an increased risk of insulin resistance and impaired angiogenesis in the Indian population, contributing to a higher susceptibility to both T2DM and diabetic foot ulcers, (Teena et al., 2020) which is consistent with our results. On the other hand, contradicting results were observed in another study where *NFE2L2* rs35652124 C allele had a protective effect against the development of retinopathy in T2DM patients as the CC genotype was more common in subjects without diabetic retinopathy (Mhaibes and Ali, 2024). Similar findings were reported in animal models, where the NRF2 factor plays an important role in preventing the progression of diabetic retinopathy. In the study, which examined mice with genetic inactivation of the *NFE2L2* gene, they observed significantly greater visual dysfunction and retinal damage compared to wild-type mice (Xu et al., 2014). These results indicate that NRF2 may contribute to the maintenance of retinal health in T2DM. However, the different results obtained for *NFE2L2* rs35652124 across studies could be attributed to various factors such as genetic differences between the studied populations, sample size, and methodologies used in the respective studies. Additionally, clinical characteristics of the included patients could also contribute to these differences, especially as we only

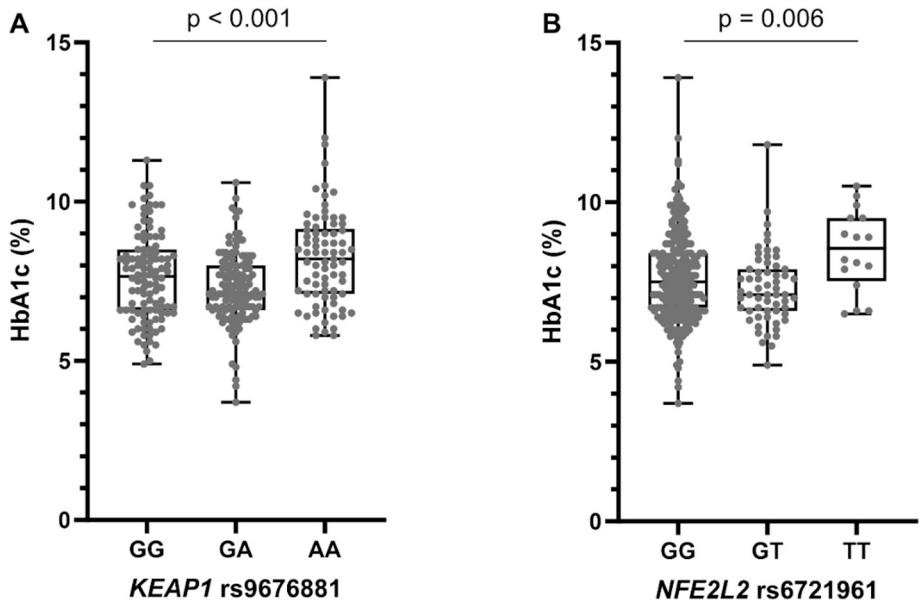

Fig. 1. The association of *KEAP1* rs9676881 (A) and *NFE2L2* rs6721961 (B) with HbA1c levels.

Table 4
Significant associations from univariable and multivariable analyses of genetic polymorphism *NFE2L2* rs3652124 with late complications of T2DM.

Genotype	Ischemic heart disease N (%)	OR (95 % CI)	P	OR (95 % CI)$_{adj}$	P$_{adj}$
TT	14 (10.1)	Ref.		Ref.	
TC	9 (6.2)	0.59 (0.25–1.40)	0.230	0.58 (0.24–1.39)	0.220
CC	7 (25.0)	2.98 (1.08–8.24)	**0.036**	2.82 (0.99–8.08)	0.053
TC + CC	16 (9.2)	0.90 (0.43–1.92)	0.794	0.88 (0.41–1.89)	0.735
Genotype	**Retinopathy N (%)**	**OR (95 % CI)**	**P**	**OR (95 % CI)$_{adj}$**	**P$_{adj}$**
TT	17 (12.2)	Ref.		Ref.	
TC	21 (14.4)	1.21 (0.61–2.40)	0.593	1.12 (0.53–2.39)	0.763
CC	8 (28.6)	2.87 (1.09–7.53)	**0.032**	2.30 (0.76–7.00)	0.141
TC + CC	29 (16.7)	1.44 (0.75–2.74)	0.272	1.29 (0.63–2.64)	0.482
Genotype	**Nephropathy N (%)**	**OR (95 % CI)**	**P**	**OR (95 % CI))$_{adj}$**	**P$_{adj}$**
TT	10 (7.2)	Ref.		Ref.	
TC	13 (8.9)	1.26 (0.53–2.98)	0.597	1.26 (0.52–3.04)	0.611
CC	6 (21.4)	3.52 (1.16–10.66)	**0.026**	3.23 (0.98–10.62)	0.054
TC + CC	19 (10.9)	1.58 (0.71–3.52)	0.262	1.54 (0.67–3.51)	0.309

Adj: Adjusted for T2DM duration (ischemic heart disease), adjusted for T2DM duration, body weight, and HbA1c levels (retinopathy), adjusted for T2DM duration and body weight (nephropathy). OR – odds ratio, CI – confidence interval. Bolded values in the table represent statistically significant results in the univariable analysis.

observed significant associations in univariable analysis and not after adjustment for clinical parameters, emphasizing the need for further research to better understand the genetic influences on retinopathy development across different populations.

Although we did not find other studies directly examining the link between the *NFE2L2* polymorphism rs35652124 and nephropathy or ischemic heart disease in T2DM patients, this polymorphism was previously associated with elevated systolic and diastolic blood pressure, cardiovascular mortality, and other kidney or cardiovascular outcomes, such as the risk of chronic kidney disease (Gómez-Garciá et al., 2022). Additionally, rs35652124 C allele was previously linked with an increased risk of acute type A aortic dissection and increased incidence of brain ischemia, which is consistent with the elevated risk of ischemic heart disease observed in our study (Zhang et al., 2021). On the other hand, the reference T allele was associated with higher blood pressure and cardiovascular mortality in hemodialysis patients (Shimoyama et al., 2014), however, it was hypothesized that this might be potentially associated with the regulation of lipoproteins and not just oxidative stress regulation (Zazueta et al., 2022) Our results nonetheless suggest a potential association between the rs35652124 polymorphism and the risk of developing micro- and macrovascular complications. This finding opens up possibilities for further research focused on validating our hypotheses in larger and more diverse populations.

Consistent with our findings, some studies conducted on animal models have demonstrated that the activation of the NRF2-KEAP1 pathway plays a crucial role in protecting against oxidative stress and chronic inflammation, which are involved in the development of late complications such as diabetic nephropathy. (Lee et al., 2024). Research on mouse models has further elucidated the role of the NRF2 signaling pathway in protecting against damage caused by oxidative stress, which is key to understanding the progression of macrovascular complications in T2DM. Targeted modulation of the NRF2 signaling pathway may mitigate oxidative stress and reduce the risk of late complications in T2DM, as demonstrated in animal model experiments (Xu et al., 2014).

4.1. *Characteristics and limitations of our study*

While our study provides valuable insights, it is important to acknowledge certain limitations. One of the primary constraints was the sample size, which may not have been sufficient to detect statistically significant effects of the selected polymorphisms on the development of individual microvascular or macrovascular complications.

We investigated two *KEAP1* (rs1048290 and rs9676881) and three *NFE2L2* (rs6706649, rs6721961, rs35652124) polymorphisms. MAFs of investigated SNPs were generally comparable with those reported in the general European population. However, a notable deviation was observed for the rs9676881 polymorphism, where the MAF in our cohort was substantially higher (45.9%) compared to that reported in the European population (37.9%). These findings may suggest specific genetic

characteristics within the studied cohort and could influence the interpretation of genetic data and its association with T2DM.

Further analysis identified deviations from HWE in three polymorphisms: rs9676881, rs6706649, and rs6721961, indicating our cohort is not necessarily representative of the general population, which could impact the reliability of observed genetic association and their interpretations. Nonetheless, these polymorphisms were retained in the analysis due to their established associations with an increased risk of T2DM and its late complications in previous studies. For instance, rs6721961 and rs6706649, located in the promoter and regulatory regions of the *NFE2L2* gene, have been linked to antioxidant responses and vascular function, both of which are critical in conditions like hypertension and cardiovascular disease (Wang et al., 2021; Jiménez-Osorio et al., 2016; Zazueta et al., 2022). Conversely, the rs9676881 polymorphism in the *KEAP1* gene, which also exhibited a deviation from HWE, may be involved in the pathogenesis of T2DM. Although no empirical studies directly confirm this hypothesis, existing literature highlights associations between other *KEAP1* polymorphisms, such as rs11085735 and rs11545829, and the development of T2DM, as well as their potential link to late complications of diabetes (Fan et al., 2022; Khalili et al., 2022).

Lastly, the impact of different antihyperglycemic treatments within the cohort may have influenced the treatment outcomes, representing an additional potential confounding factor.

5. Conclusion

Our findings revealed that the *KEAP1* rs9676881 and *NFE2L2* rs6721961 polymorphisms were associated with higher HbA1c levels, indicating poorer glycemic control in individuals carrying polymorphic alleles. Study results provide additional insights into the genetic factors that influence the pathophysiology of T2DM. Notably, the identification of potential targets within the NRF2-KEAP1 pathway present promising avenue for improving the management of late T2DM complications. Further research, particularly in European populations, is essential to improve our understanding of the complex interactions between genetic and environmental factors. Such studies may facilitate the development of personalized therapeutic strategies, ultimately enhancing the management of T2DM and mitigating its associated complications.

Ethics Statement

The study adhered to the Declaration of Helsinki and the Slovenian Code of Medical Ethics and received ethical approval from the National Medical Ethics Committee of the Republic of Slovenia at the Ministry of Health (81–01-11 and KME 0120–209/2018/8).

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CRediT authorship contribution statement

Zala Vraničar: Writing – review & editing, Writing – original draft, Investigation. **Katja Goričar:** Writing – review & editing, Formal analysis, Data curation. **Tanja Blagus:** Writing – review & editing, Investigation, Data curation. **Vita Dolžan:** Writing – review & editing, Supervision. **Jasna Klen:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2025.149631.

Data availability

The datasets generated during and/or analysed during the study are available from the corresponding author on reasonable request.

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