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REVIEW ARTICLE

Apolipoprotein E (ApoE) Gene Polymorphism and Cardiovascular Risk in Type 2 Diabetic Patients: A Systematic Review

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Abstract

Background: Apolipoprotein E gene (ApoE) polymorphism has been extensively studied in the context of lipid metabolism and cardiovascular disease (CVD) risk. Its association with Type 2 Diabetes Mellitus (T2DM) patients presents a unique subset of cardiovascular risk due to underlying metabolic changes. This systematic review aims to summarize and critically evaluate the available evidence on the impact of ApoE gene polymorphism on cardiovascular risk among T2DM patients. Methods: We used the PEO (Population, Exposure and Outcome) framework to develop our protocol, which is publicly available in the Open Science Framework (OSF) registries. Following this protocol, a systematic search was conducted in PubMed, SCOPUS, and Google Scholar to identify studies focusing on adult individuals (≥ 18 years old) diagnosed with Type 2 Diabetes Mellitus (T2DM) that reported on ApoE gene polymorphisms. Only studies published in English between 2014 and 2024 were included. Three authors independently assessed the quality of the eligible studies using the Newcastle-Ottawa Scale (NOS) for case-control, cohort, and cross-sectional designs and the qualified studies were selected for full-text screening and data extraction. Results: 16 studies satisfied the quality assessment using the Newcastle-Ottawa Scale (NOS) with satisfying NOS scores included in the final review, which comprised 1 cohort study, 2 cross-sectional studies, and 13 case-control studies. Across the studies, E3/E3

is the dominant genotype in both control and T2DM populations. E3/E4 and E4/E4 genotypes are found to be significantly elevated in T2DM cases with cardiovascular risk factors compared to controls. The Ɛ3 allele is the most common across all populations, in most studies. The Ɛ4 allele is consistently strongly associated with elevated levels of low-density lipoprotein (LDL-C), total cholesterol, and triglycerides. Conclusion: Apolipoprotein E gene polymorphism is an evident risk factor for cardiovascular complications and ApoE genotyping will be a valuable tool for stratifying T2DM patients based on cardiovascular risk.

Keywords: ApoE gene polymorphism, Cardiovascular diseases, Type 2 diabetes mellitus, Systematic review

Introduction

In recent times, shifts in lifestyle have led to a transformation in disease trends, moving away from infectious and nutrition-related illnesses toward degenerative conditions such as cardiovascular disease (CVD) [1]. Mortality due to cardiovascular disease (CVD) is rising globally, from less than 10 % of total deaths at the start of the century to nearly 30 % by the end, with diabetes mellitus, particularly Type 2 diabetes mellitus (T2DM), emerging as a major risk factor [1,2]. Several observational studies have shown that patients with T2DM are at an increased risk of developing atherosclerotic cardiovascular diseases, including coronary artery disease, stroke, and peripheral artery disease [3]. The association between diabetes and cardiovascular complications is multifactorial, involving metabolic disturbances, hyperglycemia, dyslipidemia, and endothelial dysfunction. However, genetic factors also play a pivotal role in modulating the risk of CVD in individuals with T2DM [4].

Among the genetic factors linked to cardiovascular risk in T2DM, the ApoE gene is of particular interest [5]. Apolipoprotein E is a critical component in lipid transport and a primary ligand for low-density lipoprotein (LDL) receptors, influencing cholesterol metabolism and its influence in cardiovascular diseases. Specifically, ApoE2 and ApoE4 isoforms have been associated with an increased risk of heart disease [6,7]. ApoE2 elevates atherogenic lipoprotein levels due to its reduced affinity for LDL receptors, while ApoE4 raises LDL levels as it preferentially binds to triglyceride-rich, very low-density lipoproteins, leading to the downregulation of LDL receptors. This ApoE4 variant also heightens the susceptibility to cardiovascular diseases by influencing LDL levels. Therefore, the structural features and functional differences among ApoE isoforms, play a critical role in shaping the landscape of cardiovascular diseases and associated risks. In diabetic patients, ApoE can undergo various changes and may play a role in the development and progression of cardiovascular diseases [6,7].

This systematic review aims to attempt a qualitative synthesis of the observational evidence regarding the association between ApoE gene polymorphisms and cardiovascular risk in individuals with Type 2 diabetes and will attempt to validate the predictive value of ApoE genotyping in evaluating cardiovascular risk among diabetic patients.

Materials and methods

We followed the updated 2020 “Preferred Reporting Items for Systematic Reviews” (PRISMA) guidelines for reporting the findings in this systematic review. A full version of the protocol is registered as a public record in the Open Science Framework (OSF) registry along with all supplementary files related to this review. (See protocol and supplementary files: https://doi.org/10.17605/OSF.IO/R36TS) [8,9].

Inclusion and exclusion criteria

We used the PEO (Population, Exposure and Outcome) framework to create the research questions and develop the protocol.

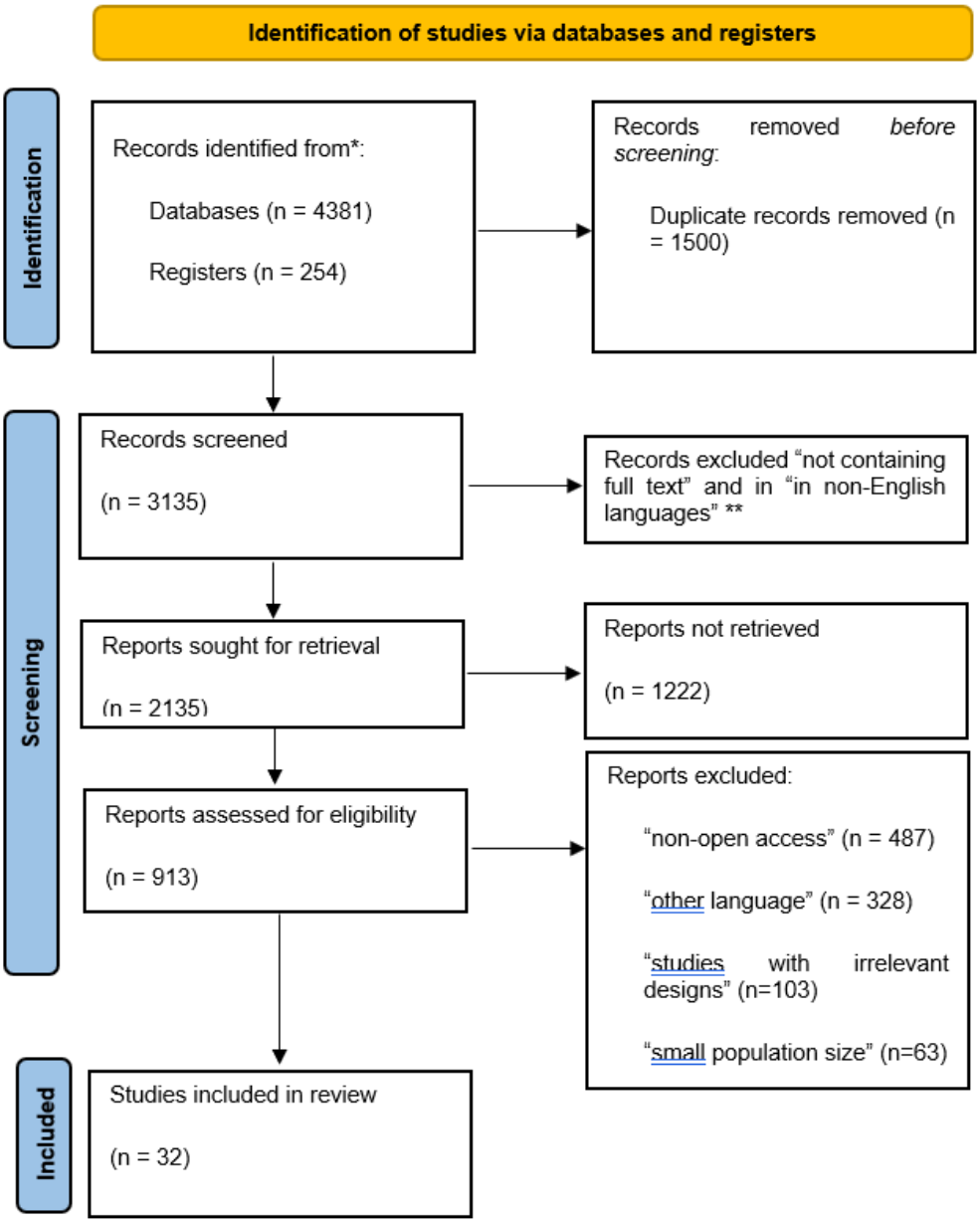
Inclusion criteria

Studies focused on adults (≥ 18 years old) diagnosed with T2DM and reported on ApoE gene polymorphisms, specifically the E2, E3, and E4 alleles. The studies evaluated cardiovascular outcomes, including primary outcomes such as myocardial infarction, stroke, and coronary artery disease, as well as secondary outcomes like changes in lipid profiles, markers of atherosclerosis, blood pressure, BMI, and HbA1c levels. The eligible study designs included

cohort studies, case-control studies, cross-sectional studies, clinical trials, and genetic association studies that assessed cardiovascular outcomes in relation to ApoE polymorphisms in T2DM patients. Only studies published in the English language and published between 2014 and 2024 were included.

Exclusion criteria

Studies involving non-human subjects, animal models, or cell-based studies, as well as studies focusing on patients who were not diagnosed with Type 2 Diabetes Mellitus or who had other forms of diabetes (such as Type 1 Diabetes), were excluded. Studies that did not assess ApoE gene polymorphisms or those that focused on genes unrelated to ApoE, were also excluded. Additionally, studies that did not report on cardiovascular outcomes or relevant cardiovascular risk factors (e.g., studies solely focusing on neurodegenerative diseases or non-cardiovascular outcomes) were excluded. Reviews, editorials, commentaries, conference abstracts, and case reports without original data, or studies lacking a clear comparison group or control relevant to ApoE genotypes, were also not considered for inclusion.



Flowchart 1 PRISMA 2020 flow diagram of the identification, selection and screening stages of the systematic reviews process.

Table 1 Data extraction form of case-control studies.

Author; Year	Groups	Age (Mean & SD)	Gender distribution (M/F)	ApoE genotype distribution (%)	Cardiovascular outcome
Nadeem *et al.* [10]	C: Control D1: Diabetes only D2: T2DM + IHD D3: T2DM + stroke D4: T2DM + IHD + stroke [Each group n = 5]	C = 46.5 - 85 D1 = 46 - 85 D2 = 48 - 80 D3 = 55.5 - 76 D4 = 63.5 - 78	18/34 (In all groups)	C: (E3/E3 = 3.8, E4/E4 = 3.8, E2/E3 = 5.7, E3/E4 = 5.7) D1: (E3/E3 =19.2, E4/E4 = 13.4, E2/E3 = 11.5, E3/E4 = 15.3) D2: (E3/E3 = 26.9, E4/E4 = 30.7, E2/E3 = 15.3, E3/E4 = 21.1) D3: (E3/E3 = 23, E4/E4 = 19.2, E2/E3 = 23, E3/E4 = 25) D4: (E3/E3 = 26.9, E4/E4 = 25, E2/E3 = 19.2, E3/E4 = 23)	Frequency of genotype E3/3 high in cases, Genotype 2/3 was associated with diabetes and stroke or diabetes involving both IHD and stroke. Genotype 3/4 was present frequently in diabetics with stroke and genotype 4/4 was present frequently in subjects with diabetes and IHD. It can be concluded that genotypes 3/3, 4/4, 2/3, and 3/4 influence the development of IHD and stroke in type 2 diabetes.
El-Lebedy *et al.* [11]	C: Control = 84 D1: T2DM = 100 D2: T2DM + CVD = 100	C = 51.8 ± 5.2 D1 = 50.9 ± 7.5 D2 = 58.3 ± 7.2	C = 45/39 D1 = 57/43 D2 = 74/26	C: (E2/E3 ≤ 12, E3/E3 ≤ 80, E3/E4 ≤ 10, ε2 ≤ 10, ε3 ≤ 90, ε4 ≤ 5) D1: (E2/E3 ≤ 10, E3/E3 = 80, E3/E4 ≤ 12, ε2 ≤ 5, ε3 = 90, ε4 ≤ 10) D2: (E2/E3 = 10, E3/E3 = 65, E3/E4 ≤ 25, ε2 ≤ 5, ε3 ≤ 85, ε4 ≤ 12)	E3/E4 genotype associated with 3.6-fold increased risk to develop CVD and ε4 allele associated with 3.2-fold increased CVD risk
Galal *et al.* [12]	C: Controls = 100 D1: T2DM = 100 D2: Obese = 100 D3: T2DM + Obese = 100	C = 31.9 ± 10.8 D1 = 31.8 ± 10.7 D2 = 33.0 ± 9.5 D3 = 34.3 ± 8.8	C = 39/61 D1 = 41/59 D2 = 48/52 D3 = 36/64	C: (E3/E3 = 58, E3/E4 = 10, E2/E2 = 12, E2/E3 = 9, E2/E4 = 7, E4/E4 = 4, Ɛ3 = 67.5, Ɛ2 = 20, Ɛ4 = 25) D1: (E3/E3 = 46, E3/E4 = 12, E2/E2 = 18, E2/E3 = 6, E2/E4 = 5, E4/E4 = 13, Ɛ3 = 55, Ɛ2 = 23.5, Ɛ4 = 21.5) D2: (E3/E3 = 52, E3/E4 = 5, E2/E2 = 24, E2/E3 = 14, E2/E4 = 2, E4/E4 = 3, Ɛ3 = 61.5, Ɛ2 = 32.0, Ɛ4 = 6.5) D3: (E3/E3 = 37, E3/E4 = 4, E2/E2 = 32, E2/E3 = 5, E2/E4 = 6, E4/E4 = 16, Ɛ3 = 41.5, Ɛ2 = 37.5, Ɛ4 = 21)	Ɛ2 and Ɛ4 alleles are independent risk factors for the elevated risk of T2DM and obesity. Moreover, the APOE Ɛ4/Ɛ4 genotype was associated with higher risk of T2DM, while the APOE Ɛ2/Ɛ2 genotype was associated with higher risk of obesity.

Author; Year	Groups	Age (Mean & SD)	Gender distribution (M/F)	ApoE genotype distribution (%)	Cardiovascular outcome
Chen *et al.* [13]	C: Controls = 351 D1: T2DM = 431 D2: T2DM + CAD = 378	C = < 60 = 219, > 60 =132 D1 = < 60 = 177, > 60 = 254 D2 = < 60 = 101, > 60 = 277	C = 226/125 D1 = 285/146 D2 = 243/277	C: (E2/E2 = 0.6, E2/E3 = 13.7, E2/E4 = 2.3, E3/E3 = 72.6, E3/E4 = 9.7, E4/E4 = 1.1, Ɛ2 = 8.5, Ɛ3 = 84.3, Ɛ4 = 7.1) D1: (E2/E2 = 0.2, E2/E3 = 12.8, E2/E4 = 1.9, E3/E3 = 73.8, E3/E4 = 10.4, E4/E4 = 0.9, Ɛ2 = 7.5, Ɛ3 = 85.4, Ɛ4 = 7.1) D2: (E2/E2 = 0.8, E2/E3 = 12.7, E2/E4 = 3.2, E3/E3 = 60.1, E3/E4 = 22, E4/E4 = 1.3, Ɛ2 = 8.7, Ɛ3 = 77.4, Ɛ4 = 13.9)	Elderly, overweight T2DM patients who carried the APOE ε3/ε4 genotype and ε4 allele have of the risk of CAD.
Zeng *et al.* [5]	C: Controls = 995 D: T2DM = 743	C = 69(56 - 84) D = 69(59 - 79)	C = 452/995 D = 350/393	C: (E2/E2 = 0.50, E2/E3 = 12.3, E2/E4 = 2.1, E3/E3 = 65.03, E3/E4 = 18.19, E4/E4 = 1.81, Ɛ2 = 6.80, Ɛ3 = 78.2, Ɛ4 = 15.01) D: (E2/E2 = 0.54, E2/E3 = 10.77, E2/E4 = 1.75, E3/E3 = 60.83, E3/E4 = 23.96, E4/E4 = 2.15, Ɛ2 = 7.74, Ɛ3 = 80.3, Ɛ4 = 11.96)	The genotype E3/E4 and allele ε4 of ApoE are associated with T2DM risk and in patients with E3/E3, E4/E4 genotype had significantly higher lipid profiles which is predisposing factor for CVD.
Sapkota *et al.* [14]	C: Controls = 1,608 D: T2DM = 1,956	C = 50.0 ± 14.5 D = 55.4 ± 11.2	C = 888/720 D = 1078/878	C: (E2/E2 = 0.1, E3/E2 = 6.8, E3/E3 = 80.6, E2/E4 = 0.8, E3/E4 = 11.3, E4/E4 = 0.3, Ɛ2 = 0.04, Ɛ3 = 0.90, Ɛ4 = 0.06) D: (E2/E2 = 0.1, E3/E2 = 6.4, E3/E3 = 79.1, E2/E4 = 0.6, E3/E4 = 13.1, E4/E4 = 0.7, Ɛ2 = 0.04, Ɛ3 = 0.89, Ɛ4 = 0.08)	No significant differences in the distribution of *APOE* genotypes were observed between T2D and CAD cases and controls.
Wang *et al.* [15]	C: Control = 243 D: T2DM with IS = 210	C = 64.58 ± 10.21 D = 65.41 ± 8.20	C = 100/110 D = 126/117	C: (E2/E2 = 1.65, E2/E3 = 11.52, E2/E4 = 2.88, E3/E3 = 7.78, E3/E4 = 12.76, E4/E4 = 0.41, Ɛ2 = 8.85, Ɛ3 = 82.92, Ɛ4 = 8.23) D: (E2/E2 = 0.95, E2/E3 = 18.57, E2/E4 = 1.90, E3/E3 = 60, E3/E4 =	The genotype ε2/ε3 of *APOE* is possibly a genetic predisposition factor for vascular complications in T2DM.

Author; Year	Groups	Age (Mean & SD)	Gender distribution (M/F)	ApoE genotype distribution (%)	Cardiovascular outcome
				17.62, E4/E4 = 0.95, Ɛ2 = 11.9, Ɛ3 = 78.1, Ɛ4 = 10.7)	
Srirojnopkun *et al.* [16]	C: Control = 275 D: T2DM = 241	C = 47.29 ± 7.97 D = 59.35 ± 9.05	C = 79/196 D = 58/183	C: (E2/E2 = 1.82, E2/E3 = 14.91, E3/E3 = 59.27, E3/E4 = 20.36, E4/E4 = 1.45, E2/E4 = 2.18, Ɛ2 = 10.36, Ɛ3 = 76.91, Ɛ4 = 12.73) D: (E2/E2 = 4.15, E2/E3 = 3.73, E3/E3 = 60.99, E3/E4 = 26.97, E4/E4 = 2.92, E2/E4 = 1.24, Ɛ2 = 6.64, Ɛ3 = 76.35, Ɛ4 = 17.01)	Ɛ4 Carriers have a significantly higher TC, and LDL-C levels compared to the E2 and E3 carriers.
Seo *et al.* [17]	C: Control = 1084 D: T2DM = 352	C = 4.93 ± 9.52 D = 59.81 ± 7.78	C = 210/142 D = 656/428	C: (E2/E2 = 1.01, E2/E3 = 9.87, E2/E4 = 0.55, E3/E3 = 74.26, E3/E4 = 13.84, E4/E4 = 1.66 Ɛ2 = 6.1, Ɛ3 = 85.1, Ɛ4 = 8.75 %) D: (E2/E2 =1.14, E2/E3 = 9.94, E2/E4 = 0.57, E3/E3 = 74.15, E3/E4 = 13.92 E4/E4 = 1.70, Ɛ2 = 6.30, Ɛ3 = 84.87, Ɛ4 = 8.82)	Study was unable to find any association between ε genotypes with T2DM susceptibility and APOE ε2 and ε3 alleles showed a significant association with the TC and LDL levels.
Ereqat *et al.* [18]	C: Control (T2DM) = 96 D: T2DM + dyslipidaemia	C = 63.1 ± 10.53 D = 62.3 ± 9.86	C = 63/45 D = 62/34	C: (E2/E2 = 0.9, E2/E3 = 10.2, E2/E4 = 0.9, E3/E3 = 80.6, E3/E4 = 7.4, Ɛ2 = 10.2, Ɛ3 = 82.8, Ɛ4 = 7) D: (E2/E2 = 4.2, E2/E3 = 8.3, E2/E4 = 2.1, E3/E3 = 78.1, E3/E4 = 7.3, Ɛ2 = 12.4, Ɛ3 = 79.6, Ɛ4 = 8)	A comparison of lipid parameters between ε3/ε3 subjects and ε4 carriers in both groups revealed no significant differences in the mean values of LDL-C, HDL-C, TG, and TC levels. But *APOE* promoter region was associated with the risk of diabetic dyslipidemia independently of the *APOE* ε4 variant which could be a potential therapeutic target to reverse the methylation of the *APOE*

Author; Year	Groups	Age (Mean & SD)	Gender distribution (M/F)	ApoE genotype distribution (%)	Cardiovascular outcome
Touré *et al.* [19]	C: Controls = 81 D1: T2DM = 90 D2: T2DM + Arterial stiffness = 31	C = 49.09 ± 8.11 5 D1 = 50.27 ± 6.36 D2 = 54.26 ± 5.22	~	C: (E2/E3 = 70.4, E2/E4 = 16.0, E2/E2 = 0, E3/E3 = 9.9, E3/E4 = 3.7, E4/E4 = 0, Ɛ2 = 86.4, Ɛ 3 = 84.4, Ɛ4 = 19.8) D1: (E2/E3 = 75.6, E2/E4 = 15.6, E2/E2 = 0, E3/E3 = 5.6, E3/E4 = 3.3, E4/E4 = 0, EƐ = 91.1, Ɛ3 = 84.4, Ɛ4 = 18.9) D2: (E2/E3 = 74.2, E2/E4 =16.1, E2/E2 = 0, E3/E3 = 9.7, E3/E4 = 0, E4/E4 = 0, Ɛ2 = 90.3, Ɛ3 = 83.9, Ɛ4 = 16.1)	ε2ε3 genotype associated with low risk for T2DM and atherosclerosis. ε3ε4 genotype associated with higher T2DM risk and possible severe atherosclerosis. ε2ε4 linked with a very high risk of T2DM and atherosclerosis in cases.
Liu *et al.* [20]	C: Controls (T2DM) = 150 D: T22DM with LEAD = 150	C = 56.0 (47.5, 63.0) D = 63.5 (54.0, 69.0)	C = 58/92 D = 90/60	C: (E2 = 24.1, E3/E3 = 67.2, E4 = 8.6, Ɛ2 = 12.1, Ɛ3 = 82.8, Ɛ4 = 5.2) D: (E2 = 8, E3/E3 = 72.7, E4 = 19.3, Ɛ2 = 4, Ɛ3 = 85.7, Ɛ4 = 10.3)	The ε4 allele of the ApoE gene is known to be related to the development of cardiovascular disease
Gonzalez-Aldaco *et al.* [21]	C1: Normal weight = 186 C2: Overweight = 138 C3: Obese = 125 D: T2DM = 168	C1 = 33.5 ± 13.1 C2 = 50.0 ± 16.5 C3 = 41.7 ± 9.0 D = 53.9 ± 9.93	C1 = 53/133 C2 = 55/83 C3 = 38/87 D = 63/105	C: (E2/E2 = 0, E2/E3 = 5.4, E2/E4 = 1.1, E3/E3 = 75.7, E3/E4 = 17.1, E4/E4 = 0.7, Ɛ2 = 3.3, Ɛ3 = 86.9, Ɛ4 = 9.8) D: (E2/E2 = 0.6, E2/E3 = 16.1, E2/E4 = 1.2, E3/E3 = 70.2, E3/E4 = 11.9, E4/E4 = 0, Ɛ2 = 9.2, Ɛ3 = 84.2, Ɛ4 = 6.6)	Normal weight or obese Ɛ*4* allele carriers had a higher level of total cholesterol and hypercholesterolemia than non- Ɛ*4* carriers. Among the T2DM patients, the Ɛ*2* carriers had abnormal HOMA-IR value than the non-Ɛ*2* carriers. Comparatively, between the T2DM patient's *vs* non-diabetics, the *E2/E3* genotype or Ɛ*2* allele conferred a higher risk for T2DM.

Table 2 Data extraction form of cross-sectional studies.

Author, Year	Study population	Age	Gender distribution (M/F)	ApoE genotype distribution (%)	Cardiovascular outcome
Zhang *et al.* [22]	ASCVD = 691 T2DM = 311	65 (57 - 75)	620/382	E2/E2 = 0.2, E2/E3 = 16.2, E2/E4 = 1.4, E3/E3 = 63.2, E3/E4 =17.6, E4/E4 = 1.4, Ɛ2 = 8.89, Ɛ3 = 80.09, Ɛ4 = 10.93)	Strong relationships between ApoE polymorphisms and lipids baseline levels. The E2 phenotypes (e2/e3, e2/e2) had significant LDL-C baseline levels than subjects with E3 (e3/e3, e2/e4) and E4 (e3/e4, e4/e4) phenotypes, and E2 phenotypes had the highest TG baseline levels.
Liu *et al.* [23]	C: Controls = 211 D1: T2DM without CVD = 247 D2: CVD without T2DM = 232 D3: T2DM + CVD = 234	C = 60.65 ± 11.72 D1 = 61.52 ± 11.61 D2 = 63.05 ± 11.79 D3 = 64.61 ± 10.95	C = 109/102 D1 = 127/119 D2 = 126/106 D3 = 119/115	C:(E2/E2 = 0.5, E3/E3 = 75.8, E4/E4 = 11.9, E2/E3 = 8.9, E2/E4 = 2.3, E3/E4 = 19.2, Ɛ2 = 6.2, Ɛ3 = 81.8, Ɛ4 = 12) D1: (E2/E2 = 0.8, E3/E3 = 74.1, E4/E4 = 0, E2/E3 = 6.5, E2/E4 = 1.2, E3/E4 = 17.4, Ɛ2 = 4.7, Ɛ3 = 86, Ɛ4 = 9.3) D2: (E2/E2 = 0.4, E3/E3 = 62.1, E4/E4 = 2.6, E2/E3 = 9.9, E2/E4 = 5.2, E3/E4 = 19.7, Ɛ2 = 7.9, Ɛ3 = 76.9, Ɛ4 = 15.1) D3: (E2/E2 = 0.4, E3/E3 = 59.4, E4/E4 = 1.7, E2/E3 = 8.5, E2/E4 = 3, E3/E4 = 26.9, Ɛ2 = 6.1, Ɛ3 = 77.1, Ɛ4 = 16.7)	APOE polymorphisms are associated with T2DM and CVD. APOE ε4 allele is indicated as an independent risk factor for both T2DM and CVD. APOE genotypes are correlated with plasma lipid profiles.

Table 3 Data extraction form of cohort studies.

Author, Year	Study population	Age	Gender distribution (M/F)	ApoE genotype distribution (%)	Cardiovascular outcome
Santos-Ferreira *et al.* [24]	444 patients	49 ± 14	259/185	E2/E2 = 2.5, E3/E2 = 9.0, E4/E2 = 1.8, E3/E3 = 63.7, E4/E3 = 20.5, E4/E4 = 2.5, Ɛ2 = 7.9, Ɛ3 = 78.5, Ɛ4 = 13.6)	No interaction was found between APOE genotypes and CV outcomes.

Search strategy

To obtain all potentially relevant sources to review we conducted a systematic search across scholarly databases including PubMed, SCOPUS and Google Scholar. For each database, a unique search strategy needs to be applied. A model search string for PubMed with MeSH terms and boolean operators: ("Apolipoprotein E" OR "ApoE" OR "ApoE polymorphism" OR "ApoE gene" OR "ApoE E2" OR "ApoE E3" OR "ApoE E4") AND ("Type 2 Diabetes Mellitus" OR "T2DM" OR "Diabetes Mellitus, Type 2" [MeSH]) AND ("Cardiovascular Disease" OR "CVD" OR "cardiovascular risk" OR "myocardial infarction" OR "stroke" OR "coronary artery disease" OR "atherosclerosis" OR "lipid profile" OR "LDL-C" OR "HDL-C" OR "triglycerides") AND (("cohort study" OR "case-control study" OR "cross-sectional study" OR "clinical trial" OR "genetic association study") OR ("cohort studies" [MeSH Terms] OR "case-control studies" (MeSH Terms) OR "cross-sectional studies" (MeSH Terms) OR "clinical trials as topic" (MeSH Terms))).

Data management

A transparent and organized data management process was ensured and the sources obtained, screening decisions, and data extraction files were stored in the OSF database according to ethical guidelines and repository policies.

Quality assessment

The quality of the selected studies was independently assessed by 3 reviewers using the Newcastle-Ottawa Scale (NOS) for case-control, cohort, and cross-sectional studies. The NOS for case-control and cohort studies evaluated criteria such as the adequacy of the case definition, representativeness of cases, selection and definition of controls, comparability of cases and controls, ascertainment of exposure, and non-response rate. For cross-sectional studies, the NOS quality evaluation assessed the representativeness of the sample, sample size, selection of study subjects, comparability between groups, assessment of outcome, and description of statistical tests.

Table 4 NOS scale quality assessment for cross-sectional studies.

Study reference	Selection (Max 3 star)	Comparability (Max 1 star)	Outcome (Max 3 Stars)	Total stars
Zhang *et al.* [22]	★★	★	★★	5/7
Liu *et al.* [23]	★★		★★	4/7

Table 5 NOS scale quality assessment for Cohort studies.

Study reference	Selection (Max 4 stars)	Comparability (Max 2 stars)	Outcome (Max 3 stars)	Total stars (Max 9 stars)
Santos-Ferreira *et al.* [24]	★★★	★	★★	6/9

Table 6 NOS scale quality assessment for case-control studies.

Study reference	Selection (Max 4 stars)	Comparability (Max 2 stars)	Exposure (Max 3 star)	Total stars (Max 9 stars)
Nadeem *et al.* [10]	★★★★	★	★★	7/9
El-Lebedy *et al.* [11]	★★★	★	★★★	7/9
Galal *et al.* [12]	★★	★	★★	5/7
Chen *et al.* [13]	★★★	★	★★	6/7
Zeng *et al.* [5]	★★★★	★	★★	7/9
Sapkota *et al.* [14]	★★★★	★	★★	7/9

Study reference	Selection (Max 4 stars)	Comparability (Max 2 stars)	Exposure (Max 3 star)	Total stars (Max 9 stars)
Wang *et al.* [15]	★★★	★	★★	6/9
Srirojnopkun *et al.* [16]	★★★	★	★★★	7/9
Seo *et al.* [17]	★★★	★	★★	6/9
Ereqat *et al.* [18]	★★★	★	★★	6/9
Touré *et al.* [19]	★★★★	★	★★	7/9
Liu *et al.* [20]	★★★	★	★★	6/9
Gonzalez-Aldaco *et al.* [21]	★★★	★	★★	6/9

Results

The PRISMA flow diagram (**Flowchart 1**) illustrates the entire review process. A systematic search was conducted according to the strategy outlined in the protocol, resulting in an initial retrieval of 20,600 studies from databases. From this initial pool, 468 records were selected for screening. After the screening process, 56 studies were chosen for quality assessment using the Newcastle-Ottawa Scale (NOS) and 16 studies with satisfying NOS scores were included in the final review, which comprised 1 cohort study, 2 cross-sectional studies, and 13 case-control studies. These 16 studies were selected for full-text reading and data extraction (**Tables 4 - 6**).

All the selected studies examined the distribution of ApoE gene polymorphisms (genotypes and alleles) in T2DM populations and their direct association with cardiovascular risk or risk factors such as coronary artery disease (CAD), stroke, dyslipidemia, and obesity.

Prevalence of genotypes in T2DM and cardiovascular risk

Across the studies, E3/E3 is the dominant genotype in both control and T2DM populations, but the distribution of other genotypes varied according to the cases and their complications. Such as E3/E4 and E4/E4 genotypes are found to be significantly elevated in T2DM cases with cardiovascular risk factors compared to controls. On the other hand, E2/E3 shows mixed results, in some studies it is associated with a protective effect against dyslipidaemia and obesity, which are risk factors for CVD. However, in others, it is linked to elevated CVD risk in T2DM, particularly when combined with obesity or stroke cases. These evidences strengthen the predictive utility of ApoE gene polymorphisms for cardiovascular risk in T2DM. It is clear that analyzing gene polymorphisms will aid in healthcare decision-making and in categorizing patients into different risk groups.

Allelic distribution

The Ɛ3 allele is the most common across all populations, in most studies. The Ɛ4 allele consistently showed a strong association with elevated levels of LDL-C, TC, and triglycerides. Whereas the Ɛ2 allele demonstrates dual effects, where in some studies they are protective in reducing LDL-C and CVD risk in some populations, and in some studies, they are linked to dyslipidaemia and obesity. These observations indicate that analyzing the ApoE gene polymorphism can provide an early understanding of atherosclerotic status and predict cardiovascular risk in individuals with T2DM.

Graph 1 Graphical representation of the genotypic and allelic distribution of the ApoE gene among different groups of studies.

Discussion

Apolipoprotein E is a key structural and functional protein involved in lipoprotein metabolism, mediating the binding and clearance of chylomicrons, very-low-density lipoproteins (VLDL), and their remnants in the liver. The 3 main alleles - ApoE2, ApoE3, and ApoE4 - arise from single-nucleotide polymorphisms in the APOE gene and lead to amino acid substitutions in the ApoE protein. These isoforms differ in their affinity for lipoprotein receptors, which ultimately influence plasma lipid levels, including LDL-C and triglycerides, both critical contributors to atherosclerosis [25].

The elevated risk of CVD complications in T2DM patients can be attributed to several factors and the results of the studies in this systematic review suggest ApoE gene polymorphism is one of the critical risk factors and this elevated risk is conferred through mechanisms involving insulin resistance, inflammation, and dyslipidemia [25].

The *APOE* gene is located on chromosome 19 and encodes the ApoE protein, which is integral to the transport and clearance of cholesterol-rich lipoproteins such as chylomicrons, very-low-density lipoproteins (VLDL), and their remnants. Three major alleles ε2, ε3, and ε4 arise from single-nucleotide polymorphisms that translate into amino acid substitutions in the protein. From these alleles, 6 common genotypes emerge (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4 and E4/E4) [26].

All the reviewed studies conducted a comprehensive genetic analysis of the ApoE genotype distribution and across the studies, ε3 allele and E3/E3 was the dominant genotype in both control and T2DM populations. The majority of the studies observed a relationship between ApoE gene polymorphisms and cardiovascular risk in T2DM subjects. The Ɛ4 allele, E3/E4 and E4/E4 genotypes were found to be significantly elevated in T2DM cases with cardiovascular risk factors compared to controls [10-12]. Whereas the Ɛ2 allele and E2/E3 genotypes shows a mixed effect where in some studies it was associated with a protective effect against dyslipidaemia and in some the very reverse [19,24].

The distribution of these alleles exhibits substantial variability among different ethnic groups and geographic regions. For instance, the ε4 allele is more prevalent in certain African and Northern European populations, whereas ε2 may be slightly more common in parts of Southeast Asia. Several factors may be accountable for this diverse distribution, such as genetic drift, founder effects, migration patterns, and natural selection, moreover, lifestyle and dietary habits are very influential. Such as high saturated fat intake in Western countries to predominantly plant-based diets in some regions of Asian countries [12,21,22,27]. Insulin resistance is the hallmark feature in T2DM which contributes to a distinct pattern of dyslipidaemia characterised by elevated triglycerides, reduced HDL, and variably elevated LDL, in all the studies that reported ApoE gene polymorphisms as a risk marker for CVD, presented *APOE* polymorphisms as an additional layer influencing the extent of lipid abnormalities and leading to subsequent atherosclerotic risk [28].

The synthesized qualitative evidence suggests that ApoE gene polymorphisms play a critical role in cardiovascular complications in T2DM through mechanisms such as lipid metabolism abnormalities, insulin resistance, dyslipidemia, and inflammatory and atherosclerotic processes [10,23]. However, caution is warranted when interpreting these findings, as some studies directly associate ApoE polymorphisms with cardiovascular disease (CVD), while others highlight that the phenotypic characteristics, such as lipid metabolism abnormalities, and inflammatory mechanisms may arise as a consequence of long-standing diabetes rather than solely due to ApoE gene variations.

The relationship between ApoE polymorphisms and cardiovascular risk is complex and influenced by multiple confounding factors, including age, sex, ethnicity, genetic background, lifestyle and behavioral factors, metabolic and clinical conditions, as well as medication and treatment regimens [22-24]. To accurately quantitatively synthesize the association between ApoE gene polymorphisms and cardiovascular risk, future studies should account for and adjust for these key confounding variables.

Despite the well-documented associations between these polymorphisms and CVD in T2DM, the magnitude of this effect is not uniform across all studies, and some studies were observed to fail to detect a significant relationship between ApoE gene polymorphism and CVD [14, 24]. Moreover, in this

study, we have attempted a qualitative summary of current evidence on the predictive role of ApoE gene polymorphism in cardiovascular risk in T2DM. The findings of this study will serve as valuable qualitative evidence for future research efforts aimed at establishing ApoE gene genotyping as a routine marker. As an extension of the current study, a quantitative analysis of the current qualitative summary can be pursued in the future.

Although routine ApoE genotyping is not yet standard in clinical practice, it is clear that it holds value as a risk marker in T2DM patient subsets, guiding targeted lipid-lowering and lifestyle interventions. Future research with large-scale, multiethnic studies, is required to clarify the complexities of gene-by-environment interactions and to verify the usefulness of introducing ApoE genotyping into clinical practice.

Conclusions

Apolipoprotein E gene polymorphisms exert significant influences on lipid metabolism and cardiovascular risk among T2DM patients and the distribution of these alleles and genotypes varies considerably among study populations. All the reviewed empirical studies support the role of the Ɛ4 allele and E3/E4, E4/E4 genotypes in increasing cardiovascular risk in T2DM patients and E2/E3 and the Ɛ2 allele are occasionally protective but have mixed effects, particularly in populations predisposed to obesity and dyslipidaemia. Future research with large-scale, multiethnic studies is required to generalize these findings and to introduce ApoE genotyping in standard clinical practice.

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