10.2478/abm-2010-0041

Asian Biomedicine Vol. 4 No. 2 April 2010; 329-335

Brief communication (Original)

The apolipoprotein E (APOE) gene and the risk of diabetic nephropathy (DN): a meta-analysis in East Asian populations

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Background: Several studies have examined the association between DN and the APOE gene, but the results have been inconsistent.

Objective: Determine whether APOE is a risk factor for DN by a meta-analysis.

Methods: A meta-analysis was performed using all findings of 16 similar case–control studies in East Asian to evaluate the effect of APOE as a risk factor for DN. Several electronic databases were searched for relevant articles up to 2009. After data collection, a meta-analysis was used to assess heterogeneity, combine results and evaluate variations by using software STATA SE 9.0. Publication bias was examined by the Egger's linear regression test and fail-safe number.

Results: The meta-analysis showed that the ε2 allele almost doubled the risk of DN in East Asians (pooled ORs [95% CI]: 1.85 [1.49-2.29]). In contrast, studies relating the ε4 allele to DN risk were very heterogeneous and the pooled ORs were 1.05 [95% CI: 0.72-1.52]. In the subgroup meta-analysis, ε4 was substantially related to an increased risk for DN in studies conducted in China (pooled ORs [95% CI]: 1.51 [1.11-2.06]), which was different from previous results. However, the higher risk of DN associated with ε4 was not found in Japanese or Korean populations (pooled ORs [95% CI]: 0.46 [0.27-0.80] and 0.58 [0.09-3.55], respectively).

Conclusion: The ε2 allele conferred a higher risk of DN in East Asians, and no significant result was obtained with the ε4 allele.

Keywords: Apolipoprotein E, diabetic nephropathy, meta-analysis, polymorphism, type 2 diabetic, mellitus

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic diseases. The complications of T2DM include specific injury to several organs, including renal lesions that are a key cause of end-stage renal disease (ESRD). The etiology of diabetic nephropathy (DN) involves various factors. Although abundant evidence shows a direct relationship between long-standing hyperglycemia and DN, hyperglycemia alone is not sufficient for this complication. Studies show that 35% patients with DM develop nephropathy independently of hyperglycemia [1-3]. Genetic factors and abnormalities of lipid metabolisms are also thought to contribute to the development and progression of DN [4-6]. The apolipoprotein E (APOE) gene encoding this pathway of lipid metabolism was found to be a candidate gene for susceptibility to DN [4].

APOE is a 299-amino acid glycoprotein that mediates the hepatic uptake of lipoproteins and reverses cholesterol transport. Thus, it plays a key role in lipid metabolism. The vast majority of studies have focused on the three most common alleles: ε2, ε3 and ε4, encoding three isoproteins: E2, E3 and E4. APOE polymorphisms change both the structure and the function of the protein. E3 is the wild-type isoprotein with normal function. The E2 isoprotein, which is defective for binding to the APOE receptor, is involved in the accumulation of triglyceride (TG)-rich

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lipoproteins and remnant particles derived from TG-rich lipoproteins in plasma. However, the E4 isoprotein, with increased binding to the APOE receptor, causes reduced TG levels and increased total cholesterol and low-density lipoprotein (LDL) cholesterol levels in plasma.

Recent data indicated that the frequency of the ε2 allele was significantly higher in patients with diabetic nephropathy than in those without nephropathy. These raised the possibility that the ε2 allele increased the risk of DN in Chinese, Japanese and Korean patients with T2DM [7-9]. However, additional few studies in East Asian populations conflicted with this result [10]. In addition, it had been reported that the ε4 allele was a protective factor for DN except for the little-mentioned study concerning the association between ε4 allele and risk for DN [2, 11, 12]. These difficulties in estimating the potentially true and modest effects of APOE genotypes on DN risk in East Asian populations may be due to ethnic distinctions or limited sample sizes in the individual studies.

In this study, we performed a meta-analysis using all findings of 16 similar case–control studies conducted until 2009 to evaluate the effect of APOE as a risk factor for DN in East Asian.

Materials and methods

Meta-analysis

For the meta-analysis, we made a comprehensive literature search of MEDLINE, CNKI, and the Chinese Wan Fang database up to 2009 using the following index terms: apolipoprotein E, APOE, APOE polymorphism, type 2 diabetes mellitus, diabetic nephropathy, diabetes with kidney diseases. Any study was considered that aimed to examine the association between APOE genotype and diabetic nephropathy. Our selection criteria required each study to clearly describe the country of origin, study design, time of publication, case and control selection criteria, sample size, genotype frequency and genotyping methods. Also, the included studies indicated the odds ratio (OR) and 95% confidence interval (CI) for DN relative to APOE genotype or provided raw data that allowed us to estimate these values. Two of the authors identified and reviewed each relevant paper. Disagreements were dealt with in discussions. The final data for our meta-analyses included 16 studies comprising 1550 diabetic patients without DN and 1660 patients with DN.

We summarized the data of those studies. The collected information include the first author's name, year of publication, country and/or region, study design, selection criteria, characteristics of DN cases, and controls, genotype distribution in cases and controls, the number of cases and controls for each APOE genotype, and the OR for diabetic nephropathy associated with the APOE genotype. At the same time, we defined carriers of the ε4 allele as those who had the ε3/ε4 and ε4/ε4 genotypes and carriers of ε2 allele as those who had the ε2/ε3 and ε2/ε2 genotypes, and we used the ε3/ε3 genotype as the referent group.

Statistical analyses

A meta-analysis was conducted using STATA SE 9.0. We used the Chi-square-based Q statistic to formally examine between-study heterogeneity. Heterogeneity was considered significant at a p-value of <0.1. Data were incorporated by using both fixed effects (Peto OR Mantel-Haenszel) and random effects (DerSimonian and Laird) models. In the absence of between-study heterogeneity, we combined results with a fixed effects model. If there existed between-study heterogeneity, random effects were more appropriate because they incorporated the between-study variance and tended to provide wider CIs. At the same time, subgroup analysis in Chinese, Japanese, or Korean subjects was employed while conducting the study.

Publication bias was evaluated by both Egger's linear regression test and the fail-safe number for P= 0.05 ($Nfs_{0.05}$) [13]. Publication bias was absent for p >0.05 in Egger's linear regression test and a zero within the 95% CI of the intercept in the publication bias plot, and higher $Nfs_{0.05}$ implied better reliability of the meta-analysis.

Results

Seventeen studies [2, 7, 9, 14-27] were identified, and these are profiled **in Table 1.** They all met the eligibility criteria. For each study, the first author, publication year, country or region, case-control group, sample size, APOE genotype, and allele distribution are all shown **in Table 1**.

Either polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or DNA sequencing was used to determine the genotypes. Of seventeen studies, one was excluded because of incomplete data. Three thousand two hundred ten subjects were included in this meta-analysis.

Table 1. Descriptive characteristic of eligible studies of T2DM and DN.

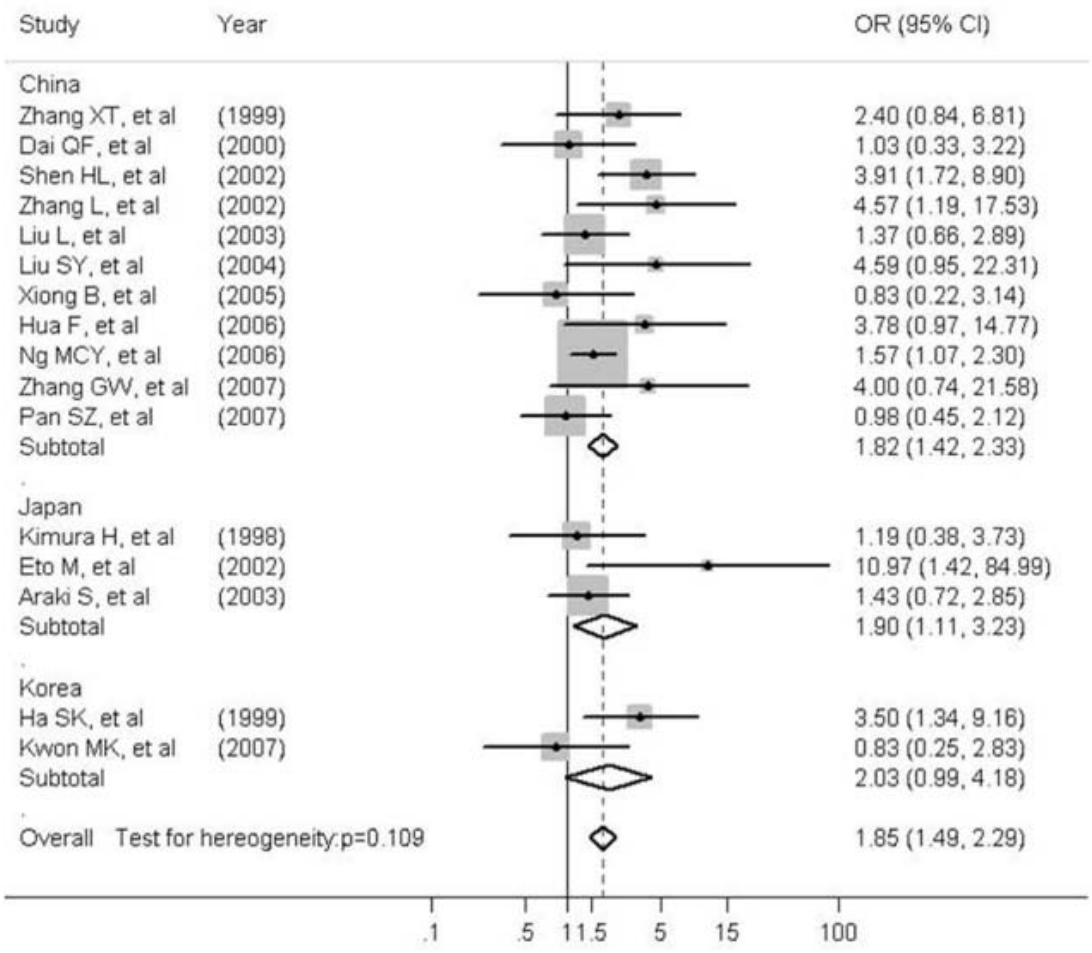
Author	year	Region or country	group	Sample size	genotypes						alleles		
					ε2/ 2	ε2/ 3	ε2/ 4	ε3/ 3	ε3/ 4	ε4/ 4	ε2	ε3	ε4
Zhang XT, et al.	1999	ShangHai	1	57	6	17	5	16	13	0	34	62	18
			2	40	0	9	2	15	14	0	11	53	16
Dai QF, et al.	2000	FuKien	1	88	0	13	1	58	14	2	14	143	19
			2	32	0	5	0	23	3	1	5	54	5
Shen HL, et al.	2002	ShangHai	1	159	1	34	2	94	28	0	38	250	30
			2	106	1	7	2	84	11	1	11	186	15
Zhang L, et al.	2002	AnHui	1	58	2	10	3	35	6	2	17	86	13
			2	56	0	3	1	40	11	1	4	94	14
Liu L, et al.	2003	ShangHai	1	218	1	36	2	137	41	1	40	351	45
			2	80	0	11	1	56	12	0	12	135	13
Liu SY, et al.	2004	HarBin	1	56	0	14	1	32	9	0	15	87	10
			2	28	0	2	0	21	5	0	2	49	5
Xiong B, et al.	2005	LanZhou	1	33	1	4	1	22	3	1	7	53	6
			2	32	1	5	1	22	2	1	8	51	5
Hua F, et al.	2006	JiangSu	1	52	1	10	0	33	7	1	12	83	9
			2	50	1	2	2	34	10	1	6	80	14
Ng MC, et al.	2006	Hong Kong	1	366	4	71	4	237	49	1	83	594	55
			2	386	4	53	5	282	39	3	66	656	50
Guo Q, et al.	2006	GanSu	1	21	3	-	4	4	-	-	-	-	-
			2	44	-	-	4	8	-	-	-	-	-
Zhang GW, et.al	2007	Zhe Jiang	1	40	2	4	1	24	9	0	9	61	10
			2	38	0	2	0	32	3	1	2	69	5
Pan SZ, et.al	2007	FuKien	1	113	1	14	1	63	32	2	17	172	37
			2	97	2	15	1	70	8	1	20	163	11
Kimura H, et.al.	1998	Japan	1	81	0	7	0	62	12	0	7	143	12
			2	96	0	6	4	63	22	1	10	154	28
Eto M, et.al.	2002	Japan	1	99	21*		-	67	11*		-	-	-
			2	59	1*		-	35	23*		-	-	-
Araki S, et.al.	2003	Japan	1	130	0	15	3	98	14	0	18	225	17
			2	299	0	24	0	225	50	0	24	524	50
Ha SK, et.al.	1999	Korea	1	74	2	14	0	47	11	0	18	119	11
			2	93	0	7	1	72	12	1	8	163	15
Kwon MK, et.al.	2007	Korea	1	36	0	5	2	27	2	0	7	61	4
			2	58	0	8	1	36	12	1	9	92	15

* carriers of the ε2 allele and the ε4 allele, -: the absence of the genotype or allele; Group 1= patients with microalbuminuria or macroalbuminuria (The group was identified as those in whom 24h urinary albumin excretion (UAE) was above 30mg), group 2= patients with normoalbuminuria

In our meta-analysis, there was no significant between-study heterogeneity between the ε2 carrier and ε3/ε3 genotypes (p >0.1) **(Fig. 1A)**. The studies showed that East Asian ε2 carriers had almost double the risk of DN (pooled ORs: 1.85 [95% CI: 1.49 to 2.29]). In contrast, studies relating ε4 carriers to DN risk were very heterogeneous (p <0.1), and the pooled ORs was 1.05 (95% CI: 0.72 to 1.52) (**Fig. 1B)**. The ε4 allele was substantially related to an increased risk for DN in studies conducted in China (pooled ORs: 1.51 [95% CI: 1.11 to 2.06]), but this higher risk was not found in Japanese or Korean populations (pooled ORs: 0.46 [95% CI: 0.27 to 0.80] and pooled ORs: 0.58 [95% CI: 0.09 to 3.55], respectively).

To evaluate of the reliability of the meta-analysis, Egger's linear regression test and $Nfs_{0.05}$ were both performed to examine publication bias. Egger's linear regression test was for the quantitative evaluation of the symmetry of the meta-analysis funnel plot. The P-values of Egger's test for ε2 and ε4 carriers were 0.14 (95% CI: -0.38 to 2.48) and 0.60 (95% CI: -3.48 to 2.10), respectively (**Table 2**). This result indicated that the meta-analysis funnel plots of ε2 and ε4 carriers were symmetrical and absent from publication bias. $Nfs_{0.05}$ is defined as the number of negative results that could reverse the significant findings. The $Nfs_{0.05}$s of the ε2 and ε4 carriers were 99 and 67, respectively (**Table 2**). The resulting data indicated that the reliability of this meta-analysis was acceptable.

(A) ε2 carriers versus persons with the ε3/ε3 genotype



(B) ε4 carriers versus persons with the ε3/ε3 genotype

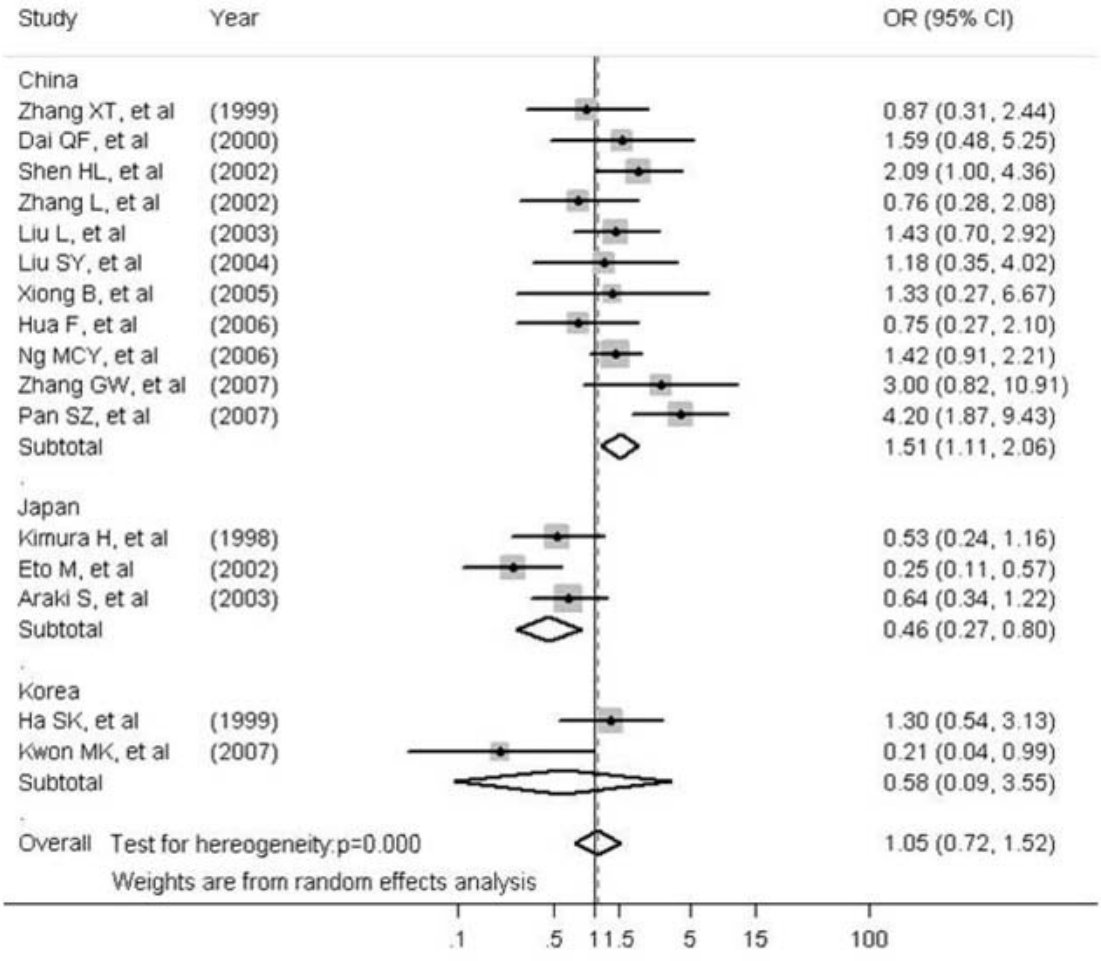


Fig. 1 Odds ratios for DN in ε2 carriers versus persons with the ε3/ε3 genotype **(A)** and in ε4 carriers versus persons with the ε3/ε3 genotype **(B).** Black circles indicate the odds ratio in each study; horizontal lines represent the 95% CI. Diamonds show the pooled estimates (with the 95% CI).

Table 2. Egger's test and fail-safe numbers ($Nfs_{0.05}$) for carriers of the ε2 and ε4 alleles.

	Coefficient	Standard error	t	p >|t|	95% CI	$Nfs_{0.05}$
ε2 carrier	1.05	0.67	1.57	0.14	-0.38,2.48	99
ε4 carrier	-0.69	1.30	-0.53	0.60	-3.48,2.10	67

Discussion

Many studies in East Asian populations have suggested that APOE ε2 and ε4 are associated with the progression of DN in T2DM patients. APOE ε2 confers an increased risk for both the onset and the progression of DN [26]. However, several studies in Korean subjects with T2DM did not confirm this association of the ε2 allele with increased risk of DN [27]. Furthermore, the favorable effect of APOE ε4 on DN remains inconsistent. Why then have studies obtained different results regarding the association between APOE polymorphisms and diabetic nephropathy? These results are possibly due to genetic, clinical, ethnic, and experimental heterogeneity combined with inadequate power from small sample sizes. These may have led to false-positive and/or

false-negative results. However, although the meta-analysis did not fully overcome the problem of genetic heterogeneity, it did increase the statistical power of the genetic evidence contained in the data sets of the pooled studies.

To our knowledge, few studies have assessed the association of APOE polymorphisms with DN in the East Asian populations. In the meta-analysis, we examined sixteen case-control studies that included 3210 subjects, and publication bias was assessed to further confirm the reliability of the meta-analysis. The meta-analysis demonstrated that carriers of the ε2 allele had an increased risk for DN. In general, ε2 allele increased the risk of DN by 1.82 times in patients with T2DM.

On the other hand, we found that carriers of ε4 in the East Asian populations did not have an increased risk of DN. There was heterogeneity between the different studies in this regard. Next, we performed the meta-analysis in three subgroups. In the Chinese subgroup, an overall OR was calculated, and this confirmed that patients with T2DM who are carriers of ε4 have a 1.51 times greater risk of DN, which was different from previous results. However, in the Japanese and Korean subgroups, the findings demonstrated that ε4 was a protective factor for DN. In previous studies, the presence of the APOE ε4 allele correlated with a protective effect on DN. This was mainly due to explanations such as the following. First, the higher levels of HDL cholesterol in the ε4 carrier might counteract the deleterious effect of high LDL cholesterol [28], which might have a main role in nephropathy development. Second, APOE is synthesized in the kidney and has a high affinity for extracellular glycosaminoglycans. The E4 isoprotein has preferential binding affinity for glycosaminoglycans and could consequently displace growth factors involved in diabetic nephropathy pathogenesis [2, 29]. Third, the E4 isoprotein was reported to be more effective at modulating remnant lipoproteins uptake and converting remnant lipoproteins to LDL. This could lead to lower plasma remnant lipoprotein levels, and it might have an important influence on renal damage in diabetes [25, 30].

However, hypercholesterolemia coupled with elevated LDL and modified LDL (glycosylated and oxidized LDL) was suggested to be a contributor to the development of diabetic nephropathy, leading to a hypothesis that the ε4 allele may be a risk factor for diabetic nephropathy. The accumulation of modified LDLs stimulated mesangial cell secretion of various chemotactic factors and adhesion molecules, resulting in monocyte infiltration [6]. In addition, studies have also confirmed that phagocytosis of modified LDLs by monocytes play a key role in the formation of mesangial foam cells, which appear to be important mediators in the pathogenesis of diabetic nephropathy [6]. Therefore, it is clear that explanation of the differential effects of this allele on DN risk will be complex. The natural environment and lifestyle are additional factors that may interact with the ε4 allele to eventually increase its adverse effects on DN.

Acknowledgements

The research was supported in part by the grants from the National Scientific Foundation of China (No. 30671110) and the National Basic Research Program of China (No. 2006CB503901). No conflict of interest exits in the submission of this manuscript.

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