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Association between apolipoprotein E (*APOE*) gene and diabetic peripheral neuropathy: A meta-analysis.

Prateek Rajkarnikar[1], Arun Bhattarai[2], Shanker K.C[3], Christodoulos Monastiriotis[4], Yan Cheng Xu[1]*

[1]Wuhan University Zhongnan Hospital, Department of Endocrinology
[2]Wuhan University Zhongnan Hospital, Department of Gastroenterology
[3]Wuhan University Zhongnan Hospital, Department of Cardiology
[4]The Democritus University of Thrace, Greece, Department of Internal Medicine

Abstract

Some studies have shown the correlation between *E4* type of Apolipoprotein E gene and diabetic neuropathy, however, there are contradicting results in recent studies. The aim of this meta-analysis was to investigate the association between *APOE4* gene and diabetic neuropathy. Database of Pubmed, Wuhan University Library, and Google were searched. A total of 56 studies were screened and 5 studies were selected for quantitative meta- analysis. This meta-analysis was performed to assess heterogeneity and combine results by using software RevMan 5.2. Strength and relationship were calculated as the odds ratio (OR) with a confidence interval (CL) of 95% using generic inverse variance data. Sensitivity analysis and Publication Bias analysis was conducted. A total of 5 studies were included for quantitative analysis with total of 914 patients (498 diabetic patients with neuropathy as case and 416 diabetic patients without neuropathy as controls). The result of our study indicated that *APOE4* carriers were likely to have high risk of diabetic neuropathy. (E4 vs. others; pooled OR with 95% confidence interval 1.80 (1.28, 2.53), $p< 0.05$ ($p =0.0007$)). In conclusion, this meta-analysis suggests that there is significant association between *APOE4* gene and occurrence of diabetic peripheral neuropathy. However, further studies are needed for more detailed analysis.

Key words: Apolipoprotein E, *APOE*, diabetic neuropathies, diabetic polyneuropathies, gene polymorphism, meta-analysis

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Correspondence email: xjl100901@whu.edu.cn

https://orcid.org/0000-0002-3298-5110

Introduction

There is a rising worldwide prevalence of diabetes, which is one of the most challenging health problems in the 21st century. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980 with prevalence rising from 4.7% to 8.5% in the adult population (1). American Diabetes Association (ADA) states that by the year 2050, one in three American adults will have diabetes if current trends continue and estimates a person diagnosed at age 50 dies six years earlier than a person without diabetes. According to IDF Atlas, nephropathy, retinopathy and neuropathy are most common microvascular complications of diabetes affecting patient's quality of life and increasing economic burden. However, if early detection of risk factors can be identified then severe consequences like end-stage renal failure, blindness and diabetic foot can be delayed or even prevented (2). It is well known that changes in small arteries and capillaries are not only responsible for micro vascular complications, but also manifests cardiovascular complications in T2DM (3).

Diabetic neuropathy is a common complication of diabetes. It has been estimated that approximately 50% of all diabetic patients will develop neuropathy in their lifetime (4). Severe neuropathies may lead to consequences like physical disability, amputations, and cardiac interventions causing burden on public health system and affecting quality of life of patients (5). The most common neuropathies are diabetic peripheral neuropathy (DPN) and cardiovascular autonomic neuropathy (CAN) (6). These neuropathies in patients having diabetes are diverse in character of their symptoms, neurological involvement, risk factors, underlying mechanisms and pathology. Hence clinical presentation and natural history of diabetic peripheral neuropathy are not entirely predictable based on diabetes duration and degree of glycaemic control. In order to target the effective diagnosis, treatment and identification of risk factors for diabetic neuropathies, more researches are needed including study of new genetic variants that can significantly contribute to these conditions. One of the candidate gene linked with these neuropathies is *APOE* gene. Association of *APOE* gene with cardiovascular disease and neurological disorders of the central and peripheral nervous system mainly Alzheimer's disease has been widely accepted (7). Studies have also shown positive association of *APOE* gene with diabetic nephropathy (8). However, significance of *APOE* gene in diabetic neuropathy is still in debate and inconclusive.

APOE was discovered in the early 1970s as a protein component of triglyceride rich lipoproteins (9). *APOE* is mapped at chromosome 19 and is a polymorphic gene containing three major alleles (*E2, E3,* and *E4*) with six possible genotypes (*E2/2, E2/3, E2/4, E3/3, E3/4,* and *E4/4).* *APOE* gene is responsible for producing a protein of 299 amino acids with three different isoforms (*ε2, ε3, ε4*) differing in two amino acid residues at positions 112 and 158 (10). The *APOE3* isoform is the most common isoform with a frequency of approximately 70–80% containing a cysteine at position 112 and an arginine at position 158, while *ε2*(5–10%) possesses cysteines at both positions and *ε4*(10–15%) possesses arginine at both positions and these two isoforms have been thought to be dysfunctional (9,10). Role of *APOE* polymorphism as risk factor for diabetes neuropathy has been interest of research since many years. However, results from studies vary from each other. The first study was done by Tsuzuki et al in 1998 showing frequency of diabetic neuropathy higher in *APOE4* than *APOE2* and *APOE3* ($p < 0.05$). However, some studies contradict with the result. Therefore, we performed this meta-analysis to provide a reliable evaluation of the association between *APO*E4 carriers and diabetic neuropathy.

Methods and materials

Data search strategy and sources

We evaluated all case-control studies that studied the association of Apolipoprotein E gene polymorphism with diabetic neuropathy. Data sources were from Pubmed, Wuhan University Library Database and Google. Keywords combinations used were "*APOE*", "Apolipoprotein E", "Diabetic Neuropathy", "DPN", "NDPN", "Polymorphism", "Genotypes", and "Alleles". Screenings of references of selected articles were also screened. Review articles relevant to our study were also searched in addition to eligible studies. We included all published/unpublished articles written in any language to avoid literature bias. No quality scoring was done.

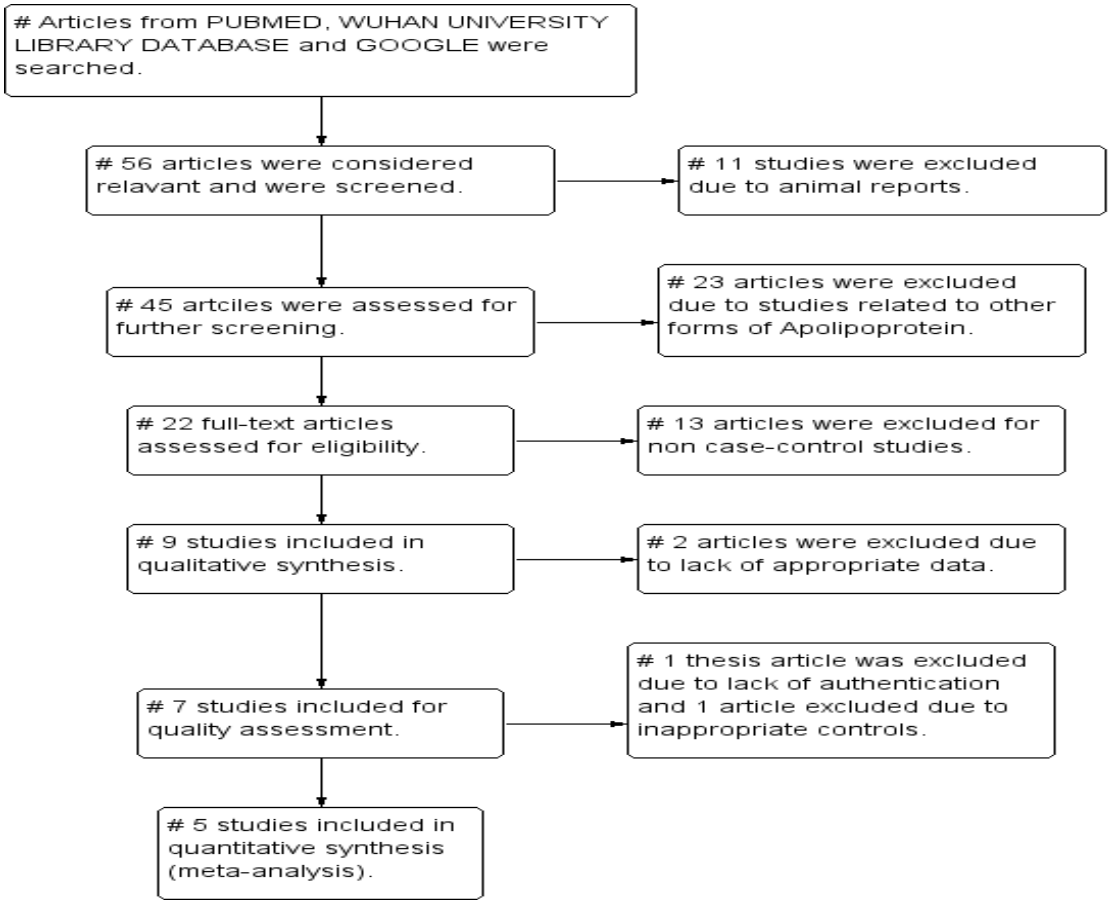

Figure 1. PRISMA selection of eligible studies.

Study Inclusion and Exclusion Criteria

The studies, which met the following criteria were included for the study. 1) The studies aimed to identify association between *APOE* polymorphism and diabetic neuropathy (diabetes of any type); 2) Only case-control studies; 3) The studies used diabetic peripheral neuropathy patients as case and diabetic without neuropathy patients as controls; 4) Only human studies. Studies were excluded if: 1) Studies were review articles; 2) Studies conducted on animals. 3) Studies done on other form of Apolipoprotein other than Apolipoprotein E. 4) Unable to contact the original author of unpublished articles.

Data Collection

Two investigators (Dr. Prateek Rajkarnikar and Dr. Arun Bhattarai) independently extracted data after analyzing eligible criteria for relevant studies using standardized data extraction table. The following information were extracted from each study: author name, journal of publication, year of publication, country of study done, *APOE* gene distribution in each group, *APOE* gene identification methods and severity assessment methods. Any disagreements were resolved by mutual discussions.

Statistical Analysis

This study compared the *APOE* gene carriers between diabetic peripheral neuropathy (DPN+) and diabetic without peripheral neuropathy (DPN-) as case and controls respectively by using the odds ratio (OR). We grouped the genotypes and calculated allele carriers as *E2 (E2/2+E2/3), E3 (E3/3)* and *E4 (E3/4+E4/4).* We excluded the data of *E2/4* because both *E2* allele and *E4* allele have different effects on occurrence of DPN. It might not be statistically correct to calculate this genotype as *E2* carrier or *E4* carrier independently. Hardy-Weinberg equilibrium (HWE) test was conducted in the control groups for each study, except for Bedlack et al. due to lack

of sufficient data. Chi-square value was calculated and p value >0.05 was considered consistent with HWE. We estimated between-study heterogeneity through all eligible comparisons by using the Chi^2 based Q statistic (11). Heterogeneity was considered significant if p value <0.10. I^2 test was done to further evaluate heterogeneity with value more than 75%, 50%, and 25% were considered evidence of high, moderate and low statistical heterogeneity respectively (12). Both fixed and random effects model were used. Data were combined using fixed-effects models (when there was no heterogeneity) with inverse-variance weights (13). Random effects are more suitable when there is heterogeneity. The odds ratio (OR) and its 95% confidence interval (CI) was used as the metric of choice. A standard X^2 test was also performed to find out the significance of the overall OR with p value ≤0.05 (14). Begg's funnel plots and Egger's linear regression test were used to evaluate publication bias. Publication bias was absent if p value>0.05 in Egger's linear regression test. All Data were analyzed by RevMan 5.2, Stata 11.0, and Microsoft Excel.

Results

Data searched on Pubmed, Wuhan University library database and Google initially yielded 56 articles. We performed screening to evaluate the inclusion and exclusion criteria. 11 articles were instantly excluded due to animal reports. Out of 45 articles, 23 articles were excluded because they studied different forms of Apolipoprotein other than *APOE* polymorphisms. Thirteen articles were excluded because they were not case control studies. Out of nine remaining articles, two were excluded because of inappropriate and incomplete data (15,16). One thesis paper was excluded due to lack of authentication (17) and one study was excluded because it did not have diabetic patients without peripheral neuropathy as controls (18). Finally, five studies were included for this meta-analysis. Four of these studies were published articles (19-22) whereas, one article was a thesis research paper, which we included for the analysis with the permission of the author (23). The information related to first author, year of publication, country, sample size of case (DPN+) and control (DPN-) for each *APOE* genotype with allele carriers are presented in table 1.

Two studies were written in English, one study written in Japanese (22), one in Greek (23) and one in Chinese language (20). The identified studies contained total of 914 patients distributed in two groups, diabetic patients with peripheral neuropathy (DPN+) group (n=498) and diabetic patients without peripheral neuropathy (DPN-) group (n=416). The aim in comparison of DPN+ vs. DPN- was to evaluate the role of *APOE4* gene on the occurrence of peripheral neuropathy in diabetic patients.
The overall effects pooled OR of *APOE4* carriers showed statistical significance. 1.80 (1.28, 2.53), p value< 0.05 (p =0.0007). A Forrest plot of comparison is shown in figure 2.

There was no heterogeneity between pooled studies (I^2 *<25% P* value for heterogeneity > 0.10) and therefore, fixed-effect model was used. We used Begg and Mazumdar test[24] for visual inspection of the funnel plots to see any asymmetry suspecting publication bias. We also used Egger's weighted regression test to statistically assess the publication bias; p value > 0.05 was considered to represent absence of publication bias. The results of both tests showed no evidence of publication bias with Begg's test p value=1 and Egger's test p value = 0.775 shown in Figure 3, 4. One study was deleted each time to see the influence of the individual study on the pooled ORs. However, the corresponding pooled ORs did not change significantly suggesting that our results are statistically correct.
This study is the first meta-analysis done to identify association of *APOE4* gene with diabetic neuropathy. Although few case control studies had been done in relation to this topic, we believe that our results have statistical significance and may encourage for more studies to be done in relevant to this report.

Table 1 Characteristics of the studies included in meta-analysis

Author Name	Year	Country	Study Group	APOE identification	DPN Severity Scoring system	Group	Total	Carriers			Odd Ratio (OR) E4 vs. others	HWE test of controls	
								E2/2+ E2/3 (E2)	E3/3 (E3)	E3/4+ E4/4 (E4)		x^2	*p*
Tsuzuki et.al	1998	Japan	DPN vs. NDPN in Type 2 diabetes	Phenotyping *APOE* IEF SYSTEM	Author devised	DPN+	48	3	30	15	1.72 [0.80, 3.69]	2.62	0.45*
						DPN-	110	10	77	23			
Voron'ko et.al	2005	Russia	DPN vs. NDPN in Type 1 diabetes	PCR	Author devised	DPN+	65	13	44	8	1.43 [0.47, 4.35]	2.41	0.49
						DPN-	67	21	40	6			
Bedlack et.al	2003	U.S.A	DPN vs. NDPN in Type 1 and Type 2 diabetes	PCR	Neuropathy Impairment Score in the Lower Limbs (NISLL)	DPN+	N/A	N/A	N/A	N/A	3.12 [1.20, 8.14]	N/A	N/A
						DPN-	N/A	N/A	N/A	N/A			
Monastirioti s et.al	2014	Greece	DPN vs. NDPN in Type 2 diabetes	PCR	Neuropathy Disability Score	DPN+	174	16	138	20	0.96 [0.45, 2.05]	1.83	0.6
						DPN-	101	12	77	12			
Wang et.al	2018	China	DPN vs. NDPN in Type 2 diabetes	PCR	Author devised	DPN+	211	35	115	61	2.27 [1.31, 3.93]	2.62	0.45
						DPN-	138	23	94	21			

***- HWE test was done with total sample population.**

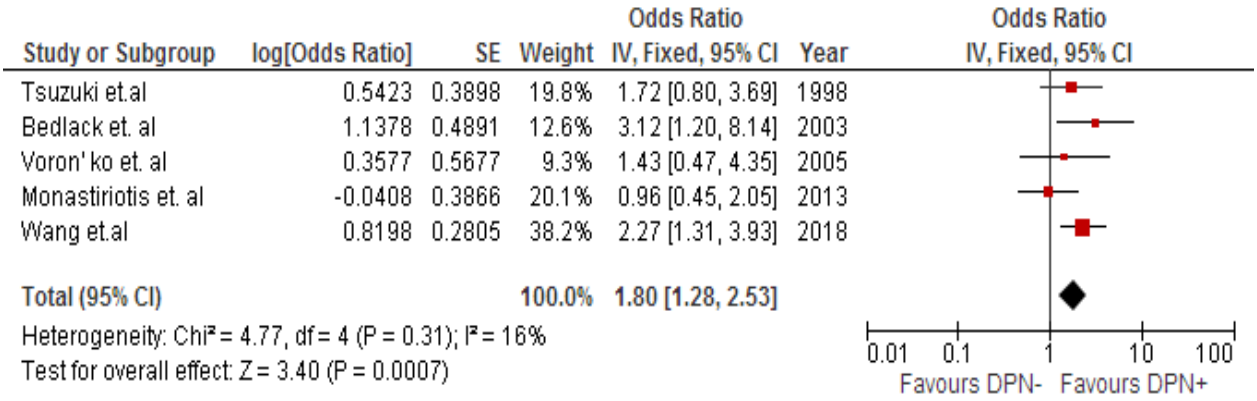

Figure 2. Forest plot illustrating the study-specific odds ratio (ORs) with 95% confidence intervals (CI) comparing *APOE4* carriers vs. others (DPN+ vs. DPN-).

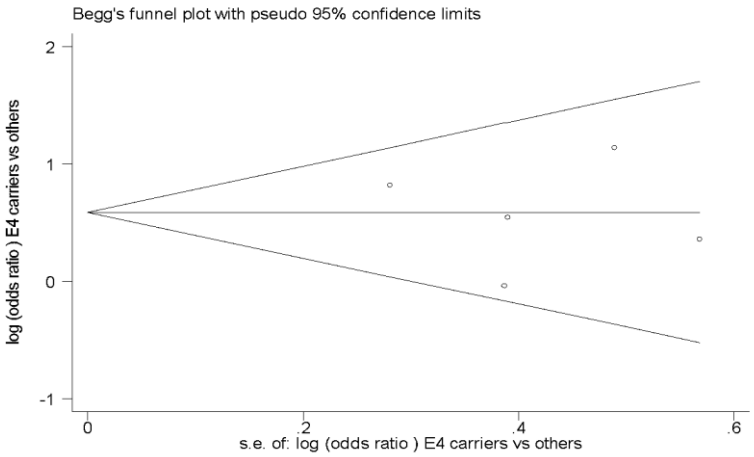

Figure 3. Begg's funnel plot for the results of meta-analysis of E4 carriers compared to others
***p* value =1**

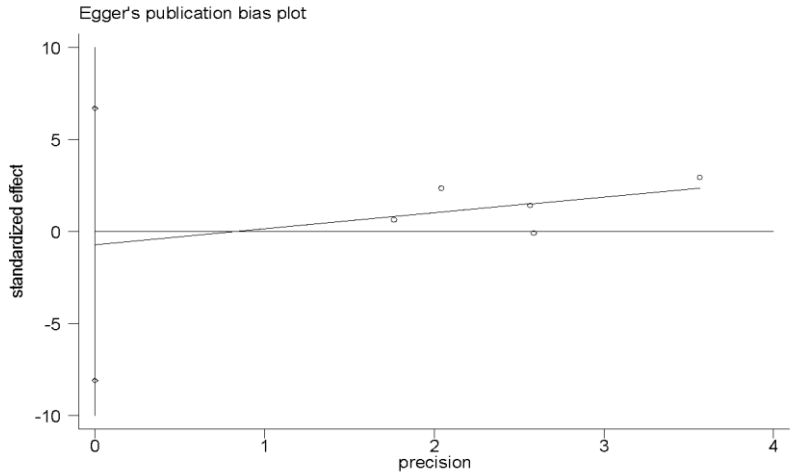

Figure 4. Egger's publication bias test for the results of meta-analysis of E4 carriers

***p* value = 0.775**

Discussion

Asymptomatic phase before the actual onset of diabetic hyperglycemia and clinical diagnosis is characteristic of T2DM. The onset of T2DM is usually slow and many years may pass before clinical diagnosis (25). This asymptomatic phase might last for 4 to 7 years in 30–50% patients who remain undiagnosed (26). Diabetic neuropathy is a common complication of diabetes. While estimates vary, depending on the methods and criteria used to diagnose diabetic neuropathy, approximately 50% of all diabetic patients will develop neuropathy in their lifetime (4). While actual etiology of DPN is not clearly understood, gene expression changes, molecular transport, inflammation, oxidative stress due to chronic hyperglycemia, hypertension, dyslipidemia, insulin resistance, and uremia are considered as the key factors contributing to its' pathogenesis (5). However, patient history and clinical presentation based on diabetes duration and degree of glycaemic control cannot entirely predict DPN. It is interesting to note that how some patients develop minimal symptoms of neuropathy after many years of diabetes and others suffer severe nerve damage as early as during pre-diabetes. This inconsistency tends to question the effect of genetic variability in pathogenesis of DPN. Identification of susceptibility genes and their association with diabetes and its complication had been studied since many years. One of the candidate gene linked with these neuropathies is *APOE* gene. *APOE* play important role in formation of many plasma-lipoprotein lipid particles like very low-density lipoproteins (VLDL), intermediate density lipoproteins (IDL), high density lipoproteins (HDL) and chylomicrons and helps in the stability and solubility of these lipoproteins during their circulation (10). Association of *APOE* gene with cardiovascular disease and neurological disorders of the central and peripheral nervous system mainly Alzheimer's disease have been widely accepted (7). Studies have also demonstrated as positive association of *APOE* gene with diabetic nephropathy (8). However, significance of *APOE* gene in diabetic neuropathy is still in debate and inconclusive. Some of the popular studies and their results are described below.

Author	Number of patients	Conclusion
Tsuzuki S et al. 1998	158	E4 is a risk factor for diabetic peripheral neuropathy
Bedlack et al. 2003	187	E4 is a risk factor for diabetic peripheral neuropathy
Zhou et al. 2005	56	E4 is not a risk factor for diabetic peripheral neuropathy
Voron'ko et al. 2005	180	E4 is not a risk factor for diabetic peripheral neuropathy
Monastiriotis et al. 2013	234	E4 is associated with severity of peripheral neuropathy
C. Monastiriotis 2014	275	E4 is not a risk factor for diabetic peripheral neuropathy
Erdogan et al. 2015	100	*APOE* is not an independent risk factor for diabetic foot
Wang et al. 2018	355	E4 is a risk factor for diabetic peripheral neuropathy

These studies show that the results are still inconclusive and further studies are needed to evaluate the *APOE* gene susceptibility in association with diabetic neuropathy. The results of our meta-analysis suggested that patients carrying *APOE4* allele might be at risk of developing diabetic neuropathy, OR (95% CI) 1.80 (1.28, 2.53), p value< 0.05 (p =0.0007). Some studies have also shown promising results of *APOE4* and association between severities of diabetic peripheral neuropathy. Tsuzuki et al. (p value <0.005) (22), Monastiriotis et al. (p value 0.0003) (16) and Bedlack et al. (p value 0.02) (19) showed *APOE4* as a risk factor for increasing severity of already diagnosed diabetic peripheral neuropathy. *APOE* genotype influence on neuropathy severity in diabetic patients might be explained by acceleration in atherosclerosis, use of growth factors, cytoskeletal stabilization, or alterations in cell adhesion caused by different allele types (27). However, Edrogan et al. (p value >0.05) (15) and Zhou et al. (p value 0.26) (18) have stated that *APOE4* has no association with DPN severity.

This is the first meta-analysis to simply analyze the association of *APOE* gene with incidence of diabetic peripheral neuropathy. However, there were many limitations. Firstly, there were limited number of studies and results of some included studies were concluded from a relatively small sample size. Secondly, we were unable to examine the interactions among *APOE* gene and other factors including other candidate genes, diabetes control, environment, which might also play an important role on development of diabetic peripheral neuropathy. Finally, we could not analyze the data to study the *APOE* gene and its relation with severity of DPN because all authors had used different severity scoring system. We tried to include all possible studies without restriction of language and publication to limit the literature bias. Further studies are needed with detailed comparisons of different *APOE* genotypes with other clinical variables using common DPN severity scoring system.

In conclusion, in spite of several limitations, our meta-analysis suggests that there is significant association between *APOE4* gene and occurrence of diabetic peripheral neuropathy. More studies are needed to provide detailed information for evaluating association of *APOE* gene in DPN.

Conflict of interest

The author declares that they have no conflicts of interest concerning this article.

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