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Original Article

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Apolipoprotein E (*APOE*) Polymorphisms and Susceptibility to Breast Cancer: A Meta-Analysis

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Purpose

Apolipoprotein E (APOE, MIM: 107741) has three functionally distinct isoforms of the protein (E2, E3, and E4), encoded by corresponding alleles ε2, ε3, and ε4, which have been well described. Findings from previous studies investigating association between *APOE* polymorphisms and breast cancer risk have been inconsistent. The present meta-analysis was conducted in order to investigate association of *APOE* polymorphisms with risk of breast cancer.

Materials and Methods

Several electronic databases were used for identification of studies containing information on *APOE* polymorphisms and breast cancer risk published up to January 2012. We identified 10 eligible studies, including 3,835 subjects (2008 patients, and 1,827 healthy controls), that reported on polymorphisms of *APOE* and risk of breast cancer. Summary odds ratios (ORs) and 95% confidence intervals (CIs) were obtained using a fixed and random-effects models.

Results

Among studies reported from Asia, an association of the ε4 allele with increased risk of breast cancer, in comparison with the ε3 allele, was observed (OR, 1.56; 95% CI, 1.19 to 2.04; p=0.001). It should be noted that allele ε2 showed no association with breast cancer risk. Among Caucasians, neither the ε4 (OR, 0.99; 95% CI, 0.83 to 1.17; p=0.917) nor the ε2 (OR, 0.92; 95% CI, 0.72 to 1.17; p=0.514) allele showed an association with susceptibility to breast cancer, when compared with the ε3 allele. Carriers of the ε4 allele (E4E4, E4E3, and E4E2 genotypes), in comparison with the E3E3 genotype, showed an association with elevated risk of breast cancer only among Asians (OR, 1.75; 95% CI, 1.23 to 2.47; p=0.002). No publication bias was detected.

Conclusion

This meta-analysis suggest that the *APOE* ε4 allele is a low-penetrant risk factor for development of breast cancer.

Key words

Apolipoproteins E, Breast neoplasms, Meta-analysis, Disease susceptibility

Introduction

Apolipoprotein E (APOE, MIM: 107741) is involved in cholesterol transport, lipid metabolism, and protein synthesis through mediation of binding of the low-density lipoprotein receptor and the *APOE* receptor of lipid particles to specific lipoprotein receptors. It is also involved in numerous other functions, including tissue repair, immune response and regulation, and cell growth and differentiation. The *APOE* gene is located on human chromosome 19q13.2; three functionally distinct isoforms of the protein (E2, E3, and E4), encoded by corresponding alleles ε2, ε3, and ε4, have been described. These isoforms, originally identified by isoelectric focusing, are defined by amino acid changes at positions 112 (rs. 429358) and 158 (rs. 7412): alleles ε2, ε3, and ε4 are defined, respectively, by cysteine/cysteine, cysteine/arginine, and arginine/arginine at these two sites. The most common isoform is ε3, with a frequency of approximately 70-80% [1,2].

Individual case-control association studies are usually small and underpowered and, thus, are unable to provide a definitive answer, even in cases involving existence of a true association. Thus, meta-analysis can

effectively combine data from several studies, resulting in increased statistical power (lower type II error rate). Findings from previous studies investigating the association between *APOE* polymorphisms and breast cancer risk have been inconsistent [3-10]. Some studies have reported an association of E4, compared with the E3E3 genotype, with increased risk of breast cancer [7,9], while other reported findings have indicated no association between *APOE* polymorphism and risk of breast cancer. To the best of our knowledge this is the first meta-analysis to investigate the association of *APOE* polymorphisms with risk of breast cancer.

Materials and Methods

1. Search strategy

Electronic databases, including MEDLINE (National Library of Medicine, Washington, DC), Scopus, EBSCOhost Research Databases, ProQuest, Scirus, Directory of Open Access Journals (DOAJ), Indian Science Abstract, Google Scholar, SAGE, Open J-Gate, High-Wire, J-STAGE, KoreaMed, and Scientific Information Database (SID) were searched for identification of studies on *APOE* polymorphisms and breast cancer published up to January 2012. Search terms included "breast cancer", polymorphisms, "apolipoprotein E" or "apoE" or "APOE" or "Apo E." In addition, references cited in the retrieved articles were screened in an effort to trace additional relevant studies.

2. Inclusion criteria

The meta-analysis was limited to articles published in the English language. Articles describing case-control design studies and their primary references, which showed no obvious overlap of cases with other studies were selected for analysis. A report by Menzel et al. [6] included two case-control groups; therefore it was included as two studies in the meta-analysis. Also, a report by Niemi et al. [4], who investigated risk associated with either breast cancer or benign breast cancer with *APOE* polymorphism, was included as two case control studies. Articles selected for meta-analysis had no overlap of subjects with other studies.

3. Data extraction

All studies were reviewed twice and a standardized form was used for data extraction. Data were collected on the authors, year of publication, country of origin, study design, source of control group (hospital based, population based), ethnicity, and numbers of *APOE* genotypes and/or *APOE* alleles among cases and controls. A database was established according to information extracted from each article. It should be noted that in the report by Menzel et al. [6], numbers of participants for each genotype of *APOE* were not reported, as with other papers as well. Also, the numbers of participants for each polymorphism of *APOE* were not equal. Therefore, the prevalence of the alleles was estimated from the row data. Table 1 lists the number of cases and control groups for each allele of the *APOE* polymorphism.

4. Statistical analysis

Odds ratio (OR) and their corresponding 95% confidence intervals (CI) of breast cancer associated with the genetic polymorphism of *APOE* were calculated for each comparison. First, I estimated the risk of the ε2 and ε4 alleles, compared with the wild-type ε3 allele. In the second step, I estimated the association between *APOE* genotypes and susceptibility to breast cancer. In this step, risks of the ε2 carriers (E2E2 and E2E3 genotypes) and ε4 carriers (E4E4, E4E3, and E4E2 genotypes) were compared with those of the E3E3 genotype.

In order to account for the possibility of heterogeneity across studies, a statistical test for heterogeneity was performed based on the Q statistic test, in which a p-value less than 0.10 suggested significant heterogeneity between studies [11]. Association was measured using random-effect or fixed-effect

Table 1. Prevalence of *APOE* alleles in studies used in the meta-analysis

Study	Ethnicity	Source of controls	Place	Controls				Cases			
				ε2	ε3	ε4	Total	ε2	ε3	ε4	Total
Moysich et al., 2000 [3]	Caucasians	Hospital	USA	43	533	88	664	34	424	62	520
Niemi et al., 2000 [4]	Caucasians	Population	Finland	29	408	97	534	17	331	74	422
Niemi et al., 2000 [4]	Caucasians	Population	Finland	29	408	97	534	22	467	109	589
Yaylim et al., 2003 [5]	Caucasians	ND	Turkey	0	40	0	40	2	61	1	64
Menzel et al., 2004 [6]	Caucasians	Population	Czech Rep.	27	237	36	300	15	156	17	188
Menzel et al., 2004 [6]	Caucasians	Hospital	Austria	53	449	176	678	31	337	64	432
Chang et al., 2005 [7]	Asians	Hospital	Taiwan	45	349	70	464	56	410	114	580
Chang et al., 2006 [8]	Asians	ND	Taiwan	24	256	16	296	34	496	52	582
Surekha et al., 2008 [9]	Caucasoid	Hospital	India	11	201	8	220	6	193	21	220
Porrata-Doria et al., 2010 [10]	Caucasians	Hospital	Puerto-Rico	61	350	47	458	59	299	52	410

APOE, apolipoprotein E; ND, not described.

Table 2. Summary of meta-analysis of case-control studies examining alleles of *APOE* polymorphism and breast cancer risk

Studies	No. of studies	ε2 vs. ε3				ε4 vs. ε3			
		Q	OR	95% CI	p-value	Q	OR	95% CI	p-value
All studies	10	6.88	0.88	0.74-1.05	0.179	36.62[a)]	0.96	0.84-1.09	0.539
All studies excluding studies 4-6	7	5.05	0.90	0.74-1.09	0.318	12.03	1.13	0.98-1.31	0.085
Asians	3	1.93	0.88	0.64-1.20	0.429	2.31	1.56	1.19-2.04	0.001
Caucasians excluding studies 4-6	3	3.07	0.92	0.72-1.17	0.514	2.03	0.99	0.83-1.17	0.917

[a)]There is significant ($p < 0.001$) heterogeneity between studies. APOE, apolipoprotein E; Q, Q-statistic; OR, odds ratio; CI, confidence interval.

Table 3. Prevalence of apolipoprotein E (*APOE*) genotypes in studies used in the meta-analysis

Study	Controls							Cases						
	E2E2	E3E2	E3E3	E4E2	E4E3	E4E4	Total	E2E2	E3E2	E3E3	E4E2	E4E3	E4E4	Total
Moysich et al., 2000 [3]	1	34	216	7	67	7	332	2	26	173	4	52	3	260
Yaylim et al., 2003 [5]	0	0	20	0	0	0	20	0	2	29	0	1	0	32
Chang et al., 2005 [7]	14	4	145	13	55	1	232	11	24	145	10	96	4	290
Surekha et al., 2008 [9]	0	9	93	2	6	0	110	0	6	84	0	19	1	110
Porrata-Doria et al., 2010 [10]	0	19	165	42	1	2	229	0	13	143	46	0	3	205

Table 4. Summary of meta-analysis of case-control studies examining genotypes of *APOE* polymorphism and breast cancer risk

Studies	No. of studies	E2 carriers vs. E3E3				E2 carriers vs. E3E3			
		Q	OR	95% CI	p-value	Q	OR	95% CI	p-value
All studies	5	5.96	1.14	0.81-1.59	0.379	7.87	1.29	1.03-1.62	0.024
Asians	2	2.42	1.53	0.91-2.58	0.108	1.31	1.75	1.23-2.47	0.002
Caucasians excluding studies 4-6	3	1.68	0.95	0.62-1.47	0.842	1.74	1.05	0.78-1.41	0.750

There is significant ($p < 0.001$) heterogeneity between studies. There is no significant heterogeneity between studies. E2 carriers indicates E2E2+E2E3 genotypes and E4 carriers indicates E4E4+E4E3+E4E2 genotypes. APOE, apolipoprotein E; Q, Q-statistic; OR, odds ratio; CI, confidence interval.

models according to the heterogeneity of the study. The fixed-effects method assumes no significant heterogeneity between results of the individual studies being pooled, whereas, the random-effects method allows for such heterogeneity. The fixed-effects method was used by Mantel and Haenszel [11] and the random-effects method was used by DerSimonian and Laird [12]. Visual inspection of Begg's funnel plots was performed for assessment of publication bias. An asymmetric plot suggested possible bias, in which case Egger's test [13] was used.

Results

We identified 10 eligible studies, including 3,835 subjects (2008 patients, and 1,827 healthy controls), that reported on polymorphisms of *APOE* and risk of breast cancer, which are summarized in Table 1 [3-10]. The forest plot of the meta-analysis of the *APOE* alleles is shown in Fig. 1. Comparison of prevalence of the ε4 vs. ε3 alleles among cases and controls showed statistically significant heterogeneity between studies ($Q=36.2$, $df=9$, $p < 0.001$). In order to find the source of heterogeneity, we stratified the studies based on the ethnicity of participants. As a result, heterogeneity showed a dramatic decrease among studies reported from Asia ($Q=2.31$, $df=2$, $p=0.315$), and a significant association was revealed. Presence of the ε4 allele indicated increased risk of breast cancer, in comparison with the ε3 allele (OR, 1.56; 95% CI, 1.19 to 2.04; $p=0.001$). It should be noted that no association of the ε2 allele with breast cancer risk was observed (Table 2).

Among other studies, heterogeneity was still observed. Excluding studies reported by Yaylim et al. [5] (due to a very small sample size) and Menzel et al. [6] (because there was not an equal number of the polymorphism at positions 112 and 158), heterogeneity showed a decrease ($p > 0.381$) (Table 2). When compared with the ε3 allele, no association of either the ε4 (OR, 0.99; 95% CI, 0.83 to 1.17; $p=0.917$) or the ε2 (OR, 0.92; 95% CI, 0.72 to 1.17; $p=0.514$) allele with susceptibility to breast cancer was observed.

From 10 studies [3-10], only five studies [3,5,7,9,10] reported frequencies of *APOE* genotypes; these are summarized in Table 3. The forest plot

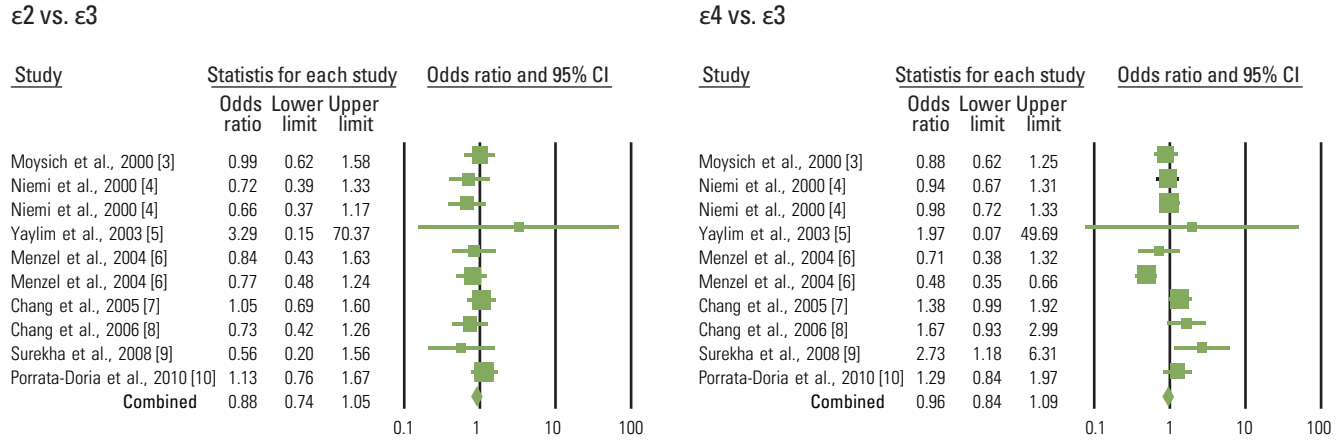

Fig. 1. Forest plot of the meta-analysis of associations between alleles of apolipoprotein E (*APOE*) polymorphism and breast cancer risk. CI, confidence interval.

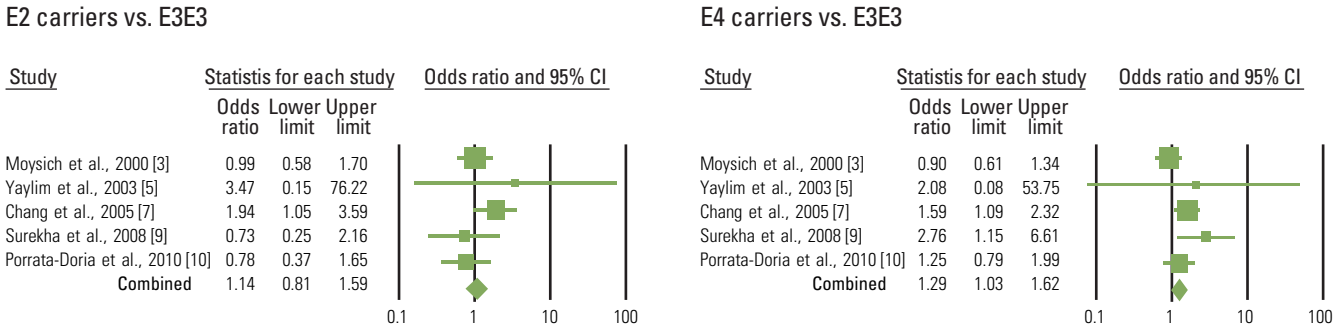

Fig. 2. Forest plot of the meta-analysis of associations between genotypes of apolipoprotein E (*APOE*) polymorphism and breast cancer risk. CI, confidence interval.

of the meta-analysis of the *APOE* genotypes is shown in Fig. 2. As shown in Table 4, carriers of the ε4 allele (E4E4, E4E3, and E4E2 genotypes), in comparison with the E3E3 genotype, showed an association with elevated risk of breast cancer only among Asians (OR, 1.75; 95% CI, 1.23 to 2.47; p=0.002).

In order to assess publication bias for reported comparisons of the alleles and genotypes of *APOE* polymorphism and breast cancer risk, Begg's funnel plots were prepared and Egger's test was performed on the set of 10 studies [3-10]. The funnel plots (Fig. 3) appeared to be symmetrical in shape, and results of statistical analysis showed no significant publication bias.

Discussion

The main finding of the present study was that carriers of the ε4 allele (E4E4, E4E3, and E4E2 genotypes), in comparison with the E3E3 genotype, showed an association with elevated risk of breast cancer only among Asians. In conclusion, findings of this meta-analysis suggest that the *APOE* ε4 allele is a low-penetrant risk factor for development of breast cancer. The *APOE* ε4 allele has been associated with increased risk of several multifactorial diseases, including hypertension [14] and coronary heart disease [15].

Based on findings from animal studies, ecologic studies, and studies of migrants from areas with low fat intake to those with high fat intake, an association of dietary fat intake with breast cancer risk has been hypothesized [16]. Previous studies have reported increased risk of breast cancer in association with elevated triglyceride (TG) levels [17]. Association of increased TG concentrations with decreased levels of sex hormone binding globulin, resulting in elevated levels of free estardiol and subsequently increased risk of breast cancer, has been reported [18]. Association of elevated TG levels with breast cancer risk among women with the *APOE* E4 genotype has been reported. The *APOE* E4 genotype has been reported to reduce TG clearance from plasma, resulting in persistently elevated TG concentrations, which could result in decreased levels of sex hormone binding globulin and elevated levels of free estradiol [3,19].

Possible association of some genetic polymorphisms with altered risk of some types of cancers only in some ethnic groups has been previously reported [20-23]. For example, increased risk of gastric cancer in associ-

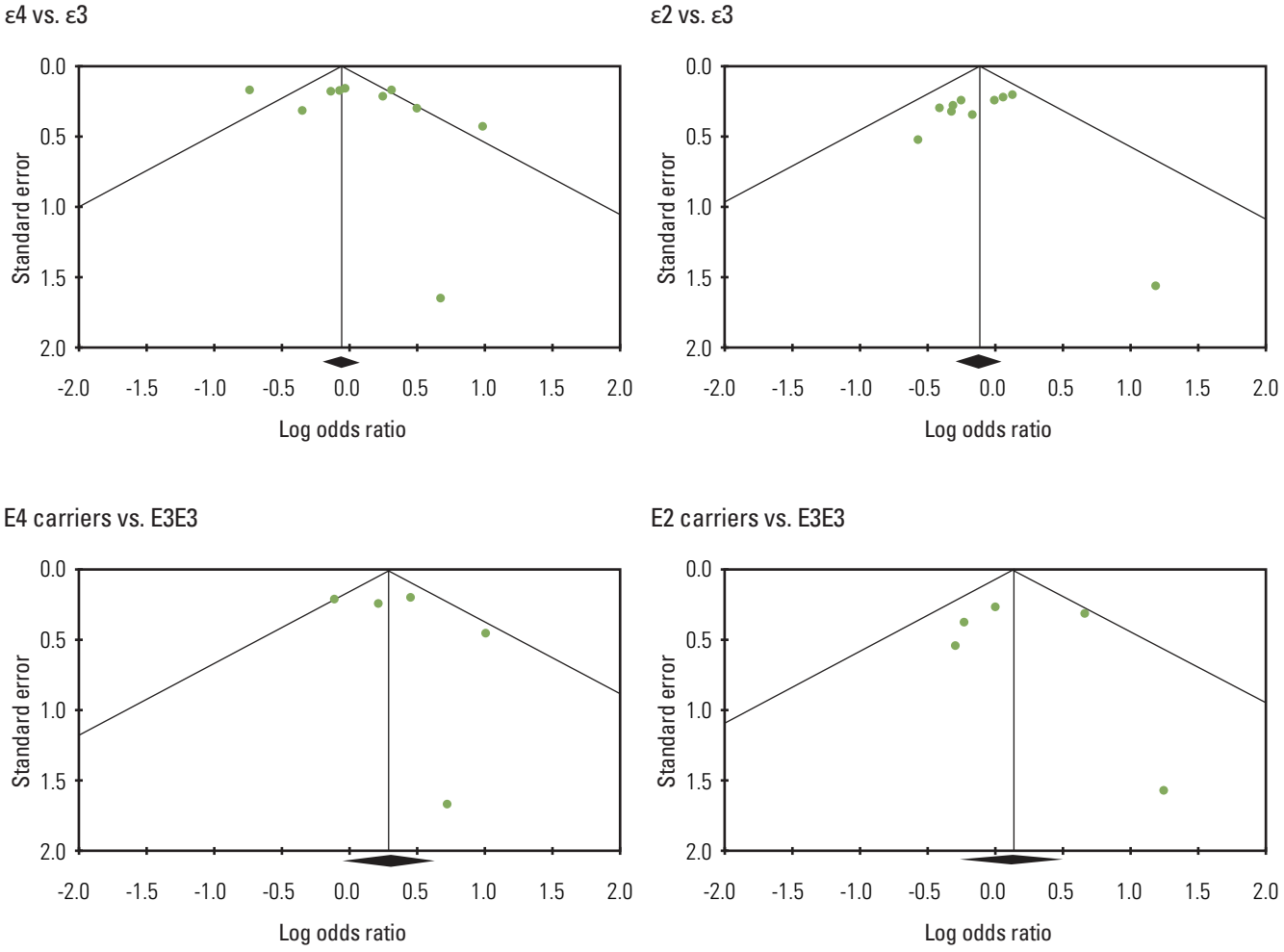

Fig. 3. Funnel plots of the meta-analysis of associations between alleles and/or genotypes of apolipoprotein E (*APOE*) polymorphisms and breast cancer risk (p-values of Egger's test were 0.556, 0.297, 0.923, and 0.488, for ε2 vs. ε3, ε4 vs. ε3, E2 carriers vs. E3E3, and E4 carriers vs. E3E3 comparisons, respectively).

ation with the GSTT1 polymorphism has been reported only among Caucasians [21]. In relation to the XRCC1 polymorphism, association of Arg399Gln with lung and breast cancers among Asians, but not among Western countries, has been reported [20,23]. Similarly, findings from the present study indicated the *APOE* ε4 allele as a risk factor for development of breast cancer in Asia.

The gene encoding *APOE* was mapped on human chromosome 19q13.2. Of particular interest, based on published meta-analysis, single nucleotide polymorphisms of several genes, including XPD, ERCC2, XRCC1, and TGFB1, located on human chromosome 19q13, have been associated with breast cancer risk [23-25]. Therefore, it is quite probable that our present finding indicates a true association. We know that the major limitation of this study is the small number of articles available for meta-analysis, as well as its geographical distribution; for example, there are no reports from China, Western Europe, etc. Future well-designed large epidemiological studies are warranted in order to provide validation for the present findings.

Conclusion

The present meta-analysis suggest that the *APOE* ε4 allele is a low-penetrant risk factor for development of breast cancer.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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