Association between apolipoprotein E gene polymorphism and the risk of recurrent pregnancy loss: A meta-analysis

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Abstract

Purpose The association between apolipoprotein E (ApoE) gene polymorphism and the risk of recurrent pregnancy loss (RPL) remains controversial. The present meta-analysis was performed to derive a precise estimate of the relationship.

Method(s) We searched the PubMed, Embase, and Web of Science data-bases for studies related to the association between the ApoE genotype and the risk of RPL. We estimated the summary odds ratio (OR) with 95% confidence interval (CI) to assess the association.

Result(s) Seven studies, including 2,090 RPL cases and 742 control samples, were identified. The results showed a significant association between ApoE E4 mutation and RPL risk (for E4 allele: OR = 1.98, 95 % CI = 1.14–3.43, P = 0.499; for E2E3 vs. E3E3: OR = 1.33, 95%CI = 1.12–1.42, P = 0.008; for E2E4 vs. E3E3: OR = 1.26, 95 % CI = 1.07–1.49, P = 0.005).

Conclusion(s) The meta-analysis suggests an association between ApoE E4 mutation and increased risk of RPL.

Keywords Polymorphism · Apolipoprotein E · Recurrent pregnancy loss · Meta-analysis

Introduction

Recurrent pregnancy loss (RPL) is a common pregnancy complication that affects at least 2 % of women who are in their reproductive age [1]. The pathophysiology of RPL is complex and poorly understood. Genetic, infective, anatomical,

endocrine, and immune defects have been postulated as the causes of RPL [2]. Genetic theory for susceptibility to RPL has been supported by genome-wide association studies [3, 4]. However, the influence of new potential candidate genes that explain the etiology of RPL and offer therapeutic targets should still be examined to come up with treatment methods for RPL. One potential candidate gene is apolipoprotein E (ApoE), the most important cholesterol precursor for steroid hormone production in steroidogenic tissues and a major influencer of the regulation of steroid hormone function [5], thereby influencing human reproduction. ApoE is a glycosylated protein encoded by three distinct alleles, namely, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. The major resulting proteins, namely, ApoE2, ApoE3, and ApoE4, significantly affect the redistribution and metabolism of lipoproteins and cholesterol. The function of chromosome 19q13 (on which the ApoE gene lies) in several autoimmune diseases, such as systemic lupus erythematosus [6], and the putative effects of ApoE on multiple sclerosis [7] have been proposed by several researchers. Several original studies have reported the relationship between ApoE polymorphism and RPL, but the results are inconclusive partially because of the possible small effect of the polymorphism on RPL risk and the small sample in each published study. Therefore, we performed a meta-analysis to derive a precise estimate of this association.

Materials and methods

Literature search

We searched all published studies (up to July 5, 2013) in the databases of PubMed, Embase, and Web of Science. These computer searches were limited to English language articles, and unpublished dissertations were excluded. The following keywords were used in the searches: (apolipoprotein E or ApoE) and (polymorphism or mutation or variant) and (recurrent pregnancy loss or RPL). Additionally, related articles were manually searched.

Capsule The ApoE E4 allele is associated with an increased risk of developing RPL and may be a marker for clinical evaluation.

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Table 1 Characteristics of the studies evaluating the effects of apoE allele on RPL risk

Study	Year	Country	Ethnicity	Cases			Controls		
				E2	E3	E4	E2	E3	E4
Agarwal	2010	India	Asian	30	359	11	36	360	4
Asgari	2013	USA	Caucasian	7	133	22	11	149	2
Goodman	2010	USA	Caucasian	8	106	16	7	58	2
Ozdemir	2011	Turkey	Caucasian	127	822	74	8	174	15
Ozdemir	2011	Turkey	Caucasian	61	479	55	15	183	7
Ozdemir	2011	Turkey	Caucasian	198	1,301	129	23	357	22
Korkmazer	2013	Turkey	Caucasian	8	66	16	12	60	18

Inclusion criteria

For the studies to be included in our meta-analysis, they must meet the following criteria: (1) the original document must be a randomized case-control study; (2) data collected must include information on ethnicity, such as Latin American, Asian, European, or African; (3) one or more of the alleles, such as $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, should be mentioned; (4) the frequency of the ApoE gene and its allele in the control group remain in the Hardy-Weinberg equilibrium.

Data extraction

Information was carefully extracted from all eligible publications independently by two investigators. If these two investigators could not reach a consensus, the result was reviewed by a third investigator. For each study, the information extracted included the first author, the year of publication, the study population (country and ethnicity), the source of the control samples, the total number of cases and control samples, and the frequency of ApoE gene polymorphism in the cases and in the control samples.

Statistical analysis

The strength of association between ApoE gene polymorphism and RPL risk was assessed by ORs with 95 % CIs, which were used to compare genotypes and alleles between cases and the control samples. E3E3 is well accepted as the “wild-type” genotype because it is the most common genotype between healthy and affected subjects at a frequency of about 67 % [1]. Therefore, genotype E3/E3 is designated as a reference category in the present study. Pooled ORs were estimated for seven genetic models (E2/E2 vs. E3/E3, E2/E3 vs. E3/E3, E2/E4 vs. E3/E3, E3/E4 vs. E3/E3, E4E4 vs. E3E3, E2 allele vs. E3 allele + E4 allele, E3 allele vs. E2 allele + E4 allele, E4 allele vs. E2 allele + E3 allele). The fixed-effects model was used in the absence of between-study heterogeneity; otherwise, the random-effects model was used. Heterogeneity was measured using Cochran’s Q statistic and

the I^2 statistic. Sensitivity was analyzed by limiting the meta-analysis to studies conforming to the Hardy-Weinberg equilibrium (HWE, $p < 0.05$ of HWE was considered significant). Publication bias was determined using both Begg’s funnel plot and Egger’s regression test ($p < 0.05$ was considered representative of statistically significant publication bias). All statistical analyses were performed using Stata 11.0.

Results

Study characteristics

Five publications met the inclusion criteria [8–12]. In Ozdemir et al., the ORs were presented separately for the three different subgroups (i.e., women with a history of RPL, RPL male partners, and RPL patients with no gender restriction) [11]. Therefore, Ozdemir et al.’s study was separately considered for subgroup analysis. Thus, seven studies, including 2,090 cases and 742 control samples, were used in the meta-analysis.

Table 2 Characteristics of the studies evaluating the effects of apoE genotypes on RPL risk

Study	Group	APOE genotypes					
		E2E2	E2E3	E2E4	E3E3	E3E4	E4E4
Agarwal	Case	9	12	0	168	11	0
	Control	8	19	0	168	5	0
Goodman	Case	/	6	2	46	14	1
	Control	/	5	2	28	2	0
Ozdemir	Case	/	116	11	353	63	/
	Control	/	8	0	83	15	/
Ozdemir	Case	/	65	6	207	49	/
	Control	/	15	0	84	7	/
Ozdemir	Case	/	181	17	560	112	/
	Control	/	23	0	167	22	/
Asgari	Case	0	7	0	58	10	6
	Control	0	11	0	68	2	0

Table 3 Meta-analysis of the association of apoE gene polymorphism with risk of RPL

Genetic contrasts	Studies	Odds ratio		Model	Heterogeneity	
		OR (95 % CI)	POR		I ² (%)	PH
E2E2 VS E3E3	2	1.13(0.45–2.84)	0.795	Fixed	0	0.978
E2E3 VS E3E3	6	1.33(1.12–1.42)	0.008	Fixed	39.2	0.062
E2E4 VS E3E3	6	1.26(1.07–1.49)	0.005	Random	72.5	<0.0001
E3E4 VS E3E3	6	1.07(0.97–1.18)	0.182	Fixed	23.3	0.236
E4E4 VS E3E3	3	2.14(0.50–9.18)	0.304	Fixed	10.6	0.327
E2 allele	7	1.19(0.72–1.97)	0.33	Random	67.2	0.006
E3 allele	7	0.64(0.47–0.86)	0.089	Random	56.9	0.031
E4 allele	7	1.98(1.14–3.43)	0.499	Random	73.4	0.001

Table 1 lists the studies identified and their main characteristics. Among the seven studies, the sample sizes ranged from 105 to 1,082. Six studies involved Caucasians, and one study involved Asians. Cases included women with a history of ≥ 2 consecutive spontaneous abortions and without a history of successful pregnancy. The control samples were mainly healthy populations with no history of pregnancy loss and at least one normal pregnancy (Table 2).

Main results of allele comparison

The main results are presented in Table 3. Forest plots are shown in Figs. 1, 2 and 3. Compared with that of the E3 allele, an association of the E2 allele with RPL risk was not detected ($OR=1.19$, 95 % $CI=0.72$ – 1.97 , $P=0.33$) with significant inter-study heterogeneity ($I^2=67.2$ %, $P=0.006$). However, we found a significant association of the E4 allele with RPL risk compared with that of the E3 allele ($OR=1.98$, 95 % $CI=1.14$ – 3.43 , $P=0.499$) with significant inter-study heterogeneity ($I^2=73.4$ %, $P=0.001$).

Main results of genotype comparison

A significant association with RPL risk was found in the genetic model of E2E3 vs. E3E3 (for $OR=1.33$, 95 % $CI=1.12$ – 1.42 , $P=0.008$) and in the genetic model of E2E4 vs. E3E3 (for $OR=1.26$, 95 % $CI=1.07$ – 1.49 , $P=0.005$) compared with wild-type E3E3. However, no association was found in the genetic model of E2E2 vs. E3E3 (for $OR=1.13$, 95 % $CI=0.45$ – 2.84 , $P=0.795$), E3E4 vs. E3E3 (for $OR=1.07$, 95 % $CI=0.97$ – 1.18 , $P=0.182$), and E4E4 vs. E3E3 (for $OR=2.14$, 95 % $CI=0.50$ – 9.18 , $P=0.304$). The main results of meta-analysis are shown in Table 3.

Sensitivity analysis

A single study involved in the meta-analysis was omitted each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not materially altered (data not shown). Thus, our results were statistically robust.

Fig. 1 Association between E2 allele and RPL risk

Fig. 2 Association between E3 allele and RPL risk

Publication bias

Begg's funnel plot and Egger's regression test were performed to assess the publication bias of the studies in literature. As shown in Figs. 4a (for E2 allele), b (for E3 allele), and c (for E4 allele), the shape of the funnel plot had no asymmetry. Egger's test was performed to provide statistical evidence of funnel plot symmetry. The results still suggested no publication bias ($P=0.293$ for E2 allele, $P=0.293$ for E3 allele, and $P=0.099$ for E4 allele).

Discussion

The present meta-analysis, which involve 2,090 cases and 742 control samples, explored the association between ApoE polymorphism and RPL risk. Individuals with the E2E3 and E2E4

genotypes had a significantly higher risk of developing RPL ($OR=1.33$ and $OR=1.26$) than those with the E3E3 genotype. Moreover, the risk of developing RPL in individuals with the E4 allele was 1.98-fold higher than that in individuals without the said allele. Thus, the E4 allele of ApoE is an independent risk factor for the development of RPL. However, ORs were all less than 2, which indicates low clinical relevance and high possibility of confounders. Given the ethnic or geographical difference in the distribution of ApoE genotypes [13], performing population-based studies with appropriate control samples are still necessary. In addition, considering the isoform specific difference of the influence of ApoE on lipid metabolism, atherosclerosis, oxidative stress, and inflammatory and immune responses [14], RPL patients with known causes of recurrent miscarriage should not be excluded from studies that aim to identify the association between ApoE gene polymorphisms and RPL.

Fig. 3 Association between E4 allele and RPL risk

Fig. 4 Funnel plot for the results of the meta-analysis of the E2 allele (a) E3 allele (b) E4 allele (c)

The etiology of the E4 allele in RPL is only partly understood and may be related to lipid metabolism. The E2 allele is associated with low serum LDL cholesterol, whereas the E4 allele is associated with high LDL cholesterol. The E4 allele is also involved in inflammatory response, platelet function, apoptosis, and the modulation of oxidative stress [15]. However, Bianca et al. [16] concluded that no association exists between ApoE polymorphisms and recurrent pregnancy loss. The possible reason for the inconsistency is that Bianca

et al. compared their results in women with recurrent pregnancy loss with those in women with a history of cardiovascular disease and in women with a family history of inherited thrombophilia. No negative control samples were involved in their study. However, the control samples in our meta-analysis were selected mainly from healthy populations with no history of pregnancy loss and had at least one normal pregnancy. A negative control group of sufficient sample size with a healthy population is necessary to arrive at a sound conclusion.

Several limitations of this meta-analysis should be acknowledged. First, the sample size in most of the included studies was small and thus could have increased the probability of false positives or false negatives. Second, few studies were included in the meta-analysis; thus, the subgroups were difficult to analyze. Third, our results were based on unadjusted estimates, whereas precise analysis should be conducted if individual data were available to allow adjustment by other covariants, including age, ethnicity, menopausal status, smoking status, drinking status, obesity, environmental factors, and other lifestyle habits.

Despite these limitations, our study demonstrates that the ApoE E4 allele is associated with an increased risk of developing RPL and may be a marker for clinical evaluation. However, large-sample studies should be conducted with standardized unbiased genotyping methods, homogeneous RPL patients, and well-matched controls. Moreover, gene–gene and gene–environment interactions should be considered in the analysis. Studies that account for these factors will enhance and broaden understanding of the association between ApoE polymorphisms and RPL risk.

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