Apolipoprotein E (Apo E) gene polymorphisms and recurrent pregnancy loss: a meta-analysis

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Received: 30 July 2013 / Accepted: 21 October 2013 / Published online: 13 November 2013
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Abstract

Background Recently, a relationship between recurrent pregnancy loss (RPL) and Apolipoprotein E (Apo E) gene polymorphisms has been proposed. In order to investigate the real association between Apo E polymorphisms and RPL, our meta-analysis was carried out.

Methods We estimated the association with RPL risk under dominant and recessive models, in combination with the OR and RR with a 95 % confidence interval (CI), which was used to assess the association between RPL and Apo E polymorphisms. **Results** According to our criterion, there were 6 studies included. The dominant model used the E4/E3 group and the OR was 1.919 (95 %CI: 1.016–3.625, $I^2=53.8$ %), the RR was 1.308 (95 %CI: 1.071–1.598) suggesting that carriers of the E4 allele would have a higher risk of causing spontaneous miscarriages. In addition, the OR was 0.727 (95 %CI: 0.566–0.932, $I^2=0.0$ %) and RR was 0.923 (95 %CI: 0.867–0.982) in a fixed model for

E3/E3 homozygotes versus other genotypes, suggesting that the Apo E3 polymorphism could be a protective factor. The OR was 1.365 (95 % CI: 1.029–1.811, $I^2=0.0$ %) in a fixed model comparing the E2 allele with the E3 allele, suggesting that Apo E2 polymorphisms may contribute to RPL as a risk factor. Furthermore, after conducting sensitivity analysis in the E4/E3 group, the results showed this to be consistent and the OR was 2.249 (95 %CI: 1.474–3.431).

Conclusions There is a close association between RPL and Apo E gene polymorphisms. For RPL, the Apo E4 polymorphism could be a risk factor, the Apo E3 polymorphism may be a protective factor and the Apo E2 polymorphism may be another potential risk factor.

Keywords Apolipoprotein E · Gene · Polymorphism · Recurrent pregnancy loss

Capsule A close association between Apo E gene polymorphisms and recurrent pregnancy loss was summarized.

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Introduction

Description of Apo E gene

The apolipoprotein E (Apo E) gene consists of four exons and three introns, with 3597 base pairs, and is located on chromosome 19q13.2 in humans [1]. Combined with ApoC1 and C2, Apo E plays a role in cholesterol-triglyceride metabolism by producing a 299-amino acid long polypeptide in the liver and brain [2–4]. With a complex structure, the Apo E gene has three major isoforms: Apo E2 (cys112, cys158), Apo E3 (cys112, arg158), and Apo E4 (Arg112, Arg158), which result in six different genotypes (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4 and E4/E4) [3]. Due to these various structures, the physiological consequences are different.

As the most attentive loci, the Apo E4 allele was found in approximately 14 % of the population [5], which was said to be related to atherosclerosis, cerebrovascular ischemia and Alzheimer's disease [6, 7]. During pregnancy, women are in the hypercoagulable state. It is reported that the E4 allele is associated with higher LDL cholesterol, which could affect the function of blood vessels and promote atherosclerosis [2], which might contribute to the thrombogenesis that is believed to affect placental flow and fetal intrauterine growth. However, this view is controversial. In addition, Apo E2, as the other notable allele, was also associated with atherosclerosis. Digesting dietary fat slowly might be one of the mechanisms which could lead to the condition of genetic disorder type III hyperlipoproteinemia, the hypercoagulable state and other vascular complications. In contrast, the Apo E3 allele is considered to be less important. It is referred to as the "neutral" genotype and is found in approximately 79 % of the population [5]. To develop disease, both genetic and environmental factors are believed to be involved [8–10].

Disease

Recurrent pregnancy loss (RPL) is defined as two or more consecutive spontaneous abortions before 20 weeks gestation [11], with various etiologies. According to a related study [12], up to 20 % of pregnant women suffer from spontaneous abortion in the first trimester, which is one of the most common causes of female infertility. At present, a few known risk factors of RPL are known: advanced maternal age, chromosomal abnormalities, abnormal infections, hereditary thrombophilia, unnatural anatomic anomalies [13, 14], hormone imbalances, and immunity to environmental and male factors. However, approximately 50–75 % of couples with RPL have no accurate cause of pregnancy loss, which is known as unexplained RPL, suggesting genetic factors to be involved in the etiology of unexplained RPL [2].

Recently, some prominent genetic polymorphisms and their pathogenic mechanisms have been studied for association with RPL, including factor V G1691A (FVL), factor XIII V34L (FXIII V34L), ACE I/D, β -fibrinogen -455G>A (β -fibrinogen), Apo E (E2, E3, and E4), MTHFR C677T and MTHFR A1298C. Almost all of these were related to thrombogenesis [15], which is said to affect placental flow and fetal intrauterine growth, even leading to miscarriage by thrombosis in the decidual vessels [2]. Among them, the Apo E gene has been found to be associated with the RPL because it might contribute to thrombogenesis, with cholesterol-triglyceride metabolism being involved promoting atherosclerosis. Therefore, some studies [2, 16, 17] have shown that the Apo E gene could be one of the risk factors for RPL, especially the high frequency of the Apo E4 genotype. Nevertheless, some other studies [1, 18–20] presented no significant association of Apo E polymorphisms with recurrent pregnancy loss. On the basis of this controversy, this meta-analysis was conducted with data published in recent years.

Materials and methods

Search criteria

The PubMed and Embase databases were applied in this search; the following key words were combined: "recurrent pregnancy loss", "recurrent spontaneous miscarriages", "habitual abortion", "fetal loss", "RPL", "abortion", "miscarriage", "Apo E", "Apo", "Apolipoprotein E4" and "Apolipoprotein". The last retrieval was conducted in April 2013. Literatures were restricted to only English-language studies. To avoid replicating analyses of the same samples, all studies were published in the primary literature, which was compared carefully. Rigorous inclusion criteria were as follows: (1) The case-control studies were about the association between the Apo E polymorphism and the risk of recurrent spontaneous miscarriages. (2) Two or more losses during pregnancy were defined as recurrent pregnancy loss. The unexplained RPL samples that had two or more continuous pregnancy losses with no other specific causes inducing the losses, such as histology and/or quantitative serum hCG levels, were classified into the case group. The controls were chosen from healthy parous women with no history of pregnancy loss and/or at least two or more normal pregnancies. (3) Studies conformed to Hardy-Weinberg equilibrium. (4) The polymerase chain reaction (PCR) analysis was used to identify genotypes by DNA. In addition, any studies which met the inclusion criteria, but where the samples came from fetal tissue material rather than maternal blood or provided no adequate information on the actual distribution of polymorphisms were excluded [21]. Review articles, case reports and case series were also not included.

For each study, we collected the following valuable information: author; year of publication; country; ethnicity; number of spontaneous abortions; number of cases and controls for each Apo E allele (E2, E3, E4); and pregnancy information about controls (Table 1).

Statistical analysis

As a special gene, Apo E has three distinct alleles in humans, namely E2, E3 and E4, which could be assembled into six different genotypes (E2E2, E2E3, E2E4, E3E3, E3E4 and E4E4). Considering these, the study was conducted using three different groups (E2E2, E2E3, E3E3; E2E2, E2E4, E4E4; and E3E3, E3E4, E4E4) following the traditional SNP polymorphism analysis. Hardy-Weinberg equilibrium (HWE; $P>0.05$) was tested [22]. In this analysis, two models (dominant model: E4/E4+E4/E3 versus E3/E3, E2/E2+E2/E3 versus E3/E3, E4/E4+E4/E2 versus E2/E2; recessive model: E4/E4 versus E4/E3+E3/E3, E2/E2 versus E2/E3+E3/E3, E4/E4 versus E4/E2+E2/E2) were applied to describe the potential association with RPL risk. Meanwhile, in order

Table 1 The essential characteristics of the studies we included

Author	Year	Country(ethnicity)	Genotype cases frequency (number of samples)								Spontaneous abortion (n)	Mean age of maternal	Genotype controls frequency (number of samples)								Source of controls	Mean age of maternal	Method
			E2/ E2	E2/ E3	E2/ E4	E3/ E3	E3/ E4	E4/ E4	E4/ E4	E4/ E4			E2/ E2	E2/ E3	E2/ E4	E3/ E3	E3/ E4	E4/ E4					
Asgari N et al.	2013	Iran(Asian)	0	7	0	58	10	6		Ranging between 2 and 8,mean 6.2	Mean 27.7	0	11	0	68	2	0		With two healthy children and no history of RPL	Mean 30	PCR		
Ozdemir O et al.	2012	Turkey(Caucasian)	0	116	11	353	63	0		2 or more	Median±Standard error: 27.8±2.1	0	15	0	83	15	0		With no history of pregnancy loss and at least one normal pregnancy	Median±Standard error : 28.9±2.2	PCR		
Agarwal M et al.	2010	North India(Asian)	9	12	0	168	11	0		Ranging between 3 and 7,mean 4	Mean 28.4	8	19	0	168	5	0		Healthy parous women with at least two live births With no history of abortions	Mean 31.9	PCR		
Goodman C et al.	2009	USA(Mix)	0	6	2	46	14	1		2 or more	Mean 34.7	0	5	2	28	2	0		With at least two live births and not more than one spontaneous abortion	Mean 37.5	PCR		
Rynekrova J et al.	2012	Czech Republic (Caucasian)	1	12	0	46	16	1		2 or more	Aged 15–44 years	7	134	11	732	166	10		With at least two healthy children and no history of abortions	Aged 25–65 years	PCR–RFLP		
Lambrinoudaki I et al.	2010	Greece(Caucasian)	0	0	0	6	0	0		2 or more	–	0	6	0	46	10	0		Healthy women with no miscarriages	—	PCR		

Mix: 41.6 % Japanese American, 17.9 % African American, 19.1 % Latino, 15.0 % White, 6.4 % Native Hawaiian

PCR-RFLP polymerase chain reaction-restriction fragment length polymorphism

to describe the further association of the polymorphism, allele analysis (E4 versus E3, E4 versus E2, E3 versus E2) was also used. In addition, the comparison was conducted by comparing the homozygotes to the other five genotypes (E2E2 versus E2E3+E3E3+E3E4+E4E4+E2E4; E3E3 versus E2E2+E2E3+E3E4+E4E4+E2E4; E4E4 versus E2E2+E2E3+E3E3+E3E4+E2E4), which might provide a new view on the function of different homozygotes. Based on the odds ratio (OR) and the pooled relative risk (RR) with a corresponding 95 % confidence interval (CI), we calculated the pooled odds which were used to analyze the effect of the association. While crossing these studies we paid attention to the statistical heterogeneity; if I^2 values were low or moderate ($I^2 < 50\%$), the fixed-effect model was considered for use, otherwise the random-effect model was adopted where the heterogeneity was high among these statistics. In order to assess heterogeneity, we used the I^2 based Q statistic for each genetic contrast to describe the weighted sum of the squared difference and a p-value less than 0.10 was considered in-homogeneous among the included studies [23].

Publication bias was assessed using Begg's unweighted regression test and funnel plots [5]. In addition, in order to further explore the source of heterogeneity, we carried out susceptibility analysis to determine the magnitude of influence on the overall summary estimate by excluding the article in which heterogeneity was of highest. All of the allele frequencies were calculated for studies reporting only genotype data. Stata 9.0 (Stata Corporation, USA) was used for analysis, and all P values were two-sided.

Result

Characteristics

With the associated keywords, there were 447 relevant studies retrieved in the initial search. With thorough evaluation, 431 studies were excluded for inappropriate research, contents that were either not relevant or did not investigate related genes with recurrent pregnancy loss, 16 were retrieved which described the association between the Apo E polymorphism and RPL, and finally, 6 were eligible for meta-analysis [15, 17, 19, 20, 23, 24]. One of the studies was excluded because the samples were not maternal blood, the tissue used was fetal tissue material [21]; five were excluded because they were presented as a "letter to the editor", meaning that the contents were controversial regarding the association between recurrent pregnancy loss and apolipoprotein E [14, 16, 25–27]; three were not included because they did not present detailed genotyping information [2, 18, 28]; and another study was excluded because it concentrated on patients with endometriosis [29]. The included studies were restricted to the English language. In total, there were 975 cases and 1553 controls in

this analysis; the case groups consisted of women who had experienced at least two unexplained consecutive spontaneous miscarriages, while the controls were limited to patients having delivered at least two healthy children, with no history of pregnancy loss or healthy parous women. We summarized the study characteristics in Table 1, and provided a list of details abstracted from the studies included in the meta-analysis in Fig. 1.

Overall data analysis in the three groups

Comprising 975 patients of RPL and 1553 normal controls, six studies were used to investigate the association between recurrent pregnancy loss and apolipoprotein E gene polymorphisms in women. The dominant model with random effects was used to analyze samples in the E4/E3 group (E4E4, E4E3, E3E3). All included studies conformed to Hardy–Weinberg equilibrium (Table 2). The pooled odds ratio estimate was 1.919 (95 %CI: 1.016–3.625, $I^2=53.8\%$), suggesting that carriers of the E4 allele would have a higher risk of suffering spontaneous miscarriages (Fig. 2), while the E4/E2 (E4E4, E4E2, E2E2) and E3/E2 (E3E3, E3E2, E2E2) polymorphism groups did not have an association in either the dominant or recessive model: the pooled OR (95 %CI) in the E4/E2 polymorphism group was 3.000 (0.165–54.566) in the dominant model and 2.219 (0.237–20.751) in the recessive model; for the E3/E2 polymorphism group, the summary OR (95 %CI) was 0.788 (0.321–1.935) in the dominant model and 0.855 (0.624–1.171) in the recessive model (Table 3). In order to increase the authenticity of these results, the pooled RR was also calculated (Table 4), which supported the hypothesis that the E4 allele would have a higher risk of causing spontaneous miscarriages; the pooled RR was 1.308 (95 %CI: 1.071–1.598, $I^2=55.9\%$) in the E4/E3 group.

A single allele (E2 or E3 or E4) comparison

In order to evaluate the genetic background of RPL and get more accurate results of genetic association studies, we carried out an analysis of the frequency of the three different alleles in comparison with each other. When the E4 allele was compared with the E3 allele, the summary OR was 2.111 (95 %CI: 1.090–4.088, $I^2=64.2\%$) and the RR was 1.977 (95 %CI: 1.077–3.631, $I^2=63.4\%$) in the random effects model, which supported the hypothesis that the E4 allele is a potential risk allele. When the E2 allele was compared with E4 allele, there was no obvious correlation and the pooled odds ratio estimate was 0.705 (95 %CI: 0.463–1.074). For the E2 allele and E3 allele, the results might have had a more profound significance which was worthy of further research and discussion; the summary OR was 1.365 (95 %CI: 1.029–1.811, $I^2=0.0\%$) and the RR was 1.326 (95 %CI: 1.021–1.722, $I^2=0.0\%$) in a fixed model (Fig. 3; Table 4).

Fig. 1 The process flow diagram of selected articles on genetic studies of APO E polymorphisms with RPL

Analysis of homozygous genes (E2/E2 or E3/E3 or E4/E4) versus other genotypes

While analyzing samples using homozygous genotypes (E2/E2 or E3/E3 or E4/E4) in comparison with the five remaining genotypes in cases and controls, the odds ratio estimate from the pooled data sets was 3.872 (95 %CI: 1.071–13.996, $I^2=0.0\%$) for E4/E4 homozygotes, 1.225 (95 %CI: 0.500–3.004, $I^2=0.0\%$) for E2/E2 homozygotes in a fixed model, and the OR was 0.727 (95 %CI: 0.566–0.932, $I^2=0.0\%$) and the RR was 0.923 (95 %CI: 0.867–0.982, $I^2=53.9\%$) for E3/E3 homozygotes (Fig. 4; Table 4), respectively. This showed that the E4 allele might confer an increased risk to RPL, while the E3 allele might be useful for protecting women by preventing

the occurrence of spontaneous abortions. In addition, no significant difference was detected in the E2 allele between patients and controls.

Sensitivity analysis and Publication bias

When the $I^2>50\%$ there seemed to be high heterogeneity among the results of all studies, we regulated the value of I^2 through random effects model correction under the necessary conditions. In addition, we carried out a sensitivity analysis in the E4/E3 group; according to the results, we removed the article with the highest relative heterogeneity [15]. Significantly, we found that the results in the odds ratio estimates were 2.249 (95 %CI: 1.474–3.431, $I^2=46.4\%$) in

Table 2 The frequency of the allele and the P value of Hardy–Weinberg equilibrium in E4/E3 group

Authors	Year	Ethnicity	The frequency of the allele						HWE(<i>P</i>)
			Case			Control			
			E2	E3	E4	E2	E3	E4	
Asgari N et al.	2013	Asian	7(4.5 %)	126(81.3 %)	22(14.2 %)	11(6.8 %)	149(92.0 %)	2(1.2 %)	0.9035
Ozdemir O et al.	2012	Caucasian	127(11.7 %)	885(81.5 %)	74(6.8 %)	15(6.6 %)	196(86.8 %)	15(6.6 %)	0.412
Agarwal M et al.	2010	Asian	30(12.1 %)	207(83.5 %)	11(4.4 %)	35(8.8 %)	360(90.0 %)	5(1.2 %)	0.8471
Goodman C et al.	2009	Mix	8(5.8 %)	112(81.2 %)	18(13.0 %)	7(9.5 %)	63(85.1 %)	4(5.4 %)	0.8502
Ryneckrova J et al.	2012	Caucasian	14(9.2 %)	120(78.9 %)	18(11.9 %)	159(7.5 %)	1764(83.2 %)	197(9.3 %)	0.8639
Lambrinoudaki I et al.	2010	Caucasian	0(0 %)	12(100 %)	0(0 %)	6(4.8 %)	108(87.1 %)	10(8.1 %)	0.4632

HWE Hardy–Weinberg equilibrium

Fig. 2 Meta-analysis with a dominant model (E4/E4+E4/E3 versus E3/E3) and random-effects for the association between RPL risk and the Apo E4 polymorphism. The name of the first author and year of publication for each study was shown. Results of individual and summary odds ratio

(OR) estimates, 95 % confidence interval (CI) and weights (W) of each study revealed the association between the RPL risk and the Apo E4 polymorphism. Horizontal lines represent 95 %CI and dotted vertical lines represent the value of the summary OR

the dominant model with fixed effects, which especially showed that the value of I^2 dropped from 53.8 % to 46.4 %.

Using tests for publication bias, Egger's funnel plot was estimated and shown to be symmetrical, which did not show significant bias by analysis using the Begg's test ($P=0.381$).

Discussion

Description

There were 6 studies about the Apo E polymorphisms in RPL aggregated into meta-analyses. Based on 975 patients and

Table 3 the data of meta-analysis about models

Models	Cases	Controls	OR	95 % CI	I^2	P for heterogeneity
Dominant (E3/E3+E3/E2 versus E2/E2)	840	1330	0.788	0.321–1.935	0.00 %	0.61
Recessive (E3/E3 versus E3/E2+E2/E2)	840	1330	0.855	0.624–1.171	15.10 %	0.317
Dominant (E4/E4+E4/E2 versus E2/E2)	31	38	3	0.165–54.566	NA	NA
Recessive (E4/E4 versus E4/E2+E2/E2)	31	38	2.219	0.237–20.751	0.00 %	0.829
Dominant (E4/E4+E4/E3 versus E3/E4)	799	1335	1.919	1.016–3.626	53.80 %	0.055
Recessive (E4/E4 versus E4/E3+ E3/E3)	799	1335	0.263	0.072–0.967	0.00 %	0.392
E2 allele versus E3 allele	1648	2873	1.365	1.029–1.811	0.00 %	0.418
E2 allele versus E4 allele	329	466	0.705	0.463–1.074	76.00 %	0.002
E4 allele versus E3 allele	1605	2873	2.111	1.090–4.088	64.20 %	0.016
E2E2 versus (E2E3+E3E3+E3E4+E4E4+E2E4)	975	1553	1.225	0.500–3.004	0.00 %	0.627
E3E3 versus (E2E2+E2E3+E3E4+E4E4+E2E4)	975	1553	0.727	0.566–0.932	0.00 %	0.515
E4E4 versus (E2E2+E2E3+E3E3+E3E4+E2E4)	975	1553	3.872	1.071–13.996	0.00 %	0.375

NA not appropriate

Table 4 the data of meta-analysis about models

Models	Cases	Controls	RR	95 % CI	I^2	P for heterogeneity
Dominant (E3/E3+E3/E2 versus E2/E2)	840	1330	0.786	0.547–1.129	31.4 %	0.227
Recessive (E3/E3 versus E3/E2+E2/E2)	840	1330	0.989	0.953–1.027	24.8 %	0.248
Dominant (E4/E4+E4/E2 versus E2/E2)	31	38	0.833	0.539–1.288	<i>NA</i>	<i>NA</i>
Recessive (E4/E4 versus E4/E2+E2/E2)	31	38	0.975	0.423–2.246	0.0 %	0.530
Dominant (E4/E4+E4/E3 versus E3/E4)	799	1335	1.308	1.071–1.598	55.9 %	0.045
Recessive (E4/E4 versus E4/E3+ E3/E3)	799	1335	2.151	0.877–5.274	15.1 %	0.308
E2 allele versus E3 allele	1648	2873	1.326	1.021–1.722	0.0 %	0.436
E2 allele versus E4 allele	329	466	0.746	0.505–1.103	76.3 %	0.002
E4 allele versus E3 allele	1605	2873	1.977	1.077–3.631	63.4 %	0.018
E2E2 Versus (E2E3+E3E3+E3E4+E4E4+E2E4)	975	1553	1.216	0.520–2.844	0.0 %	0.622
E3E3 versus (E2E2+E2E3+E3E4+E4E4+E2E4)	975	1553	0.923	0.867–0.982	53.9 %	0.054
E4E4 versus (E2E2+E2E3+E3E3+E3E4+E2E4)	975	1553	3.790	0.964–14.899	0.0 %	0.386

NA not appropriate; RR: Pooled relative risk

1553 controls, our integrated results showed that the Apo E polymorphisms were significantly associated with recurrent spontaneous abortion, and our findings demonstrate that the E2 and E4 alleles of the Apo E polymorphism could be major risk factors for RPL; however, the E3 allele might play an opposite role, which may be useful for protecting women during pregnancy.

With various etiologies, it is said that RPL has been found to be related to thrombophilic genes [15]. The Apo E gene also belongs to the group of thromboplastic genes and is mainly involved in the cholesterol-triglyceride metabolism process. Many studies were focused on vascular diseases such as atherosclerosis, ischemic cerebrovascular disease and Alzheimer's disease and the involvement of the Apo E gene. However, recent

Fig. 3 Meta-analysis with fixed model (E2 allele versus E3 allele) for the association between RPL risk and the Apo E2 polymorphism

Fig. 4 Meta-analysis with fixed model (E3E3 versus E2E2+E2E3+E3E4+E4E4+E2E4) for the association between RPL risk and the Apo E3 polymorphism

studies were mainly focused on the relationship of RPL with conflicting results. As a large sample and credibility research, the meta-analysis investigating the association between the Apo E polymorphism and RPL was indispensable and resulted in a more powerful conclusion regarding their relationship.

The results and sensitivity analysis

Considering that the Apo E gene has three isoforms: E2, E3 and E4 which form six different genotypes, we carried out different angles of algorithms with the included data for a comprehensive analysis. In the E4/E3 group, the summary OR was 1.919 (95 %CI: 1.016–3.625, $I^2=53.8\%$) and the RR was 1.308 (95 %CI: 1.071–1.598, $I^2=55.9\%$), but there was no association found in the E4/E2 and E3/E2 groups. This suggests that carriers of the E4 allele have a higher risk of RPL which is supported by Chelsi Goodman et al. [16] and Gonca Imir Yenicesu et al. [2] in their recent studies. Taking into account the results showing that genetic association studies could be affected by the frequency of alleles, evaluating the genetic background of RPL was necessary. The frequency of the E4 allele in patients was higher than in healthy women in every study included (Table 2). When the E4 allele was compared with the E3 allele, the summary OR was 2.111 (95 %CI: 1.090–4.088, $I^2=64.2\%$) and the RR was 1.977

(95 %CI: 1.077–3.631, $I^2=63.4\%$), which showed that E4 allele is a higher risk factor of RPL. The pooled OR was 1.365 (95 %CI: 1.029–1.811, $I^2=0.0\%$) and the RR was 1.326 (95 %CI: 1.021–1.722, $I^2=0.0\%$) for the E2 and E3 alleles, which showed that the E2 allele may be associated with RPL as another risk factor and needed more further research. There was no obvious correlation comparing the E4 allele and E2 allele. In addition, the results of homozygous gene research groups also supported that the Apo E4 polymorphism contributed to recurrent pregnancy loss as a risk factor, as the summary OR was 3.872 (95 %CI: 1.071–13.996, $I^2=0.0\%$); however, the result of comparing E2/E2 homozygotes versus other genotypes was not significant between patients and controls, which did not support the previous result. Furthermore, E3/E3 homozygosity might be a protective factor for RPL: the summary OR was 0.727 (95 %CI: 0.566–0.932, $I^2=0.0\%$) and the RR was 0.923 (95 %CI: 0.867–0.982, $I^2=53.9\%$). In analysis by synthesis, the Apo E4 polymorphisms could contribute to recurrent pregnancy loss as a risk factor, while the Apo E3 polymorphisms could contribute to the disease as a protective factor and the Apo E2 polymorphisms may be associated with RPL as a potential risk factor which should need more research for further discussion.

Among the various studies, we observed different outcomes. A few other studies [1, 19, 24] have analyzed the

prevalence of the Apo E genotypes among the patients experiencing RPL, but all of them concluded that there was no association between Apo E polymorphisms and recurrent pregnancy loss. However, differences in patient selection have been suggested as possible reasons for the different outcomes. On this topic, a heated discussion about the control group selection in some studies has been carried out, for example, regarding selecting controls suffering from cardiovascular disease, where the higher prevalence of Apo E4 was expected. In addition, we only included 6 articles in this meta-analysis and could not rule out the influences of small sample size; therefore, the overall conclusions need many more samples for support.

In addition, we carried out a sensitivity analysis to assess the studies which most affected the results [15] for the E3/E4 group. The results in the article with high relatively heterogeneity, which we removed, showed that the value of I^2 dropped from 53.8 % to 46.4 % with fixed effects. The results were also shown to have a relationship with the RPL (OR=2.249, 95 %CI: 1.474–3.431). The consistent results indicate that the conclusion is stable and reliable. Traced back to the removed article, the differences in the races and samples became the potential causes of heterogeneity.

Limitations

We identified powerful studies and tests in the meta-analysis, which suggests the potential association of the risk of RPL and the Apo E polymorphism. As with all meta-analyses, there are also limitations in this analysis.

(1) According to the inclusion and exclusion criteria, the analyzed studies were broadly similar, but it was not possible to fully eliminate the heterogeneity of these articles. For example, the gestational age used in defining abortion for patients among studies ranges from 10 weeks to the second trimester, which are clearly not the same. Further, the examinations of each group patients with unexplained miscarriage are different, because there are distinctions in medical levels for different countries. In addition, some controls have at least two healthy children, others at least have one normal pregnancy and the other studies included healthy groups who only declared no history of pregnancy loss. At the same time, the maternal ages in selected studies also have some potential differences. Absolutely, the selection bias might expand this factor mentioned above, which would lead to the heterogeneity of the results because of a lack of systemic samples. (2) The studies we included consisted of different races such as Caucasian, Asian and other ethnicities; the genetic heterogeneity among these ethnic populations could provide another important bias for this meta-analytic study, and genetic effects may be decided by different populations through different genetic models. (3) The included studies from the selected databases were limited by English language. Articles published in other

languages, located in other databases or unpublished studies could have been missed.

Conclusions

RPL is one of the most common reproductive problems in humans. It may open a new road to decreasing the incidence of detecting Apo E polymorphisms. Our meta-analysis demonstrates that there is a close association between recurrent pregnancy loss and Apo E gene polymorphisms. It is also worth pointing out that the Apo E4 polymorphism could be considered a risk factor in RPL through the analysis of the present results, the Apo E3 polymorphism may be a protective factor for pregnancy loss and the Apo E2 polymorphism may be another potential factor in the RPL risk. In conclusion, the real association between the Apo E2 polymorphism and RPL risk needs further investigation, especially regarding gene-disease-environment interactions.

Acknowledgement This study was partially supported by grants from the Guangxi Health department (Gui Wei Zhong 2008035).

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