Association between *IL-6* –174 G/C, *IL-6* –634 G/C, and *IFN- γ* +874 A/T polymorphisms and susceptibility to recurrent pregnancy loss: a meta-analysis

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Received: 19 July 2015 / Accepted: 26 August 2015 / Published online: 4 September 2015
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Abstract

Objective The aim of this study was to determine whether interleukin-6 (*IL-6*) –174 G/C, *IL-6* –634 G/C, and interferon- γ (*IFN- γ*) +874 A/T polymorphisms are associated with susceptibility to recurrent pregnancy loss (RPL).

Methods We conducted a literature search using PubMed and EMBASE databases and performed a meta-analysis using fixed- or random-effects models.

Results A total of 15 articles met the study inclusion criteria. When all study subjects were considered together, meta-analysis showed no association between RPL and the *IL-6* –174 GG+GC genotype (odds ratio [OR]=0.794, 95 % confidence interval [CI]=0.542–1.163, $p=0.236$). However, stratification of the data by ethnicity indicated an association between this genotype and RPL in non-Caucasians (OR=0.528, 95 % CI=0.302–0.925, $p=0.028$), but not in Caucasian populations. Moreover, meta-analysis revealed an association between RPL and the *IL-6* –634 GG+GC genotype in all study subjects (OR=0.556, 95 % CI=0.383–0.806, $p=0.002$), while stratification by ethnicity revealed a negative association between this genotype and RPL in Asian (OR=0.545, 95 % CI=0.371–0.800, $p=0.002$) but not Middle Eastern populations. Furthermore, a relationship between the *IFN- γ* +874 A allele and RPL was identified in non-Caucasians (OR=1.403, 95 % CI=1.133–1.734, $p=0.002$), but not in Caucasians.

Capsule Our meta-analysis demonstrates that *IL-6* –174 G/C, –634 G/C, and *IFN- γ* +874 A/T polymorphisms are associated with susceptibility to RPL, particularly in non-Caucasians.

Conclusions This meta-analysis demonstrates that *IL-6* –174 G/C, *IL-6* –634 G/C, and *IFN- γ* +874 A/T polymorphisms are associated with susceptibility to RPL, particularly in non-Caucasians.

Keywords *IL-6* · *IFN- γ* · Polymorphism · RPL · Meta-analysis

Introduction

Recurrent pregnancy loss (RPL) is defined as two or more losses of pregnancies having lasted less than 20 weeks, and involves a multifactorial etiology. Although a number of etiological factors have been identified, the cause of RPL remains unclear in approximately 50 % of cases [1]. These unexplained occurrences may be due to immunological and genetic factors [2].

Pro-inflammatory and anti-inflammatory cytokines are known to be involved in the pathogenesis of RPL [3], and successful pregnancy may be dependent on a balance between T-helper 1 (Th1) and T-helper 2 (Th2) immunity [4]. Specifically, a change of cytokine balance in favor of Th2 cytokines such as interleukin-6 (*IL-6*) is considered essential for maintaining a normal pregnancy [5]. In addition, abnormal immune reactivity in the context of the Th1/Th2 paradigm has been observed in RPL [5] and there is evidence of a diminished Th2 immune response to placental antigens in women with this condition [6]. Trophoblast antigens activate lymphocytes, resulting in the production of embryotoxic cytokines such as interferon- γ (*IFN- γ*), which inhibit embryonic and fetal development [7]. *IL-6*, involved in the inflammatory response and in the modulation of immune responses including B cell and T cell differentiation, functions as both a pro- and anti-inflammatory cytokine [8]. Significantly higher levels of *IL-6*

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are present in healthy pregnant women compared to those with RPL [9], while the opposite is true for IFN- γ [10].

In part, the production of cytokines is under genetic control, and polymorphisms, including those of *IL-6* and *IFN- γ* , are associated with variations in their expression. The *IL-6* gene is located on chromosome 7p21, and of the several known polymorphisms in its promoter region, -174 G/C (rs1800795) and -634 G/C (rs1800796) have been the most frequently studied in RPL. *IL-6* -174 G/C and -634 G/C polymorphisms are functionally significant and are known to exhibit weak linkage disequilibrium. This cytokine is regulated principally at the transcriptional level by regulatory elements in its 5' flanking region [11], within which, the -174 G/C polymorphism acts as an important regulator of transcription [12]. Likewise, a functional polymorphism (rs2430561) of the *IFN- γ* gene located on chromosome 12q24, consisting of an A to T substitution at position +874 from the translation start site in the first intron and coinciding with a putative NF- κ B binding site, is known to increase IFN- γ levels [13]. This polymorphism is correlated with IFN- γ levels, the T allele being associated with increased production [13].

Based on their functional significance, the roles of *IL-6* -174 G/C, *IL-6* -634 G/C, and *IFN- γ* +874 A/T polymorphisms in RPL susceptibility have been examined, with conflicting results [14–28]. This disparity may have been caused by small sample sizes, low statistical power, ethnic variability between studies, and/or clinical heterogeneity. To overcome the limitations of individual studies, resolve inconsistencies, and reduce the likelihood of random errors being responsible for false-positive or false-negative associations, we employed a meta-analysis approach [29–31]. Our aim in this study was to determine whether *IL-6* -174 G/C, *IL-6* -634 G/C, and *IFN- γ* +874 A/T polymorphisms are associated with RPL risk in different ethnicities.

Methods

Identification of eligible studies and data extraction

A literature search was conducted to identify studies having examined associations between *IL-6* or *IFN- γ* polymorphisms and RPL susceptibility. PubMed and EMBASE citation indices

Table 1 Characteristics of the studies included in the meta-analysis

Study	Ethnicity	Polymorphism	Number		RPL			Control			<i>p</i> value for association ^a	HWE
			Case	Control	GG	GC	CC	GG	GC	CC		
Camil, 2014 [14]	Caucasian	<i>IL-6</i> -174 G/C	69	64	60	9	0	56	8	0	0.928	Yes
Demirturk, 2014 [15]	Turkish	<i>IL-6</i> -174 G/C	113	113	72	36	5	100	11	2	4.8×10^{-5}	No
Bahadori, 2014 [16]	Middle Eastern	<i>IL-6</i> -174 G/C	85	104	36	43	6	44	51	9	0.866	Yes
Parveen, 2013 [17]	Asian	<i>IL-6</i> -174 G/C	200	300	120	67	13	223	67	10	4.8×10^{-5}	Yes
von Linsingern, 2005 [18]	Latin American	<i>IL-6</i> -174 G/C	57	74	21	26	10	40	31	3	0.009	Yes
Prigoshin, 2004 [27]	Caucasian	<i>IL-6</i> -174 G/C	38	54	35 ^b		3	49 ^b		5	0.819 ^b	NA
Saijo, 2004 [19]	Asian	<i>IL-6</i> -174 G/C	76	93	76	0	0	93	0	0	1.000	NA
Daher, 2003 [20]	Caucasian	<i>IL-6</i> -174 G/C	44	108	39 ^b		5	99 ^b		9	0.559 ^b	NA
Unfried, 2003 [21]	Caucasian	<i>IL-6</i> -174 G/C	161	124	66	72	23	43	58	23	0.199	Yes
					GG	GC	CC	GG	GC	CC		
Alkjuriji, 2013 [22]	Middle Eastern	<i>IL-6</i> -634 G/C	65	65	53	9	3	54	8	3	0.844	No
Ma, 2011 [23]	Asian	<i>IL-6</i> -634 G/C	162	156	0	46	116	11	52	93	0.002	Yes
El-Hamid, 2011 [24]	Middle Eastern	<i>IL-6</i> -634 G/C	40	30	33	5	2	27	3	0	0.203	Yes
Saijo, 2004 [19]	Asian	<i>IL-6</i> -634 G/C	76	93	18 ^b		58	37 ^b		56	0.028 ^b	NA
					AA	AT	TT	AA	AT	TT		
Zastavna, 2014 [25]	Caucasian	IFN- γ +874 A/T	163	60	56	79	28	15	26	19	0.025	Yes
Parveen, 2013 [17]	Asian	IFN- γ +874 A/T	200	300	85	82	33	160	110	30	0.004	Yes
Kamali, 2005 [26]	Middle Eastern	IFN- γ +874 A/T	132	132	37	63	32	44	65	23	0.162	Yes
Prigoshin, 2004 [27]	Caucasian	IFN- γ +874 A/T	40	53	8	26	6	22	19	12	0.345	Yes
Daher, 2003 [20]	Caucasian	IFN- γ +874 A/T	46	104	16	18	12	39	50	15	0.243	Yes
Babbage, 2001 [28]	Caucasian	IFN- γ +874 A/T	41	63	11	19	11	20	32	11	0.313	Yes

RPL recurrent pregnancy loss, HWE Hardy–Weinberg equilibrium, NA not available

^a Allele contrast

^b GG+GC

were used to locate articles published before June 2015, in which the presence of *IL-6* or *IFN- γ* polymorphisms had been determined in RPL patients and controls. Combinations of keywords such as “interleukin-6,” “interferon,” “polymorphism,” “recurrent miscarriage,” and “recurrent pregnancy loss” were entered as both Medical Subject Headings and Text Words. In addition, all references cited in retrieved articles were reviewed to identify additional studies not indexed by PubMed and EMBASE. Studies were included in the analysis if they met all of the following criteria: (i) they were case–control studies; (ii) they included patients with RPL, which was defined as two or more unexplained pregnancy losses in the first two trimesters of pregnancy; and (iii) they included genotype or allele data concerning *IL-6* –174 G/C, *IL-6* –634 G/C, or *IFN- γ* +874 A/T polymorphisms. No language restriction was applied. The following were excluded: (i) studies containing overlapping data; (ii) those in which genotype or allele data could not be ascertained; (iii) reviews; and (iv) studies in which family members had been included and the analysis was based on linkage considerations. Information relating to the methods and results of the included investigations was extracted by two independent

researchers. Disagreements were resolved by consensus or the adjudication of a third researcher. The following information was extracted from each study: author(s), year of publication, ethnicity of the study population, number of cases and controls, and genotype and allele frequencies of *IL-6* and *IFN- γ* polymorphisms.

Evaluation of statistical associations

We performed meta-analyses using allelic contrast, homozygote contrast, and recessive and dominant models. *IL-6* polymorphism allele frequencies in the relevant studies were determined using the allele counting method. The chi-square test was used to establish whether observed genotype frequencies in the control groups conformed to Hardy–Weinberg equilibrium (HWE). Point estimates of risks, odds ratios (ORs), and 95 % confidence intervals (CIs) were determined for each study, and Cochran’s Q statistic was used to assess within- and between-study variation and heterogeneity [32]. The heterogeneity test was used to assess the probability of the null hypothesis that all studies were evaluating the same effect.

Table 2 Meta-analysis of the association between the *IL-6* –174 G/C and –634 G/C polymorphisms and RPL

Polymorphism	Population	No. of studies	Subject no.		Test of association			Test of heterogeneity		
			Case	Control	OR	95 % CI	<i>p</i> value	Model	<i>p</i> value	<i>I</i> ²
<i>IL-6</i> –174 G/C G vs. C allele	All subjects	6	685	779	0.681	0.436–1.062	0.090	R	0.000	91.1
	Caucasian	2	230	188	1.214	0.881–1.672	0.237	F	0.615	0
	Non-Caucasian	4	455	591	0.550	0.342–0.884	0.214	R	0.005	76.6
GG+GC vs. CC (dominant)	All subjects	7	714	861	0.794	0.542–1.163	0.263	F	0.145	37.1
	Caucasian	3	259	270	1.128	0.670–1.897	0.650	F	0.329	0
	Non-Caucasian	4	455	591	0.528	0.302–0.925	0.026	F	0.200	35.4
GG vs. GC+CC (recessive)	All subjects	6	685	779	0.648	0.392–1.072	0.091	R	0.001	76.5
	Caucasian	2	230	188	1.234	0.796–1.913	0.347	F	0.570	0
	Non-Caucasian	4	455	591	0.504	0.296–0.860	0.012	R	0.016	70.9
GG vs. CC	All subjects	6	685	779	0.698	0.458–1.065	0.096	R	0.011	66.1
	Caucasian	2	230	188	1.062	0.693–1.627	0.784	F	0.818	0
	Non-Caucasian	4	455	591	0.586	0.344–0.996	0.048	R	0.019	69.6
<i>IL-6</i> –634 G/C G vs. C allele	All subjects	3	267	251	0.586	0.414–0.829	0.003	F	0.405	0
	Middle Eastern	2	105	95	0.760	0.388–1.456	0.422	F	0.312	1.65
	Asian	1	162	156	0.532	0.354–0.799	0.002	NA	NA	NA
GG+GC vs. CC (dominant)	All subjects	4	343	344	0.556	0.383–0.806	0.002	F	0.793	0
	Middle Eastern	2	105	95	0.737	0.171–3.131	0.680	F	0.439	0
	Asian	2	238	249	0.545	0.371–0.800	0.002	F	0.598	0
GG vs. GC+CC (recessive)	All subjects	3	267	251	0.632	0.302–1.322	0.223	F	0.113	54.0
	Middle Eastern	2	105	95	0.773	0.360–1.661	0.510	F	0.534	0
	Asian	1	162	156	0.039	0.002–0.665	0.025	NA	NA	NA
GG vs. CC	All subjects	3	267	251	0.386	0.106–1.404	0.148	F	0.131	50.8
	Middle Eastern	2	105	95	0.720	0.169–3.072	0.657	F	0.434	34.0
	Asian	1	162	156	0.035	0.002–0.600	0.021	NA	NA	NA

F fixed-effect model, *R* random-effect model, *NA* not available

When a significant Q statistic ($p < 0.10$) indicated heterogeneity across studies, a random-effects model was used in the meta-analysis, while a fixed-effects model was employed in the absence of such heterogeneity. The fixed-effects model assumes that genetic factors have similar effects on RPL susceptibility across all studies and that observed variations between studies are caused by chance alone [33]. The random-effects model however assumes substantial diversity among different investigations and assesses both within-study sampling errors and between-study variance [34]. We quantified the effect of heterogeneity using I^2 , which is equal to $100 \times (Q - df) / Q$, where “Q” represents Cochran’s Q and “df” the degrees of freedom [35]. This measure ranges between 0 and 100 % and represents the proportion of inter-study variation attributable to heterogeneity rather than chance. I^2 values of 25, 50, and 75 % were defined as low, moderate, and high estimates, respectively. Statistical tests were carried out using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, NJ, USA).

Evaluation of publication bias

Funnel plots are often used to detect publication bias. However, because of the limitations of this method, which requires a range of studies of varying sizes and involves subjective

judgments, publication bias was evaluated using Egger’s linear regression test [36].

Results

Studies included in the meta-analysis

We identified 100 reports using electronic and manual searches and selected 18 for full-text review based on their title and abstract details. Subsequently, three documents were excluded for being review articles [37–39]. Thus, 15 relevant studies met the inclusion criteria [14–28], of which, eight were related to the *IL-6* -174 G/C polymorphism and involved 859 patients and 1018 controls, four concerned the *IL-6* -634 G/C polymorphism and included 343 patients and 340 controls, while six referred to the *IFN-γ* +874 A/T polymorphism and comprised 622 patients and 712 controls (Table 1).

Meta-analysis of the association between *IL-6* -174 G/C and -634 G/C polymorphisms and RPL

A summary of the findings of our meta-analyses concerning the association between *IL-6* -174 G/C and -634 G/C

Fig. 1 Odds ratios (ORs) and 95 % confidence intervals (CIs) from individual studies and pooled data for the association between the GG+GC genotype of the *IL-6* -174 G/C (a) and -634 G/C (b) polymorphisms and RPL in Caucasian and non-Caucasian populations

Table 3 Meta-analysis of the association between the IFN- γ +874 A/T polymorphism and RPL

Polymorphism	Population	No. of studies	Subject no.		Test of association			Test of heterogeneity		
			Case	Control	OR	95 % CI	p value	Model	p value	I ²
IFN- γ +874 T vs. A allele	All subjects	6	622	712	1.188	0.905–1.559	0.214	R	0.029	59.8
	Caucasian	4	290	280	1.073	0.702–1.640	0.746	R	0.041	63.7
	Non-Caucasian	2	459	432	1.403	1.135–1.734	0.002	F	0.499	0
TT+AA vs. AA (dominant)	All subjects	6	622	712	1.318	1.041–1.670	0.022	R	0.155	37.6
	Caucasian	4	290	280	1.190	0.664–2.135	0.559	R	0.089	53.9
	Non-Caucasian	2	459	432	1.457	1.082–1.961	0.013	F	0.567	0
TT vs. TA+AA (recessive)	All subjects	6	622	712	1.200	0.716–2.013	0.489	R	0.013	65.4
	Caucasian	4	290	280	0.981	0.428–2.197	0.962	R	0.017	70.4
	Non-Caucasian	2	459	432	1.658	1.114–2.468	0.013	F	0.697	0
TT vs. AA	All subjects	6	622	712	1.373	0.802–2.351	0.248	R	0.035	58.3
	Caucasian	4	290	280	1.120	0.481–2.610	0.792	R	0.042	63.5
	Non-Caucasian	2	459	432	1.895	1.226–2.928	0.004	F	0.621	0

F fixed-effect model, R random-effect model

polymorphisms and RPL is provided in Table 2. When all study subjects were considered together, no association was found between RPL and the -174 GG+GC genotype (OR=0.794, 95 % CI=0.542–1.163, $p=0.263$). However, stratification of the data by ethnicity revealed an association between this genotype and RPL in non-Caucasian (OR=0.528, 95 % CI=0.302–0.925, $p=0.026$) but not Caucasian study subjects (OR=1.128, 95 % CI=0.670–1.897, $p=0.650$; Fig. 1). Meta-analyses using the recessive model and homozygote contrast identified the same pattern, suggesting that the $IL-6$ -174 G allele protects against the development of RPL, at least in non-Caucasian populations. Our meta-analysis also found an association between RPL and the $IL-6$ -634 GG+GC genotype in all study subjects (OR=0.556, 95 % CI=0.383–0.806, $p=0.002$), while stratification by ethnicity revealed a negative association between this genotype and RPL in Asians (OR=0.545, 95 % CI=0.371–0.800, $p=0.002$), but not Middle Eastern subjects (OR=0.737, 95 % CI=0.171–3.131, $p=0.680$; Fig. 1). Under allelic contrast, the same result was observed

(OR=0.532, 95 % CI=0.354–0.799, $p=0.002$), suggesting that the $IL-6$ -634 G allele reduces the risk of developing RPL in Asian populations.

Meta-analysis of the association between the IFN- γ +874 A/T polymorphism and RPL

No allelic association between the IFN- γ +874 A/T polymorphism and RPL was discerned in the data taken as a whole (OR=1.188, 95 % CI=0.905–1.559, $p=0.214$; Table 3). However, ethnicity-specific meta-analysis indicated an association between the +874 A allele and RPL in non-Caucasians (OR=1.403, 95 % CI=1.135–1.734, $p=0.002$), but not in Caucasians (OR=1.073, 95 % CI=0.702–1.640, $p=0.746$; Fig. 2, Table 3). Use of the recessive model and homozygote contrast resulted in the same pattern, that is, a significant association between the +874 A allele and RPL in non-Caucasian study subjects.

Fig. 2 Odds ratios (ORs) and 95 % confidence intervals (CIs) from individual studies and pooled data for the association between the GG+GC genotype of the IFN- γ +874 A/T polymorphism and RPL in Asian and Middle Eastern populations

Heterogeneity and publication bias

Some between-study heterogeneity was observed in our meta-analyses; however, none was evident under the dominant model in non-Caucasian and Asian subgroups (Tables 2 and 3). In addition, excluding studies containing control groups that deviated from HWE did not affect our results [15, 22]. Funnel plots show no asymmetry, and Egger's regression test showed no evidence of publication bias (Egger's regression p values > 0.1).

Discussion

In this meta-analysis, we combined data from published studies to evaluate genetic associations between *IL-6* -174 G/C, *IL-6* -634 G/C, and *IFN- γ* +874 A/T polymorphisms and RPL. Our results show that *IL-6* -174 G/C and -634 G/C variants are associated with susceptibility to RPL in non-Caucasian and Asian populations, and a negative association was detected between the GG+GC genotypes of these polymorphisms and this disease. In addition, *IFN- γ* +874 A/T polymorphism is associated with RPL risk in non-Caucasian population. The reason behind this disparity between ethnic groups is unclear but may be due in part to the frequency at which these polymorphisms are present in each population. For example, the *IL-6* -174 C allele has not been observed in Japanese subjects [19] and is known to be rare in Chinese and Korean populations [40, 41], while this allele is common in Caucasians. The studies in Korean and Chinese populations did not examine other alleles that may be associated with RPL [40, 41], but the study in Japanese population has shown that women carrying the -634G allele of the *IL-6* gene have a decreased risk of RPL [19]. In addition, the *IL-6* -634 C allele is not widespread in Middle Eastern groups [22] but is prevalent among Asians [23], a population in which the *IFN- γ* +874 AA genotype is also more common compared to other non-Caucasians [17]. The results of our study are consistent with findings regarding the functional significance of *IL-6* and *IFN- γ* polymorphisms [13, 42]. Thus, *IL-6* -174 G/C, *IL-6* -634 G/C, and *IFN- γ* +874 A/T variants may contribute to RPL risk by affecting the expression of their corresponding proteins.

This investigation differs from previous meta-analyses assessing the relationship between the aforementioned polymorphisms and RPL risk performed by Medica et al. [43] and Bombell et al. [44] in several ways. In the present study, an extra nine studies [14–17, 22–25], 1097 RPL patients, and 1192 controls were included, and additional meta-analyses were performed to examine the potential association between the -634 G/C *IL-6* polymorphism and RPL. Moreover, we carried out further ethnicity-specific analysis. Previous meta-analyses have failed to establish an association between the

IL-6 -174 G/C and *IFN- γ* +874 A/T polymorphisms and RPL risk [43, 44]. However, our investigation revealed a relationship between these polymorphisms and RPL in non-Caucasian populations, but not in Caucasians.

The present study has some limitations that should be noted. First, heterogeneity, confounding factors, and publication bias might have distorted the analysis. Although Egger's regression test showed no evidence of publication bias (Egger's regression p values > 0.1), it is possible that it does not have enough power due to the sample size. However, funnel plots show no asymmetry, suggesting no evidence of publication bias. Second, since our ethnicity-specific meta-analyses only included data from Caucasian, Middle Eastern, and Asian patients, our findings may not be applicable beyond these groups. Further studies would be needed to gain an understanding of disease risk associations in other ethnicities. Finally, the number of available studies was too small to draw definitive conclusions regarding the association between the polymorphisms under investigation and RPL, particularly in specific ethnic groups.

In conclusion, this meta-analysis demonstrates that *IL-6* -174 G/C, *IL-6* -634 G/C, and *IFN- γ* +874 A/T polymorphisms are associated with susceptibility to RPL, especially in non-Caucasian populations. The relative importance of these polymorphisms in the development of RPL may thus be dependent on ethnicity. These findings suggest a need for further investigations into the association between *IL-6* and *IFN- γ* polymorphisms and RPL susceptibility in different ethnic groups, with a view to elucidating their roles in the pathogenesis of this disease.

Acknowledgments This research received no specific grant from any public, commercial, or not-for-profit sectors funding agency.

Conflict of interest The authors declare that they have no competing interests.

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