Meta-analysis of the human leukocyte antigen-G (*HLA-G*) 14bp insertion/deletion polymorphism as a risk factor for preeclampsia

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Short Title: Meta-analysis of HLA-G 14bp I/D polymorphism risk for preeclampsia

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Conflicts of interest notification page

1. Noel Pabalan declares no conflict of interest
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4. Ann-Charlotte Iversen declares no conflict of interest

Authors´ contributions

N. Pabalan designed the study, collected data, analyzed data, assessed quality of the studies and wrote the paper

H. Jarjanazi collected and analyzed the data as well as assessed quality of the studies

Chen Sun contributed to design of the study, data analysis and writing of the paper

Ann-Charlotte Iversen designed the study, contributed to data analysis and wrote the paper

Abstract

The non-classical major histocompatibility complex, human leukocyte antigen (HLA)-G, plays an important role in pregnancy. *HLA-G* mediates proper interaction between maternal immune cells and fetal trophoblasts invading the uterine wall, to ensure successful placental development and function. Several *HLA-G* gene variants have been shown to be associated with development of preeclampsia (PE), but the reported associations of the *HLA-G* 14bp (base pair) insertion/deletion (I/D) polymorphism (rs66554220) with PE are inconsistent. In this meta-analysis of *HLA-G* 14bp I/D in each member of the family triad, we estimated risk (odds ratio [OR], 95% confidence interval) of associations with PE based on nine published offspring, nine mother and three father case-control studies.

No significant increased risk associations between PE and *HLA-G* 14bp I/D were detected in any of the family triad members (offspring: OR = 1.08-1.21, P = 0.57-0.74; mothers: OR = 1.11-1.28, P = 0.07-0.44; fathers: OR = 1.09-1.65, P = 0.07-0.70). Of the 20 comparisons performed, 14 (70%) were non-heterogeneous and seven of these had zero heterogeneity (I^2 = 0%). Sensitivity treatment confirmed robustness for the overall lack of association for *HLA-G* 14bp I/D. In subgroup analysis, significant association between *HLA-G* 14bp I/D and PE was shown in offspring from primipara (OR = 1.66-1.95, P = 0.04) and European Caucasian pregnancies (OR = 1.37-2.03, P = 0.02-0.03). However, heterogeneity and sensitivity tests suggest that further investigation is needed to determine if *HLA-G* 14bp I/D is involved in trophoblast *HLA-G* expression and PE development in these subgroups.

Key words: *HLA-G*, meta-analysis, polymorphism, preeclampsia

Introduction

Preeclampsia (PE) is a complex inflammatory disorder that occurs in 5–7% of pregnancies (1), and may have severe outcome for both mother and child. The clinical manifestation of PE is onset of proteinuria and elevated blood pressure in the latter half of the pregnancy. It is generally agreed that the initial stage of PE involves improper placental development in early pregnancy (2). During placenta development, fetal extravillous trophoblasts invade the maternal uterine wall and help transform the uterine spiral arteries into wide, low-resistant arteries to ensure sufficient blood flow to the placenta (2). In preeclamptic pregnancies, trophoblast invasion is reduced and the spiral arteries are not properly remodeled, resulting in a shallow placenta. As the fetus grows and requires a fully functional placenta, the insufficient preeclamptic placenta releases increasing amounts of stress signals to the maternal circulation, eventually leading to systemic inflammation with endothelial dysfunction and the clinical signs of PE in the mother (3).

The fetal extravillous trophoblasts invading the maternal uterine wall are foreign to the maternal immune cells, and to ensure a proper immune balance at the feto-maternal interaction site, extravillous trophoblasts express a specialized set of major histocompatibility complex molecules, including human leukocyte antigen (HLA)-G class Ib (4).

Although the definite functional role has not been established (5), HLA-G expressed by extravillous trophoblasts is involved in optimal trophoblast invasion and spiral artery remodeling, and reduced or aberrant HLA-G expression has been linked to development of PE (6). Recent studies have indicated that the 14 base pair (bp) insertion/deletion (I/D) polymorphism (rs66554220) in the 3-untranslated region of exon 8 of the *HLA-G* gene affects HLA-G transcription (7). This leads to abnormal or decreased HLA-G protein expression and may result in pathological pregnancies (7). Although the genetic mechanisms underlying susceptibility to PE remain unclear, it has been shown that family history increases the risk and that both maternal and paternal/fetal genes contribute to disease development (8, 9). The importance of addressing the genetic contribution of all family triad

members is particularly obvious for investigating a role for HLA-G, which function is mainly assigned to fetal trophoblasts. The *HLA-G* 14bp I/D polymorphism is considered a candidate for PE risk; however, some studies have reported a positive association to PE for both fetal (10-12) and maternal *HLA-G* 14bp I/D genotypes (13-15), while others have shown no association to PE in either family triad member (16-21). The inconsistency of results may be attributed to insufficient statistical power due to small sample size. Meta-analysis is a powerful approach that pools results of independent analyses and can increase statistical power and resolution. Given the conflicting findings in literature, we conducted a meta-analysis of all available data on the *HLA-G* 14bp I/D gene polymorphism in relation to PE risk to investigate a possible genetic association in all members of the family triad. We tested robustness of this association with sensitivity analysis. We also sought to determine the variables that potentially alter this association with modifier analysis using subgroups.

Materials and methods

Selection of studies

Figure 1 outlines the strategy used for literature search following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (22). Using the terms, "*HLA-G* polymorphism", "preeclampsia" and "human leukocyte antigen", we searched MEDLINE using PubMed, EMBASE and Science Direct for association studies in English as of April 8th, 2015. Studies were eligible if they had genotype data with a case-control design addressing PE. The literature search yielded 91 citations, which were subjected to screening and a series of exclusions, resulting in 11 articles for inclusion in the meta-analysis as listed in Table 1 (10, 11, 13-21). Of the 11 included articles, five (11, 14, 16, 20, 21) presented data either for offspring or mother only. However, Vianna *et al*'s maternal data from European and African cohorts were considered as two studies (20). Three articles (10, 18, 19) had data for mother and offspring, thus were considered six studies. Data in three

articles (13, 15, 17) were for the family triads (offspring, mother and father) and were considered as nine studies. Thus the total number of studies in our meta-analysis was nine studies on offspring (10, 11, 13, 15-19, 21), ten studies on mothers (from nine articles) (10, 11, 13-15, 17-20) and three studies on fathers (13, 15, 17).

Data extraction

Two investigators independently extracted data and reached consensus on all items. The following information was obtained from each publication: first author's name, publishing year, country of origin, and *HLA-G* 14bp I/D genotype data of cases and controls. We also calculated frequencies of the variant allele as well as deviations of controls from the Hardy-Weinberg Equilibrium (HWE).

Quality assessment of the studies

Quality of the studies was evaluated with use of the Newcastle–Ottawa Scale (23). Each study was assessed based on three broad perspectives: selection, comparability, and exposure with a score ranging from 0 to 9. Study quality scores for high, medium and poor were ≥7, 4-6 and <4, respectively.

Meta-analysis

Risks of PE for the *HLA-G* 14bp I/D polymorphism were estimated for each study with odds ratio (OR) and 95% confidence interval for association with the variant II (+/+) genotype compared to the wild-type DD (-/-) genotype. To address importance of the *HLA-G* 14bp I/D heterozygous genotype, recessive (II *vs* ID +DD), dominant (II +ID *vs* DD) and co-dominant (I *vs* D) effects were estimated. To compare effects on the same baseline, raw data for genotype frequencies were used to calculate study-specific estimates. Pooled ORs were obtained using either the fixed (24) (in the absence of heterogeneity) or random (25) (in its presence) effects models. Heterogeneity between studies was

estimated using the χ^2-based Q test (26) and quantified with the I^2 statistic which measures degree of inconsistency among studies (27). Given the low power of this test (28), significance threshold was set at $P = 0.10$ (28). Sources of heterogeneity were examined with subgroup analysis (27). The two subgroups examined were: (i) Primipara pregnancies with four offspring studies (13, 16, 18, 19) and four studies on mothers (13, 18-20), and (ii) European Caucasians with four offspring studies (11, 13, 16, 18) and three studies on mothers (13, 18, 20). Sensitivity analysis involving omission of one study at a time and recalculating the pooled OR was used to test for robustness of the summary effects. Data were analyzed using Review Manager 5.3 (Copenhagen: Nordic Cochrane Centre, Cochrane Collaboration, 2014) and SigmaStat 2.3 (Systat Software, San Jose, CA). Significance was set at $P \leq 0.05$ in all calculations except heterogeneity estimation. Publication bias was not investigated because the number of component studies for each triad member was < 10. At this number, qualitative and quantitative tests have low sensitivity (29).

Results

Characteristics of the studies

Table 1 summarizes features of the 11 articles included in the meta-analysis. Four and seven studies were population and hospital-based, respectively. Five of the 11 were matched case-control studies. The Newcastle–Ottawa Scale indicated the following features of the component studies: (i) methodological quality was variable due to the wide range of scores from 3 to 9; (ii) there were more articles of medium quality (n = 6 at score 4-6) than of high quality (n = 4 at score 7-9); and (iii) the mean (6.3 ±1.9) indicated an overall moderate quality of the studies.

Studies in the meta-analysis were categorized according to the family triad; offspring (552 cases/860 controls), mother (831 cases/1,085 controls) and father (242 cases/200 controls) (Table 2).

Table 2 shows frequency of the minor allele which was generally II (+/+) in all studies, except Lin et al (19), Humphrey et al (17) and Quach et al (21), where DD (-/-) seemed to be the minor allele. All control subjects showed expected HWE, except for the offspring data of Zhang et al. (15) and mother data of Jahan et al (14). Subgroup analysis of primipara pregnancies for offspring (156 cases/218 controls) and mothers (239 cases/361 controls) in Table 3 showed expected HWE and that the minor allele was II (+/+) in all groups.

Meta-analysis of risk associations for the *HLA-G* 14bp I/D polymorphism and PE

Table 4 summarizes the risk associations of the *HLA-G* 14bp I/D polymorphism with PE for all members of the family triads. There were no significant association between any genotype of the *HLA-G* 14bp I/D polymorphism and PE for any family triad member; offspring (OR = 1.08-1.21, P = 0.57-0.74), mother (OR = 1.11-1.28, P = 0.07-0.44) and father (OR = 1.09-1.65, P = 0.06-0.70).

In subgroup analysis of offspring *HLA-G* 14bp I/D genotypes (Table 5), significant associations to PE were shown for European Caucasian offspring with homozygous (OR = 2.03, P = 0.02) and co-dominant genotype (OR = 1.37, P = 0.03), and for offspring of primipara pregnancies with homozygous (OR = 1.95, P = 0.04) and dominant genotype (OR = 1.66, P = 0.04). No significant risk associations to PE were observed among mothers in these subgroups or in mothers of African descent (data not shown).

Figure **2** shows the forest plot of the offspring homozygous *HLA-G* 14bp I/D associations and points out a wide confidence interval indicating low precision for the Moreau et al study (11) included in the overall offspring analysis (Figure 2A) and the European Caucasian subgroup analysis (Figure 2B). Figures 2B and 2C further show that the weight percentage contribution of almost half (44-48%) of the offspring subgroup analyses came from the Bermingham et al study (16).

Heterogeneity

Table **4** shows that of the 12 overall comparisons, all four offspring effects (33%) were heterogeneous (I^2 = 50-62%), all in the offspring group. Zero heterogeneity (I^2 = 0%) was observed in three of the four mother effects and in all father effects. Of the offspring subgroup comparisons in Table **5**, two of the eight effects were heterogeneous (I^2 = 64-66%).

Sensitivity analysis

We used sensitivity treatment to explore change in direction of association (increased or decreased risk for PE) and whether non-significant effects became significant resulting from serial omission of included studies (Table 6). In subgroup analysis of offspring *HLA-G* 14bp I/D genotypes, removal of Bermingham et al (16) that had the lowest NOS (Table 1) and Hylenius et al (13) resulted in loss of significant association to PE in both European Caucasian and primipara subgroups, and a number of other omissions (11, 18, 19) also affected the offspring association to PE (Table 6). One study changed the lack of genotype associations in the mothers (20) and no omissions changed the lack of association to PE for the father *HLA-G* 14bp I/D genotypes (Table 6).

Discussion

With a combined sample size of 3,770 (1,625 cases /2,145 controls), this meta-analysis shows robust data for lack of association between the *HLA-G* 14bp I/D polymorphism and development of PE in all members of the family triad. Subgroup analysis indicated significant associations to PE for the offspring of European Caucasian and primipara pregnancies, but heterogeneity and sensitivity analysis clearly revealed less robust data. Therefore, existing studies are not sufficient to conclude any positive associations between the HLA-G 14bp I/D polymorphism and PE.

In the PE pathogenesis it is hypothesized that maternal immune cells do not interact properly with fetal cells in the placenta (2, 3). Studies have demonstrated a role of *HLA-G* in maternal-fetal

immune interaction [reviewed in (5, 6)], and the reduced extravillous cytotrophoblast invasion in placental development in PE has been associated with reduced *HLA-G* expression (30, 31). The 14bp I/D polymorphism in the 3-untranslated region of the *HLA-G* gene is associated to stability and splicing pattern of *HLA-G* mRNA, thus results in modulation of *HLA-G* protein expression with possible clinical significance (32, 33). Since both mother and fetus contribute to PE risk, thereby also involving paternal genes (34), we examined relevance of the *HLA-G* 14bp I/D polymorphism among all family triad members. We compared risk contributions of the family triad in a meta-analysis of several smaller studies with conflicting results. No overall association was detected between the *HLA-G* I/D genotype and development of PE in any of the three family members. This does not contradict the importance of *HLA-G* for proper placental development, but underlines that a relation to PE cannot be explained by this specific gene polymorphism in *HLA-G*. A combined study of other HLA-G gene polymorphisms as performed by Quach et al (21), provides a novel approach to further investigate this important matter.

This meta-analysis should be interpreted within the context of its limitations. First, variable quality of the component studies was determined by the Newcastle–Ottawa Scale. Second, incompatible data disallowed examination of associations by disease severity, although data in the majority (73%) of the included studies were on severe PE (13, 15, 17-21). Two studies (15, 20) presented genotypic data on severity of PE, but did not seem combinable because (a) Zhang et al´s (15) subjects were Han Chinese and data for individual family triad members were not presented, and (b) Vianna et al's (20) subjects were Brazilian. Third, given the multiplicity of comparisons for different genetic models, race and primipara subgroups, with the unavoidable flexibility of choosing and defining the correlates, associations may have been detected by chance alone. Fourth, deviation of two studies (14, 15) from HWE may point to methodological weaknesses, such as biased selection of subjects, genotyping errors and population stratification (35), and may have biased summary outputs. However, this limitation was addressed with sensitivity analysis and the HWE-deviating studies did not

alter any summary effects. Fifth, sensitivity treatment showed compromised robustness of the offspring subgroup effects. Finally, we have calculated that the actual sample size of each triad member used in the meta-analysis are large enough to detect an increased risk for preeclampsia at OR=1,363 for the mothers, and OR=1,412 for the offspring, but for the fathers the sample size was underpowered. The limitation that the father effects were underpowered was countered by the strengths of consistency and statistical homogeneity across the genetic models.

Despite these weaknesses, this meta-analysis has a number of strengths: First, the statistical homogeneity of pooled effects in mothers and fathers indicate comparability of the studies. Second, the lack of risk effects was consistent across the overall genetic models and triads. Third, controls were uniformly defined (i.e. healthy), as was the diagnosis of PE, thus minimizing non-differential misclassification bias. Lastly, we meta-analyzed data from each member of the family triad, which is a strength because PE development may involve father, mother and fetal genetic polymorphisms (36).

The importance of our findings is underlined by the fact that the individual studies included in this meta-analysis did not have adequate statistical power, but when combined using the meta-analysis approach, a clear lack of association to PE for the *HLA-G* 14bp I/D polymorphism was revealed. Likewise, associations with specific *HLA-G* alleles may exist which are due to linkage disequilibrium with other disease modifying alleles and are not reproduced in other populations (7). For these reasons, subgroup analysis of geographically separate populations, as performed here, is a way to overcome population specific factors. This meta-analysis reports results that were not apparent from an examination of the individual studies. We find that meta-analysis is a useful tool in examining broad trends in genetic associations to disease, avoiding possible misleading conclusions based on small single-population studies.

In conclusion, this is to our knowledge the first meta-analysis that addresses association of the *HLA-G* 14bp I/D polymorphism with PE. The results show no association for the *HLA-G* 14bp I/D gene polymorphism with development of PE for any of the triad family members, and underline the

importance of performing larger studies to reveal biologic relevance when only smaller and conflicting findings are available. Our data does not question the role of *HLA-G* in development of PE, but confirms that the *HLA-G* 14bp I/D genotype does not seem to influence PE development. Only for offspring subgroups of Caucasian or primipara pregnancies an association to PE may exist, but this must first be investigated in a focused study of sufficient statistical power.

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Figure 1 Flow diagram for the study selection process

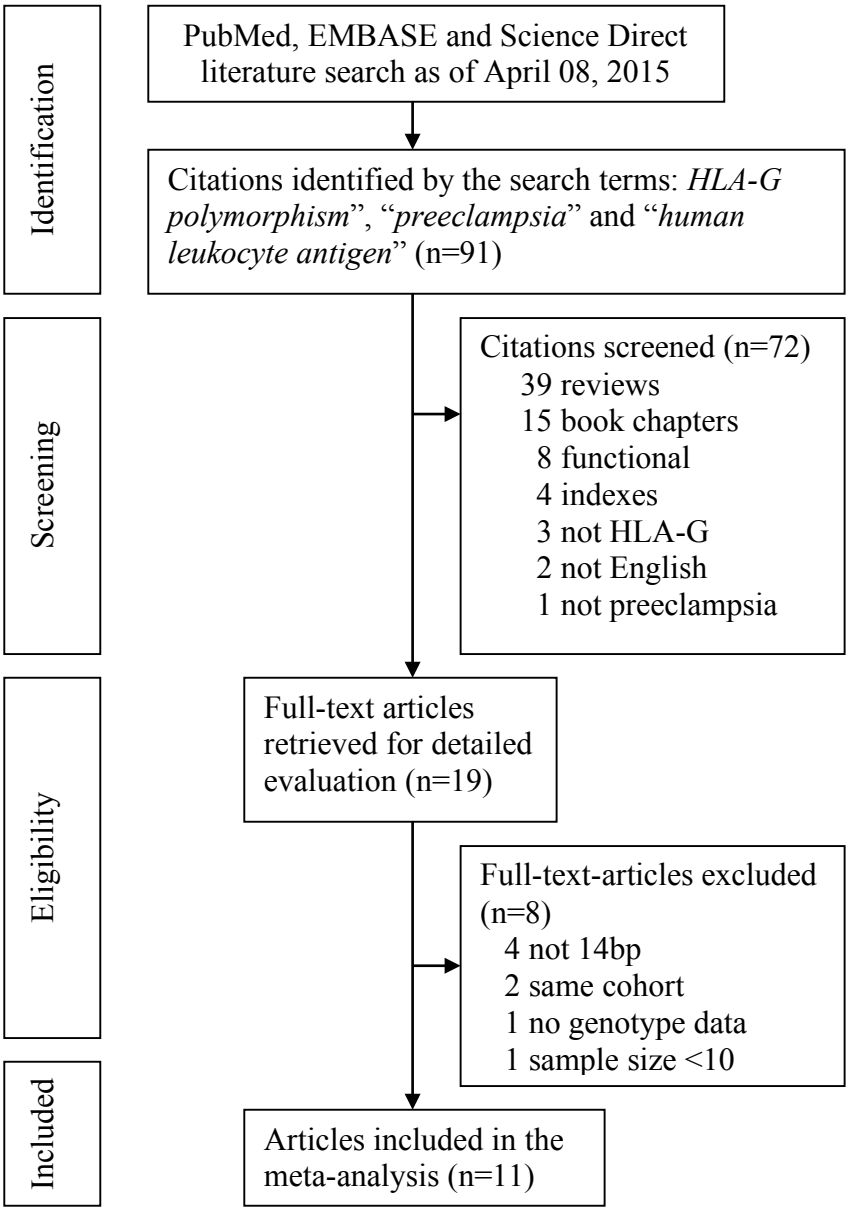
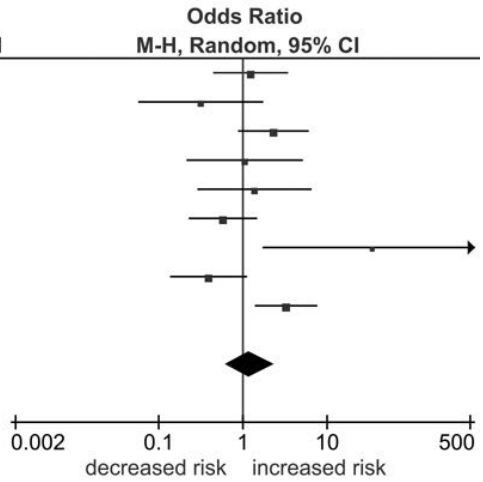


Figure 2 Homozygous *HLA-G* 14bp I/D effects (II [+/+] *vs* DD [-/-]) among offspring. Diamond denotes the pooled odds ratio (OR). Squares indicate the OR in each study, with square sizes directly proportional to the weight contribution (%) of each study. Horizontal lines represent 95% confidence intervals (CI). The Z test for overall effect indicates significance if $P < 0.05$ and the chi-square test indicates heterogeneity thus using the random-effects model if $P < 0.10$, otherwise, the fixed-effects model was used.

A. Overall

Study	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Bermingham 2000	12	24	17	38	13.3%	1.24 [0.44, 3.44]
Humphrey 1995	3	7	19	27	8.5%	0.32 [0.06, 1.75]
Hylenius 2004	13	33	11	50	13.8%	2.30 [0.88, 6.06]
Iversen 2008	6	16	4	11	9.2%	1.05 [0.21, 5.16]
Lin 2006	10	13	17	24	9.4%	1.37 [0.29, 6.54]
Loisel 2013	7	25	57	142	14.1%	0.58 [0.23, 1.48]
Moreau 2008	5	5	8	34	3.9%	34.29 [1.71, 686.32]
Quach 2014	9	24	23	38	13.1%	0.39 [0.14, 1.12]
Zhang 2012	30	98	8	67	14.7%	3.25 [1.38, 7.65]
Total (95% CI)		**245**		**431**	**100.0%**	**1.21 [0.62, 2.36]**
Total events	95		164			

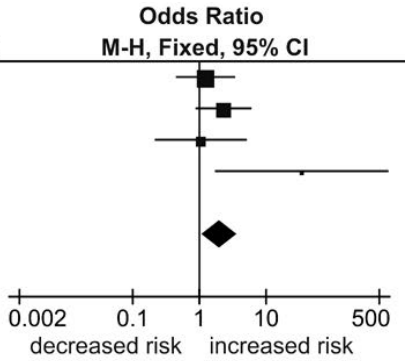
Heterogeneity: Tau² = 0.59; Chi² = 20.97, df = 8 (P = 0.007); I² = 62%
Test for overall effect: Z = 0.57 (P = 0.57)



B. European Caucasians

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Bermingham 2000	12	24	17	38	43.7%	1.24 [0.44, 3.44]
Hylenius 2004	13	33	11	50	35.2%	2.30 [0.88, 6.06]
Iversen 2008	6	16	4	11	19.7%	1.05 [0.21, 5.16]
Moreau 2008	5	5	8	34	1.4%	34.29 [1.71, 686.32]
Total (95% CI)		**78**		**133**	**100.0%**	**2.03 [1.12, 3.69]**
Total events	36		40			

Heterogeneity: Chi² = 5.05, df = 3 (P = 0.17); I² = 41%
Test for overall effect: Z = 2.32 (P = 0.02)



C. Primipara

Study or Subgroup	Case Events	Case Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Bermingham 2000	12	24	17	38	47.9%	1.24 [0.44, 3.44]
Hylenius 2004	12	22	5	36	12.6%	7.44 [2.10, 26.32]
Iversen 2008	3	10	4	11	19.4%	0.75 [0.12, 4.66]
Lin 2006	10	13	17	24	20.1%	1.37 [0.29, 6.54]
Total (95% CI)		**69**		**109**	**100.0%**	**1.95 [1.03, 3.67]**
Total events	37		43			

Heterogeneity: Chi² = 6.32, df = 3 (P = 0.10); I² = 53%
Test for overall effect: Z = 2.06 (P = 0.04)

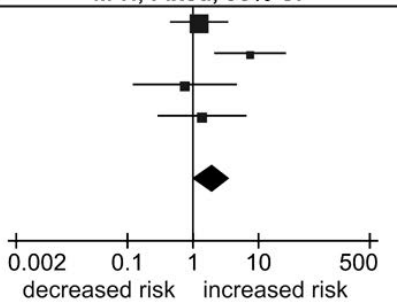


Table 1 Characteristics of the included articles on associations of the *HLA-G* 14bp I/D polymorphism with preeclampsia

First author year (reference)	Country of origin	Study design	N	Studied groups	Controls	DNA Source	Matched cases and controls	NOS
Humphrey 1995 (17)	Australia	PB	3	Triad[1]	Normotensive[3]	blood	no mention	6
Hylenius 2004 (13)	Denmark	PB	3	Triad	healthy	blood	yes	9
Zhang 2012 (15)	China	HB	3	Triad	normal[3]	blood	no mention	6
Lin 2006 (19)	China	HB	2	OM	healthy	blood	yes	6
Iversen 2008 (18)	Norway	HB	2	OM	healthy	blood	yes	8
Loisel 2013 (10)	USA	PB	2	OM	healthy	blood	yes	9
Vianna 2007 (20)	Brazil	HB	2[2]	M	healthy	blood	yes	7
Bermingham 2000 (16)	Ireland	PB	1	O	healthy	blood	no mention	3
Moreau 2008 (11)	France	HB	1	O	normal[4]	placenta	no mention	4
Jahan 2014 (14)	India	HB	1	M	healthy	blood	no mention	5
Quach 2014 (21)	Canada	HB	1	O	non-preeclamptic	placenta	no mention	6

PB, Population-based; HB, Hospital-based; N, number of studies; O, Offspring; M, Mother; DNA, Deoxyribonucleic Acid; NOS, Newcastle-Ottawa Scale. [1] Triad means offspring, mother and father; [2] Vianna et al (20) supplied European-derived and Afro-derived mothers considered as two studies; [3] Humphrey et al (17) described controls as normotensive women; [3] Zhang et al (15) described controls as normal pregnancies; [4] Moreau (11) described controls as normal placentas

Table 2 Genotype frequencies of the *HLA-G* 14bp I/D polymorphism in pregnancies with and without preeclampsia

		Genotypes										
		Sample sizes			DD		DI		II			
First author year (reference)	Ethnicity	Case	Control	Total	Case	Control	Case	Control	Case	Control	maf	HWE
Offspring (552 cases / 860 controls) 95.6*												
Hylenius 2004 (13)	Caucasian	57	98	155	20	39	24	48	13	11	0.36	0.51
Iversen 2008 (18)	Caucasian	29	28	57	10	7	13	17	6	4	0.45	0.23
Bermingham 2000 (16)	Caucasian	70	74	144	12	21	46	36	12	17	0.47	0.84
Moreau 2008 (11)	Caucasian	23	60	83	0	26	18	26	5	8	0.35	0.71
Humphrey 1995 (17)	Caucasian	13	47	60	4	8	6	20	3	19	0.62	0.49
Quach 2014 (21)	No mention	47	68	115	15	23	9	15	30	23	0.56	0.39
Zhang 2012 (15)	Asian	240	158	398	68	59	142	91	30	8	0.34	**0.0003**
Lin 2006 (19)	Asian	26	46	72	3	7	13	22	10	17	0.61	0.98
Loisel 2013 (10)	African-American	47	281	328	18	85	22	139	7	57	0.45	0.99
Mother (831 cases / 1,085 controls) 99.1*												
Hylenius 2004 (13)	Caucasian	57	98	155	17	34	29	47	11	17	0.41	0.91
Iversen 2008 (18)	Caucasian	20	34	54	7	14	11	14	2	6	0.38	0.45
Humphrey 1995 (17)	Caucasian	29	25	54	3	5	19	18	7	12	0.64	0.13
Zhang 2012 (15)	Asian	240	158	398	80	64	132	81	28	13	0.34	0.07
Lin 2006 (19)	Asian	80	144	224	9	18	43	81	28	45	0.59	0.05
Jahan 2014 (14)	Asian	206	206	412	26	30	145	143	35	24	0.49	**0.001**
Vianna 2007 (20)	Euro-derived	105	113	218	35	38	55	63	15	22	0.44	0.64
Vianna 2007 (20)	Afro-derived	50	36	86	17	15	25	16	8	5	0.36	0.83
Loisel 2013 (10)	African-American	44	271	315	13	91	22	132	9	48	0.42	0.99
Father (242 cases / 200 controls) 55.1*												
Hylenius 2004 (13)	Caucasian	57	98	155	18	36	29	48	10	14	0.39	0.75
Humphrey 1995 (17)	Caucasian	21	15	36	5	2	9	10	7	3	0.53	0.19
Zhang 2012 (15)	Asian	164	87	251	54	30	78	47	32	10	0.39	0.19

I/D, insertion/deletion; DD, 14bp deletion/deletion; DI, 14bp deletion/insertion; II, 14bp insertion/insertion; maf, minor allele frequency; HWE, Hardy-Weinberg Equilibrium; Values in **bold** are not in HWE; *statistical power for the combined studies for each member of the triad

Table 3 Genotype frequencies of the *HLA-G* 14bp I/D polymorphism in primipara pregnancies with and without preeclampsia

		Genotypes							
		DD		DI		II			
First author year (reference)	Ethnicity	Case	Control	Case	Control	Case	Control	maf	HWE
Primipara Offspring (156 cases / 218 controls)									
Hylenius 2004 (13)	Caucasian	10	31	18	34	12	5	0.31	0.29
Iversen 2008 (18)	Caucasian	7	7	10	17	3	4	0.45	0.23
Bermingham 2000 (16)	Caucasian	12	21	46	36	12	17	0.47	0.84
Lin 2006 (19)	Asian	3	7	13	22	10	17	0.39	0.98
Primipara Mother (239 cases / 361 controls)									
Hylenius 2004 (13)	Caucasian	12	29	20	28	8	13	0.39	0.19
Iversen 2008 (18)	Caucasian	6	14	6	14	2	6	0.38	0.45
Vianna 2007 (20)	Euro-derived	35	38	55	63	15	22	0.44	0.64
Vianna 2007 (20)	Afro-derived	17	15	25	16	8	5	0.36	0.83
Lin 2006 (19)	Asian	9	18	43	81	28	45	0.41	0.05

DD, 14bp deletion/deletion; DI, 14bp deletion/insertion; II, 14bp insertion/insertion; maf, minor allele frequency, HWE, Hardy-Weinberg Equilibrium

Table 4 The *HLA-G* 14bp I/D polymorphism risk association with preeclampsia

	Sample size		Test of association			Test of heterogeneity			
Genetic model	Cases (%)	Controls (%)	OR	95% CI	P^a	χ^2	P^b	I^2 (%)	Analysis Model
Offspring (n = 9)									
Homozygous	95/245 (38.8)	164/431 (38.1)	1.21	0.62-2.36	0.57	20.97	0.007	62	R
Recessive	95/552 (17.2)	164/860 (19.1)	1.08	0.68-1.73	0.74	15.97	0.04	50	R
Dominant	402/552 (72.8)	593/860 (69.0)	1.08	0.71-1.65	0.72	16.50	0.04	52	R
Codominant	497/1,104 (45.0)	757/1,720 (44.0)	1.08	0.81-1.44	0.61	21.32	0.006	62	R
Mother (n = 9)									
Homozygous	143/350 (40.9)	192/501 (38.3)	1.28	0.93-1.75	0.13	3.56	0.89	0	F
Recessive	143/831 (17.2)	192/1085 (17.7)	1.11	0.86-1.43	0.44	8.5	0.39	6	F
Dominant	624/831 (75.1)	777/1085 (71.6)	1.23	0.99-1.54	0.07	6.17	0.63	0	F
Codominant	767/1662 (46.1)	969/2172 (44.6)	1.11	0.97-1.27	0.13	3.5	0.9	0	F
Father (n = 3)									
Homozygous	49/126 (38.9)	27/95 (28.4)	1.55	0.85-2.86	0.16	0.35	0.84	0	F
Recessive	49/242 (20.2)	27/200 (13.5)	1.65	0.96-2.82	0.07	0.48	0.79	0	F
Dominant	165/242 (68.2)	132/200 (66.0)	1.09	0.72-1.65	0.70	0.92	0.63	0	F
Codominant	214/484 (44.2)	210/544 (38.6)	1.30	0.99-1.70	0.06	1.56	0.46	0	F

Odds ratio (OR) and 95% confidence interval (CI) of association with the variant II (+/+) genotype compared with the wild-type DD (-/-) genotype. Recessive (II *vs* ID+DD), dominant (II ID *vs* DD) and co-dominant (I *vs* D) effects were also assessed. N, number of studies; P^a, $P \leq 0.05$; P^b, P ≤ 0.10

Table 5 Subgroup offspring summary effects for *HLA-G* 14bp I/D polymorphism and preeclampsia

	Sample size		Test of association			Test of heterogeneity			
Genetic model	Cases (%)	Controls (%)	OR	95% CI	P^a	χ^2	P^b	I^2 (%)	Analysis Model
European Caucasian (n = 4)									
Homozygous	36/78 (46.2)	40/133 (30.0)	**2.03**	**1.12-3.69**	**0.02**	5.05	0.17	41	F
Recessive	36/179 (20.1)	40/260 (15.4)	1.33	0.81-2.19	0.26	4.25	0.24	30	F
Dominant	137/179 (76.5)	167/260 (64.2)	1.57	0.64-3.82	0.32	8.36	0.04	64	R
Codominant	173/358 (48.3)	207/520 (39.8)	**1.37**	**1.04-1.80**	**0.03**	6.13	0.11	51	F
Primipara (n = 4)									
Homozygous	37/69 (53.6)	43/109 (39.4)	**1.95**	**1.03-3.67**	**0.04**	6.32	0.1	53	F
Recessive	37/156 (23.7)	43/218 (19.7)	1.41	0.55-3.61	0.47	8.78	0.03	66	R
Dominant	124/156 (79.5)	152/218 (69.7)	**1.66**	**1.02-2.72**	**0.04**	3.24	0.36	8	F
Codominant	161/312 (51.6)	195/436 (44.7)	1.33	0.99-1.78	0.06	6.33	0.1	53	F

Odds ratio (OR) and 95% confidence interval (CI) of association with the variant II (+/+) genotype compared with the wild-type DD (-/-) genotype. Recessive (II *vs* ID+DD), dominant (II+ID *vs* DD) and co-dominant (I *vs* D) effects were also assessed. N, number of studies; Values in **bold** show significant association to preeclampsia; P^a, $P \leq 0.05$; P^b, $P \leq 0.10$; R, random-effects model; F, fixed-effects model

Table 6 Sensitivity analysis for the studies that resulted in gain or loss of significance after omission

Original summary effects					Resulting pooled OR				
GM	OR	95% CI	P^a	NOA	Omitted study (reference)	OR	95% CI	P^a	Effect of study omission
Offspring Primipara									
H	1.95	1.03-3.67	0.04	SIR	Hylenius (13)	1.16	0.54-2.51	0.71	loss of significance
					Iversen (18)	2.32	0.72-7.51	0.16	
					Bermingham (16)	2.20	0.54-9.07	0.27	
					Lin (19)	2.03	0.53-7.80	0.30	
Offspring European Caucasian									
H	2.03	1.12-3.69	0.02	SIR	Hylenius (13)	2.10	0.47-9.50	0.33	loss of significance
					Moreau (11)	1.58	0.83-3.00	0.16	
R	1.33	0.81-2.19	0.26	P >0.05	Bermingham (16)	2.00	1.06-3.79	0.03	gain in significance
C	1.37	1.04-1.80	0.03	SIR	Bermingham (16)	1.56	0.88-2.76	0.13	loss of significance
					Hylenius (13)	1.42	0.75-2.68	0.28	
					Iversen (18)	1.55	0.96-2.53	0.08	
					Moreau (11)	1.19	0.88-1.61	0.26	
Mother Overall									
H	1.28	0.93-1.75	0.13	P >0.05	Vianna (20)	1.41	1.00-2.00	0.05	gain in significance
D	1.23	0.99-1.54	0.07			1.37	1.07-1.74	0.01	

GM: genetic model; D: dominant (II+ID *vs* DD); C: co-dominant (I *vs* D); H: homozygous (II [+/+] *vs* DD [-/-]); R; recessive (II *vs* ID+DD); OR: odds ratio; CI: confidence interval; NOA: nature of association; SIR: significant increased risk; P^a: $P \leq 0.05$