



Polymorphisms and Plasma Level of Transforming Growth Factor-Beta 1 and Risk for Preeclampsia: A Systematic Review

Xun Li, Lin Shen, Hongzhan Tan*

Department of Epidemiology and Health Statistics, School of Public Health, Central South University, Changsha, Hunan, China

Abstract

Background: Transforming growth factor-beta 1 (TGF- β 1) is thought to be involved in the pathogenesis of preeclampsia (PE), but the results are inconsistent among studies. This article aims to compile an overview of the studies about the associations of TGF- β 1 polymorphism and plasma level with PE risk and to provide recommendations for future research.

Methods and Results: The databases PubMed, Embase and Web of Science were searched up to December 2013. Five studies investigating the associations of four polymorphisms with the risks of PE were involved. A meta-analysis was conducted for the 869T>C polymorphism and PE risk. The results show that genotype TT of 869T>C polymorphism is a protective factor of PE (pooled odds ratio = 0.73, 95% CI: 0.56, 0.95). Eight case-control studies reported the plasma level of TGF- β 1. The substantial heterogeneity among studies may be attributed to the differences in the blood sample processing and the TGF- β 1 analysis kits. The results suggest that plasma TGF- β 1 level in the second trimester was significantly lower in the PE group than in the normal pregnancy group, but was significantly higher in the PE group during the third trimester.

Conclusions: The current results support that the TGF- β 1 869 T>C polymorphism was associated with the risk of PE. However, the number of eligible studies is small and more studies are needed to clarify whether this association can be detected on larger sample sizes and different populations. Owing to the heterogeneity between studies, no conclusion on the association between plasma TGF- β 1 level and PE risk can be drawn from this review. Further studies about the TGF- β 1 levels at different stages of pregnancy and the development of TGF- β 1 assay methodology are required to reveal the role of TGF- β 1 in the pathological development of PE.

Citation: Li X, Shen L, Tan H (2014) Polymorphisms and Plasma Level of Transforming Growth Factor-Beta 1 and Risk for Preeclampsia: A Systematic Review. PLoS ONE 9(5): e97230. doi:10.1371/journal.pone.0097230

Editor: Colette Kanellopoulos-Langevin, Xavier Bichat Medical School, INSERM-CNRS - Université Paris Diderot, France

Received: January 8, 2014; **Accepted:** April 16, 2014; **Published:** May 13, 2014

Copyright: © 2014 Li et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by Hunan Provincial Innovation Foundation for Postgraduate (CX2013B098) awarded to the first author. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: tanhz99@qq.com

Introduction

Preeclampsia (PE), defined as the presence of hypertension accompanied by proteinuria first appearing after 20 weeks of gestation, is a major cause of maternal and perinatal morbidity and mortality [1]. PE affects approximately 5%–8% of all pregnancies [2]. The pathophysiology of PE is not fully understood despite great efforts. The challenge to prediction, prevention and management of PE is still unsolved.

PE is characterized by abnormal vascular response to placentation that is associated with increased systematic vascular resistance, enhanced platelet aggregation, activation of the coagulation system and endothelial cell dysfunction [3]. Transforming growth factor (TGF)- β 1 is a multifunctional cytokine involved in the regulation of trophoblast invasion, proliferation, and differentiation [4,5]. Growing evidence indicates that TGF- β 1 can be involved in the pathogenesis of PE, possibly through activation of an endothelial cell pathway [6] or regulation of systemic inflammation [7,8]. Several genetic variants (869 T>C, 509 C>T, 800G>A and 915 G>C) related to the expression level

of TGF- β 1 have been investigated for their possible association with risk of PE, but the results are inconsistent among studies [9–16]. The lack of reproducibility of genetic association studies is probably due to population diversity, small sample sizes in individual studies, or false-negative results with inadequate statistical power [17,18]. The association between the plasma TGF- β 1 level and the risk of PE has been reported. Plasma TGF- β 1 level is significantly higher or lower in PE patients compared with normotensive people [19–30]. Many factors can affect the reported results of plasma TGF- β 1 levels, such as the assay methodology and the gestational age at sampling. Considering the insufficient evidence and inconsistent results about the association of genetic variants of TGF- β 1 and the plasma TGF levels with PE risks, a meta-analysis or systematic review is important and necessary to assess the association. The aims of this study are to overview the association studies of TGF- β 1 polymorphism and plasma levels with risk of PE, and to provide recommendations for future research.

Methods

Literature Search

The databases PubMed, Embase and Web of Knowledge were searched up to December 2013 for studies evaluating TGF- β 1 gene polymorphisms in PE patients. The following keywords were used: (preeclampsia OR pre-eclampsia) AND (polymorphism OR variant) AND (transforming growth factor-beta 1 OR TGF beta 1 OR TGF β 1). In addition, the names of specific polymorphisms were combined with the topic “Preeclampsia”. Online databases were also searched systematically to find studies that examined the relationship between plasma TGF- β 1 level and PE risks. The following keywords were used: (preeclampsia OR pre-eclampsia) AND (transforming growth factor-beta 1 OR TGF beta 1 OR TGF β 1) AND (plasma OR level OR concentration). All reference lists from the main reports and relevant reviews were hand-searched for additional eligible studies.

Eligible Studies and Data Extraction

Studies to be included into this systematic review and meta-analysis should meet the following criteria: (1) studies investigated the relationship between PE and at least one genetic variant of TGF- β 1; or for the systematic review of TGF-beta 1 plasma concentration and risk of PE, studies included sufficient data for determining TGF- β 1 plasma level; (2) case-control studies with a case group of PE women and a control group of healthy women with uncomplicated pregnancies; (3) PE was defined as elevated blood pressure (with systolic blood pressure \geq 140 mmHg and/or diastolic blood pressure \geq 90 mmHg) accompanied with proteinuria measured in at least a semi-quantitative way (\geq 300 mg in a 24-hr urine collection and/or \geq 1+ on dipstick testing), in line with International Society for Study of Hypertension in Pregnancy criteria [31]; (4) original papers contained independent data. The studies without essential information or with overlapped data were excluded.

Data were extracted independently by two reviewers in consultation with a third reviewer. For each included study, the following information was extracted: first author, year of publication, study population (country and ethnicity), and numbers of patients and controls. For genetic association studies, information was extracted from the frequency of genotypes, Hardy-Weinberg equilibrium status and genotyping methods. For studies investigating plasma TGF- β 1 level, information was extracted from method of plasma TGF- β 1 measurement, and means and standard deviation (SD) of TGF- β 1 level in each group. If standard error of mean (SEM) was reported, SD was calculated as $SD = SEM \times \sqrt{n}$.

Statistical Methods

A meta-analysis was performed for any genetic variant that was significantly associated with PE in at least one study and has available data from at least two independent studies. Frequency of the genotypes and alleles between the PE group and the control group were compared using chi-square or Fisher exact test. The association between the polymorphism and risk of PE was estimated by calculating the pooled odds ratio (OR) and 95% confidence interval (CI), according to a general genetic model, a dominant genetic model, a recessive genetic model and an allelic model [32–34]. The significance of the pooled OR was determined using Z test, and $p < 0.05$ was considered statistically significant.

Heterogeneity among studies was assessed using Q-test and I^2 statistic [35,36]. CIs for I^2 were also calculated [37]. For Q-test, $p < 0.1$ was considered significant. With the presence of substantial

heterogeneity (Q-test, $p < 0.1$, and $I^2 > 50\%$), the random effect model was used as the pooling method, and otherwise, the fixed effect model was used. Compliance with the Hardy-Weinberg Equilibrium (HWE) among the controls in each study was checked using Chi-square test. An assessment of publication bias was planned if more than seven studies were included using funnel plot and Begg's test; $p < 0.05$ was considered as representative of statistically significant. All analyses were performed on STATA 12.0 (Stata Corporation, College Station, TX).

A meta-analysis was planned for the association studies of plasma TGF- β 1 level and PE. However, substantial heterogeneity was found among studies and therefore only a qualitative systematic review was conducted.

Results

Main Characteristics of All the Available Studies

Our literature search identified five eligible studies for the association between TGF- β 1 polymorphisms and PE risk, with a total of 608 cases and 758 controls concerning four genetic variants of TGF- β 1. Kim *et al.* (2009) [9] investigated two variants (869 T>C and 915 G>C); Aguilar-Duran *et al.* (2013) [12] investigated three variants (869 T>C, 800 G>A, and 509 C>T); Stanczuk *et al.* (2007) [13] investigated two variants (869 T>C and 915 G>C); Lima *et al.* (2009) [15] investigated two variants (869 T>C and 915 G>C); and Feizollahzadeh *et al.* (2012) [10] investigated two variants (800 G>A and 509 C>T). Therefore, 869 T>C was reported in four studies, 915 G>C in three, 800 G>A in two, and 509 C>T in two. The detailed characteristics of the included studies are shown in Table 1. Among the four genetic variants, only 869 T>C was found significantly associated with PE in at least one study and provided repeated studies for estimation of pooled OR, therefore only one genetic variant was eligible for the meta-analysis. The selection of studies is shown in Figure 1 (a).

For the association between plasma TGF- β 1 level and PE risk, eight case-control studies with a total of 627 cases and 650 controls were included (Table 2). The process of study selection is shown in Figure 1 (b).

Association between 869 T>C Polymorphisms and PE Risk

The meta-analysis for 869 T>C included four studies with a total of 466 cases and 618 controls. Significant associations were observed in genetic models for TT vs. TC (OR = 0.74, 95% CI [0.56, 0.99]), TT vs. CC (OR = 0.70, 95% CI [0.49, 0.99]), in the dominant genetic model (TT vs. (TC+CC)) (OR = 0.73, 95% CI [0.56, 0.95]), under the fixed effects model (Table 3, Figure 2). Publication bias may not be detected owing to the small number of available studies. A sensitivity analysis was performed by omitting one study at a time and then calculating the pooled OR of the remaining studies. After the study of Kim *et al.* (2009) [9] or Aguilar-Duran *et al.* (2013) [12] was omitted, the association between 869 T>C and PE became insignificant, suggesting that those two studies largely affected the combined results (results not shown).

Association between TGF- β 1 Level and PE Risk

Plasma TGF- β 1 levels and risk of PE were reported in eight studies, of which two studies were sampled in the second-trimester, five in the third-trimester, and one was after delivery (Table 2).

The two studies measuring TGF- β 1 levels during the second trimester showed that plasma TGF- β 1 levels were significantly lower in the PE group (3.2 and 2.9 ng/mL) than in the normal pregnancy group (5.3 and 4.7 ng/mL) ($p < 0.05$ for each study).

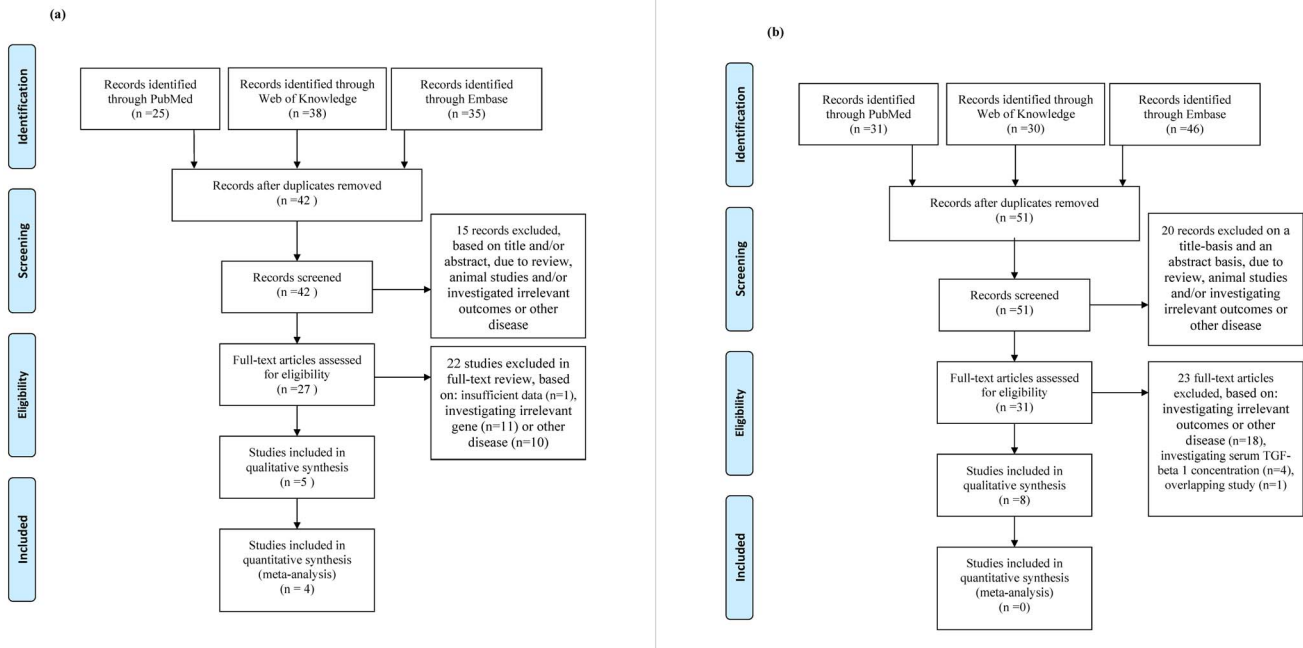


Figure 1. PRISMA flow diagram of study selection process (a) articles involving TGF-beta 1 genetic variants and (b) articles involving plasma concentration of TGF-beta1.
doi:10.1371/journal.pone.0097230.g001

One of them (Clausen *et al.* 2002) estimated the PE risk by calculating OR and showed that during the second trimester, women with the highest levels experienced a decreased risk of PE compared to those with the lowest quartile of level (OR = 0.2, 95% CI [0.03, 0.7]).

The five studies measuring the TGF-β 1 levels during the third trimester all showed that plasma TGF-β 1 levels were significantly higher in the PE group than in the normal group. Of them, two studies reported PE risk according to upper quartile versus lower quartile of third-trimester plasma TGF-β 1 (OR = 7.2, 95% CI [2.2, 23.8] and OR = 2.5, 95% CI [1.2, 5.6]);

In the study reporting the after-delivery plasma TGF-β 1 level [21], maternal plasma TGF-beta 1 concentrations were higher in cases than in controls, falls just short of standard levels of statistical significance (23.2±1.3 versus 20.3±1.0 ng/ml, *p* = 0.06; OR = 2.5, 95% CI [1.2, 5.6], upper quartile versus lower quartile).

Discussion

Main Findings

Our meta-analysis shows that TGF-β 1 869 T>C was associated with PE and TT genotype of 869T>C polymorphism was a protective factor of PE. The association between other three TGE-β 1 variants (915 G>C, 800G>A and 509 C>T) and PE risk was not significant in anystudy. Plasma TGF-β 1 level in second trimester was found significantly lower in the PE group, but this association was altered in the third trimester.

Interpretation

TGF-β family is a member of the TGF superfamily which is a collection of structurally-related multifunctional cytokines. TGF-β has three isoforms: TGF-β 1, 2, and 3. Since the first description in 1983 [38], TGF-β 1 has been suggested to be associated with a wide range of pathophysiological processes, including apoptosis of vascular endothelial cells, immune adaptation, as well as

embryonic growth and development[39–41]. PE is pathophysiologically characterized by endothelial cell damage and dysfunction [42]. A hypothesis indicates that one or more factors released from the placenta may lead to endothelial activation and a multi-systematic disorder [25,43]. In searching for those triggers, a possible mechanistic pathway of endothelial cell activation by TGF-β 1 has been described [6]. Another hypothesis proposed that endothelial dysfunction is a part of the intravascular inflammatory reaction and PE is actually an excessive maternal inflammatory response to pregnancy [44]. The balance between regulatory T cells (Tregs) and Th17 cells is thought to be important in the development of maternal systemic inflammation in PE patients [7,8,45]. Tregs can induce tolerance [46,47] and Th17 cells induce inflammation or rejection [48]. The prevalence of peripheral Tregs is decreased following PE [49–51] but that of Th17 cells is increased [7,52] compared to healthy pregnant women. Since TGF can induce the differentiation of Tregs [53] and inhibit that of Th17 cells [54], the increased Th17/Treg ratio in PE patients is possibly mediated by down regulation TGF-β signaling [7]. Thus, TGF-β becomes a candidate factor that is potentially relevant to the origin of PE.

The coding region of TGF-β 1 gene is located on chromosome 19q13, and contains seven exons and six introns [4,9]. Several polymorphisms in the TGF-β 1 gene have been reported. 869 T>C, 509 C>T and 915 G>C are significantly associated with higher, and 800G>A with lower, circulation levels of TGF-β 1 [55–57]. TGF 915 G>C polymorphism is related to left bentricular hypertrophy in hypertensives [58]. 896 T>C is associated with essential hypertension in Chinese [59]. Those observations indicate that polymorphisms in the TGF-β 1 gene may be involved in the pathological development of hypertension. Our meta-analysis suggests that the 869 T>C polymorphism is associated with risk of PE. However, the number of included studies is small. The results of sensitivity analysis showed that this significant association was largely affected by two studies (Kim

Table 1. Detailed characteristics of all eligible studies for the association with TGF gene polymorphisms and PE.

Gene polymorphisms	Author	Year	Country	Case	Control	Genotype*	Control	P for HWE
869 T>C	Kim ^a [9]	2009	Korean	164	182	Case	80/78/24	0.47
	Aguilar-Duran ^a [12]	2013	Mexico	175	253	Case	64/120/69	0.68
	Stanczuk ^b [13]	2007	Zimbabwe	49	86	Case	34/35/17	0.16
	Lima ^b [15]	2009	Brazilian	78	97	Case	25/56/16	0.10
915 G>C	Stanczuk ^b [13]	2007	Zimbabwe	44	76	Case	66/10/0	0.54
	Lima ^b [15]	2009	Brazilian	78	97	Case	82/15/0	0.41
800 G>A	Feizollahzadeh ^b [10]	2012	Iranian	142	140	Case	98/40/2	0.35
	Aguilar-Duran ^b [12]	2013	Mexico	175	253	Case	234/18/1	0.32
	Feizollahzadeh ^b [10]	2012	Iranian	142	140	Case	35/62/43	0.19
509 C>T	Aguilar-Duran ^b [12]	2013	Mexico	175	253	Case	56/126/71	0.97

*Genotype for 869 T>C, TT/TC/CC; 915 G>C, GG/GC/CC; 800 G>A, GG/GA/AA; 509 C>T, CC/CT/TT.

^aSignificant differences was found in allelic frequencies between preeclamptic and control groups.

^bNo significant differences was found in allelic frequencies between preeclamptic and control groups.
doi:10.1371/journal.pone.0097230.t001

Table 2. Characteristics of studies on plasma TGF-beta 1 levels and PE included in the systematic review.

Study	Year	Country	Cases	Control	Gestational stage by sampling	Test	TGF (ng/ml) ^a		P-value
							PE	Control	
Clausen [30]	2002	Caucasian	71	71	Second trimester	ELISA-Quantikine kit-R&D Systems	3.2 (2.0–6.1)	5.3 (3.8–7.1)	0.01
Lim [23]	2009	Korean	60	124	Second trimester	ELISA-Quantikine kit-R&D Systems	2.9±1.3	4.7±2.4	<0.001
Djurovic [25]	1997	Norway	154	76	Third trimester	ELISA-Quantikine kit-R&D Systems	5.63±1.68	4.67±1.33	0.000
Naicker [22]	2002	African	42	30	Third trimester	MEDGENIX- ELISA- Biosource Europe SA	4.9±0.9	1.9±0.54	<0.0001
Madazli [28]	2003	Turkey	35	33	Third trimester	EIA-DRG Instruments GmbH, Germany	0.041±0.005	0.018±0.001	<0.001
Muy-Rivera [21]	2004	Peru	100	100	Third trimester	ELISA-Quantikine kit-R&D Systems	18.4±1.0	13.0±0.8	<0.001
Peracoli [24]	2008	Brazil	33	36	Third trimester	ELISA-Quantikine kit-R&D Systems	10.41±2.07	7.01±3.29	<0.05
Enquobahrie [27]	2005	Africa	132	180	After delivery	ELISA-Quantikine kit-R&D Systems	23.2±1.3	20.3±1.0	0.06

^aData are presented as mean±standard deviation or median (25–75 percentile).
doi:10.1371/journal.pone.0097230.t002

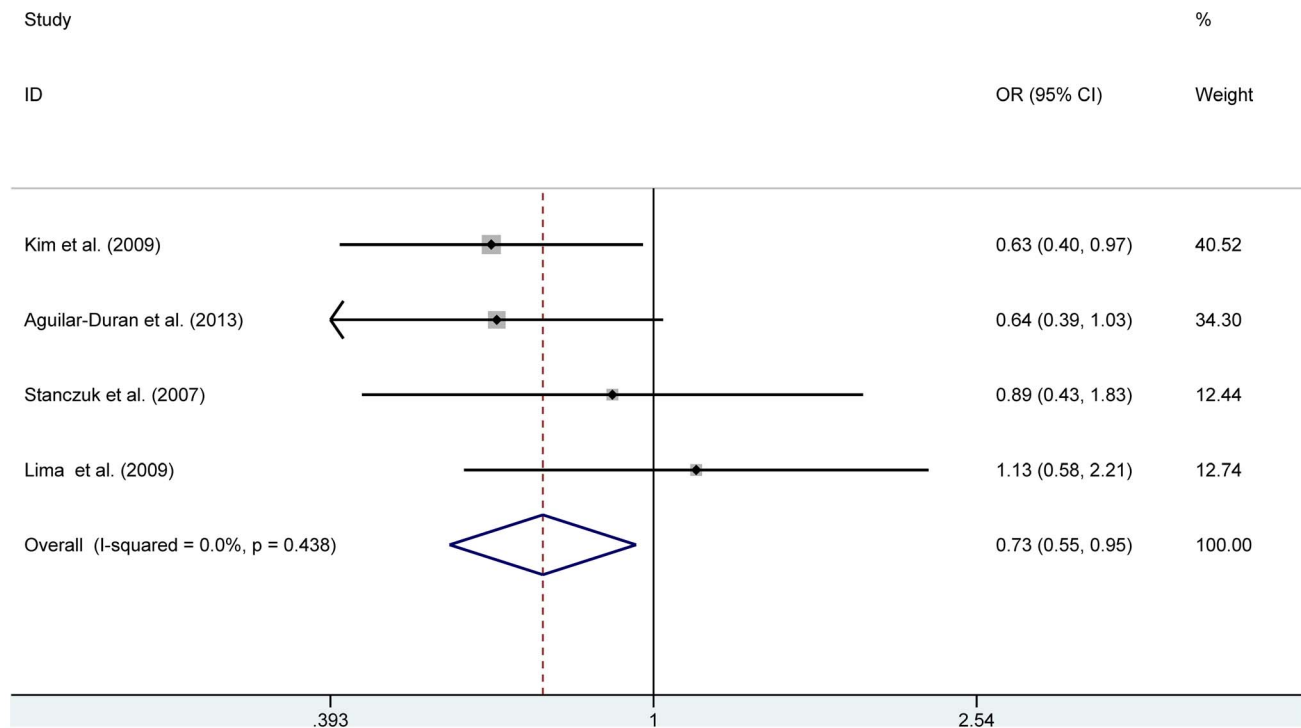


Figure 2. Meta-analysis for the association of 869 T>C polymorphism with PE risk under a dominant model (TT vs. (TC+CC)) using a fixed-effects model.

doi:10.1371/journal.pone.0097230.g002

et al. and Aguilar-Duran *et al.*), and therefore, the results of this meta-analysis should be interpreted with caution. Considering the small sample sizes and the relatively small numbers of studies on 509 C>T, 915 G>C and 800 G>A and risk of PE, the insignificant results do not absolutely rule out the presence of real associations with those single nucleotide polymorphisms (SNPs) and PE.

The identification of PE susceptible variants can provide new insight into its etiology. Moreover, it is an important step to individualize treatment and prevention programs according to the genetic profiles and/or clinical manifestation. The search for susceptible genes has led to an increased number of published studies associating genetic factors with PE. However, attempts to replicate these findings yielded inconsistent results. In a meta-analysis including 192 genetic association studies, 25 replicated genetic variants were identified [60]. Another meta-analysis identified 542 genetic association studies and included 22 independent meta-analyses [61]. But both studies did not include TGF- β 1 gene in the meta-analysis. Our results showed that the missed 869 T>C gene was significantly associated with risk of PE.

The reported mean plasma TGF- β 1 level ranged from 18 pg/mL to 20.3 ng/mL in normal pregnancy women. This considerable variability may be due to the differences in procedures for obtaining and processing blood samples, in the TGF- β 1 analysis kits, as well as in population characteristics. Platelet is a rich source of TGF- β 1, and platelet degranulation could happen during plasma preparation, leading to overestimation of the TGF plasma level [62]. Two included studies [22,24] used platelet-depleted plasma and others did not indicate whether platelet was depleted. Leukocytes also contain a large amount of TGF- β 1 and it can be speculated that during plasma preparation, leukocytes secrete TGF- β 1 into the plasma [63]. Therefore, different procedures for plasma preparation between studies are a potential source of

variation in reported TGF- β 1 levels. The assay methodology could be another important source of variation. Difficulties of TGF- β 1 measurement in complex biological fluids were discussed in detail in the review of Grainger *et al.* [62]. Different assays were designed to detect certain types of TGF- β 1 complexes which made some TGF- β complexes undetected by this assay. Grainger *et al.* (2000) compared the TGF- β 1 levels in the plasma of healthy subjects from 16 studies and the reported mean (or median) level ranged from less than 0.1 ng/ml to more than 25 ng/ml. Till now, there is no consensus on the plasma TGF- β 1 levels in normal humans, and no particular assay method was recommended for universal application. Although plasma TGF- β 1 was proposed as a biomarker for assessment of PE severity and significant associations were observed, heterogeneity between studies remains [25]. Thus, it may not be suitable to be introduced as a biomarker before a better assay methodology and sample preparation protocol could be developed.

To improve the comparability of results between studies, each study should provide details about the chosen assay methodology, the form of detected TGF- β 1 (active form or total), and the preparation procedure of plasma samples. Specific protocols designed to minimize contamination from platelets are available [64] and can be adopted in further studies to reduce the variation in detected TGF- β 1 levels between studies.

For individual studies in which the protocol and assay methodology should be identical in the PE group and the control group, the detected differences between groups should reflect the real differences to some degree. The plasma TGF- β 1 levels during the third trimester were significantly higher in the PE group than in the control group in all five studies, but the other two studies investigating the second trimester showed an altered association, indicating that the circulating level of TGF- β 1 during pregnancy may change in a special trend (first decreased and then elevated) in

Table 3. Main results of the pooled ORs in meta-analysis for the association between the 869 T>C polymorphism and PE.

Genetic model	Sample size		Test of heterogeneity			Test of association	
	Cases	Controls	Q	P (Q)	I ² (%) (95% CI)	OR* (95% CI)	P (Z)
TT vs. TC	364	492	3.39	0.335	11.5 (0–86)	0.740 (0.556–0.985)	0.039
TC vs. CC	341	415	2.73	0.435	0.0 (0–85)	0.993 (0.725–1.361)	0.966
TT vs. CC	227	329	1.49	0.685	0.0 (0–85)	0.695 (0.488–0.991)	0.045
TT vs. (TC+CC)	466	618	2.71	0.438	0.0 (0–85)	0.726 (0.555–0.951)	0.020
CC vs. (TT+TC)	466	618	2.52	0.471	0.0 (0–85)	1.134 (0.843–1.526)	0.406
T vs. C	466	618	0.73	0.866	0.0 (0–85)	0.849 (0.642–1.124)	0.253

*Pooled with fixed effect model.
doi:10.1371/journal.pone.0097230.t003

PE patients. If true, whether the decreased TGF- β 1 level during second trimester is responsible for the increased Th17/Tregs ratio and triggered the systemic inflammation in PE patients, and whether the elevated TGF- β 1 level during third trimester is one trigger or consequence of PE are unclear. Therefore, its role in the pathogenesis of PE remains intriguing and further research is needed to investigate the TGF- β 1 level throughout gestation, especially in first and second trimesters.

To the best of our knowledge, this is the first systematic review that evaluates the relationships of genetic variants and plasma level of TGF- β 1 with risk of PE. However, this study has some limitations. First, the number of studies included in the meta-analysis is comparably small (only four) and could not avoid publication bias. Although the genetic variants in PE have been investigated by hundreds of studies, TGF- β 1 is not a popular candidate gene since only five studies were identified after literature search. This is partly because TGF- β 1 was firstly identified as a candidate gene of PE as late as in 2007 [13] and its possible role in the pathogenesis of PE was described only recently [6]. Compared with other widely studied candidate genes (e.g. methylenetetrahydrofolate reductase gene C667T polymorphism first investigated in 1997 [65,66] and now has more than 51 repeated studies [67]), the TGF- β 1 gene is a younger and less-studied one. Although the results of our meta-analysis suggest that TGF- β 1 869 T>C polymorphism was associated with risk of PE, this result was mainly determined by the study of Kim *et al.* (2009) and Aguilar-Duran *et al.* (2013). Therefore, further studies are needed. Second, it is unsuitable to conduct a meta-analysis because of significant heterogeneity among studies on plasma TGF- β 1 level and PE risk [68]. The substantial heterogeneity is probably due to the complexity of measuring methodology of TGF- β 1 level and this can also be an obstacle for further application of TGF- β 1 as a clinical indicator. However, studies show significant differences in TGF- β 1 plasma levels between PE patients and normal pregnant women, indicating that TGF- β 1 may play a role in the pathogenesis of PE. Nevertheless, this issue should be investigated by further studies.

Conclusions

The results of this meta-analysis support that the TGF- β 1 869 T>C polymorphism is associated with the risk of PE. However, further research is needed to clarify whether this association can be detected in studies with larger sample sizes and different populations. It is difficult to conclude from the existing association studies on plasma TGF- β 1 level and PE risk owing to the between-study heterogeneity. Nevertheless, much evidence supports that the third trimester plasma TGF- β 1 level is significantly higher in PE patients compared with the control group. Further studies measuring the TGF- β 1 level at different stages of pregnancy are required to reveal the role of TGF- β 1 in the pathological development of PE.

Supporting Information

Checklist S1 PRISMA Checklist (DOC)

Author Contributions

Conceived and designed the experiments: HT XL. Performed the experiments: XL LS. Analyzed the data: XL LS. Contributed reagents/materials/analysis tools: HT XL LS. Wrote the paper: XL HT.

References

- Walker JJ (2000) Pre-eclampsia. *The Lancet* 356: 1260–1265.
- Clark SL, Belfort MA, Dildy GA, Herbst MA, Meyers JA, et al. (2008) Maternal death in the 21st century: causes, prevention, and relationship to cesarean delivery. *American journal of obstetrics and gynecology* 199: 36. e31–36. e35.
- Moser M (2001) Working group report on high blood pressure in pregnancy. *The Journal of Clinical Hypertension* 3: 75–88.
- Clark DA, Coker R (1998) Molecules in focus Transforming growth factor-beta (TGF- β). *The International Journal of Biochemistry & Cell Biology* 30: 293–298.
- Simpson H, Robson SC, Bulmer JN, Barber A, Lyall F (2002) Transforming growth factor beta expression in human placenta and placental bed during early pregnancy. *Placenta* 23: 44–58.
- Chen Q, Chen L, Liu B, Vialli C, Stone P, et al. (2010) The role of autocrine TGF β 1 in endothelial cell activation induced by phagocytosis of necrotic trophoblasts: a possible role in the pathogenesis of pre-eclampsia. *J Pathol* 221: 87–95.
- Toldi G, Rigo J, Jr., Stenczer B, Vasarhelyi B, Molvarec A (2011) Increased prevalence of IL-17-producing peripheral blood lymphocytes in pre-eclampsia. *Am J Reprod Immunol* 66: 223–229.
- Saito S (2010) Th17 cells and regulatory T cells: new light on pathophysiology of preeclampsia. *Immunology and Cell Biology* 88: 615–617.
- Kim SY, Lim JH, Park SY, Yang JH, Kim MY, et al. (2010) Transforming growth factor-beta 1 gene polymorphisms in Korean patients with pre-eclampsia. *Am J Reprod Immunol* 63: 291–298.
- Feizollahzadeh S, Taheripannah R, Khani M, Farokhi B, Amani D (2012) Promoter region polymorphisms in the transforming growth factor beta-1 (TGF β 1) gene and serum TGF β 1 concentration in preeclamptic and control Iranian women. *J Reprod Immunol* 94: 216–221.
- Wilson ML, Desmond DH, Goodwin TM, Miller DA, Ingles SA (2009) Maternal and fetal variants in the TGF-beta3 gene and risk of pregnancy-induced hypertension in a predominantly Latino population. *Am J Obstet Gynecol* 201: 295 e291–295.
- Aguilar-Duran M, Salvador-Moysén J, Galaviz-Hernandez C, Vázquez-Alaniz F, Sandoval-Carrillo AA, et al. (2014) Haplotype analysis of TGF- β 1 gene in a preeclamptic population of northern Mexico. *Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health* 4: 14–18.
- Stanczuk GA, McCoy MJ, Hutchinson IV, Sibanda EN (2007) The genetic predisposition to produce high levels of TGF-beta1 impacts on the severity of eclampsia/pre-eclampsia. *Acta Obstet Gynecol Scand* 86: 903–908.
- Daher S, Sass N, Oliveira LG, Mattar R (2006) Cytokine genotyping in preeclampsia. *Am J Reprod Immunol* 55: 130–135.
- de Lima TH, Sass N, Mattar R, Moron AF, Torloni MR, et al. (2009) Cytokine gene polymorphisms in preeclampsia and eclampsia. *Hypertens Res* 32: 565–569.
- Bell MJ, Roberts JM, Founds SA, Jayabalan A, Terhorst L, et al. (2013) Variation in endoglin pathway genes is associated with preeclampsia: a case-control candidate gene association study. *BMC Pregnancy Childbirth* 13: 82.
- Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K (2002) A comprehensive review of genetic association studies. *Genetics in Medicine* 4: 45–61.
- Cardon LR, Bell JI (2001) Association study designs for complex diseases. *Nature Reviews Genetics* 2: 91–99.
- Huber A, Hefler L, Tempfer C, Zeisler H, Lebrecht A, et al. (2002) Transforming growth factor-beta 1 serum levels in pregnancy and preeclampsia. *Acta obstetrica et gynecologica Scandinavica* 81: 168–171.
- Hennessy A, Orange S, Willis N, Painter DM, Child A, et al. (2002) Transforming growth factor-beta 1 does not relate to hypertension in preeclampsia. *Clin Exp Pharmacol Physiol* 29: 968–971.
- Muy-Rivera M, Sanchez SE, Vadachkoria S, Qiu C, Bazul V, et al. (2004) Transforming growth factor-beta1 (TGF-beta1) in plasma is associated with preeclampsia risk in Peruvian women with systemic inflammation. *Am J Hypertens* 17: 334–338.
- Naicker T, Khedun SM, Moodley J (2002) Transforming growth factor beta(1) levels in platelet depleted plasma in African women with pre-eclampsia. *J Obstet Gynaecol* 22: 279–282.
- Lim JH, Kim SY, Park SY, Lee MH, Yang JH, et al. (2009) Soluble endoglin and transforming growth factor-beta1 in women who subsequently developed preeclampsia. *Prenat Diagn* 29: 471–476.
- Peracoli MT, Menegon FT, Borges VT, de Araujo Costa RA, Thomazini-Santos IA, et al. (2008) Platelet aggregation and TGF-beta(1) plasma levels in pregnant women with preeclampsia. *J Reprod Immunol* 79: 79–84.
- Djurovic S, Schjeldet R, Wisloff F, Haugen G, Husby H, et al. (1997) Plasma concentrations of Lp(a) lipoprotein and TGF-beta1 are altered in preeclampsia. *Clin Genet* 52: 371–376.
- Benian A, Madazli R, Aksu F, Uzun H, Aydin S (2002) Plasma and Placental Levels of Interleukin-10, Transforming Growth Factor- β 1, and Epithelial-Cadherin in Preeclampsia. *Obstet Gynecol* 100: 327–331.
- Enquobahrie DA, Williams MA, Qiu C, Woelk GB, Mahomed K (2005) Maternal plasma transforming growth factor-beta1 concentrations in preeclamptic and normotensive pregnant Zimbabwean women. *J Matern Fetal Neonatal Med* 17: 343–348.
- Madazli R, Aydin S, Uludag S, Vildan O, Tolun N (2003) Maternal plasma levels of cytokines in normal and preeclamptic pregnancies and their relationship with diastolic blood pressure and fibronectin levels. *Acta Obstetrica Et Gynecologica Scandinavica* 82: 797–802.
- Szarka A, Rigó J, Lázár L, Bekó G, Molvarec A (2010) Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC immunology* 11: 59.
- Clausen T, Djurovic S, Reseland JE, Berg K, Drevon CA, et al. (2002) Altered plasma concentrations of leptin, transforming growth factor-beta(1) and plasminogen activator inhibitor type 2 at 18 weeks of gestation in women destined to develop pre-eclampsia. *Circulating markers of disturbed placentation?* *Placenta* 23: 380–385.
- Brown MA, Lindheimer MD, de Swiet M, Assche AV, Moutquin J-M (2001) The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). *Hypertension in pregnancy* 20: ix–xiv.
- Zhang C, Bao W, Rong Y, Yang H, Bowers K, et al. (2013) Genetic variants and the risk of gestational diabetes mellitus: a systematic review. *Hum Reprod Update* 19: 376–390.
- Lewis CM (2002) Genetic association studies: design, analysis and interpretation. *Briefings in bioinformatics* 3: 146–153.
- Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, et al. (2011) Basic statistical analysis in genetic case-control studies. *Nat Protoc* 6: 121–133.
- Whitehead A, Whitehead J (1991) A general parametric approach to the meta-analysis of randomized clinical trials. *Statistics in medicine* 10: 1665–1677.
- Higgins JP, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Stat Med* 21: 1539–1558.
- Thorlund K, Imberger G, Johnston BC, Walsh M, Awad T, et al. (2012) Evolution of heterogeneity (I²) estimates and their 95% confidence intervals in large meta-analyses. *PLoS One* 7: e39471.
- Assoian R, Komoriya A, Meyers CA, Miller DM, Sporn MB (1983) Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. *Journal of Biological Chemistry* 258: 7155–7160.
- Bierie B, Moses HL (2010) Transforming growth factor beta (TGF- β) and inflammation in cancer. *Cytokine & Growth Factor Reviews* 21: 49–59.
- Mantel P-Y, Schmidt-Weber CB (2011) Transforming growth factor-beta: recent advances on its role in immune tolerance. *Suppression and Regulation of Immune Responses*: Springer. 303–338.
- Dobaczewski M, Chen W, Frangogiannis NG (2011) Transforming growth factor (TGF)- β signaling in cardiac remodeling. *Journal of molecular and cellular cardiology* 51: 600–606.
- Kanasaki K, Kalluri R (2009) The biology of preeclampsia. *Kidney international* 76: 831–837.
- Lyall F, Greer IA (1994) Pre-eclampsia: a multifaceted vascular disorder of pregnancy. *Journal of hypertension* 12: 1339–1346.
- Redman CW, Sacks GP, Sargent IL (1999) Preeclampsia: an excessive maternal inflammatory response to pregnancy. *American journal of obstetrics and gynecology* 180: 499–506.
- Santner-Nanan B, Peek MJ, Khanam R, Richarts L, Zhu E, et al. (2009) Systemic Increase in the Ratio between Foxp3+ and IL-17-Producing CD4+ T Cells in Healthy Pregnancy but Not in Preeclampsia. *The Journal of Immunology* 183: 7023–7030.
- Sakaguchi S (2005) Naturally arising Foxp3-expressing CD25+ CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nature immunology* 6.
- Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. *Cell* 133: 775–787.
- Korn T, Bettelli E, Oukka M, Kuchroo VK (2009) IL-17 and Th17 Cells. *Annual review of immunology* 27: 485–517.
- Toldi G, Saito S, Shima T, Halmos A, Veresh Z, et al. (2012) The Frequency of Peripheral Blood CD4+ CD25high FoxP3+ and CD4+ CD25– FoxP3+ Regulatory T Cells in Normal Pregnancy and Pre-Eclampsia. *American Journal of Reproductive Immunology* 68: 175–180.
- Steinborn A, Haensch GM, Mahnke K, Schmitt E, Toerner A, et al. (2008) Distinct subsets of regulatory T cells during pregnancy: is the imbalance of these subsets involved in the pathogenesis of preeclampsia? *Clinical Immunology* 129: 401–412.
- Sasaki Y, Darmochwal-Kolarz D, Suzuki D, Sakai M, Ito M, et al. (2007) Proportion of peripheral blood and decidual CD4+ CD25bright regulatory T cells in pre-eclampsia. *Clinical & Experimental Immunology* 149: 139–145.
- Darmochwal-Kolarz D, Kludka-Sternik M, Tabarkiewicz J, Kolarz B, Rolinski J, et al. (2012) The predominance of Th17 lymphocytes and decreased number and function of Treg cells in preeclampsia. *Journal of reproductive immunology* 93: 75–81.
- Chen W, Jin W, Hardegen N, Lei K-j, Li L, et al. (2003) Conversion of peripheral CD4+ CD25– naive T cells to CD4+ CD25+ regulatory T cells by TGF- β induction of transcription factor Foxp3. *The Journal of experimental medicine* 198: 1875–1886.
- Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F (2007) Interleukins 1 β and 6 but not transforming growth factor- β are essential for the differentiation of interleukin 17–producing human T helper cells. *Nature immunology* 8: 942–949.

55. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, et al. (1998) Genotypic variation in the transforming growth factor-[beta] 1 gene: association with transforming growth factor-[beta] 1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation* 66: 1014–1020.
56. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, et al. (1999) Genetic control of the circulating concentration of transforming growth factor type β 1. *Human molecular genetics* 8: 93–97.
57. Syrris P, Carter ND, Metcalfe JC, Kemp PR, Grainger DJ, et al. (1998) Transforming growth factor-1 gene polymorphisms and coronary artery disease. *Clin Sci* 95: 659–667.
58. Xu HY, Hou XW, Wang LF, Wang NF, Xu J (2010) Association between transforming growth factor beta1 polymorphisms and left ventricle hypertrophy in essential hypertensive subjects. *Mol Cell Biochem* 335: 13–17.
59. He F, Zhao D, Deng F, Zhong H, Shi X, et al. (2010) Association of TGF- β 1 gene polymorphisms in exon1 and blood levels with essential hypertension. *Blood pressure* 19: 225–233.
60. Staines-Urias E, Paez MC, Doyle P, Dudbridge F, Serrano NC, et al. (2012) Genetic association studies in pre-eclampsia: systematic meta-analyses and field synopsis. *Int J Epidemiol* 41: 1764–1775.
61. Buurma AJ, Turner RJ, Driessen JH, Mooyaart AL, Schoones JW, et al. (2013) Genetic variants in pre-eclampsia: a meta-analysis. *Hum Reprod Update*.
62. Grainger DJ, Mosedale DE, Metcalfe JC (2000) TGF- β in blood: a complex problem. *Cytokine & growth factor reviews* 11: 133–145.
63. Kehrl J, Wakefield L, Roberts A, Jakowlew S, Alvarez-Mon M, et al. (1986) Production of transforming growth factor beta by human T lymphocytes and its potential role in the regulation of T cell growth. *The Journal of experimental medicine* 163: 1037–1050.
64. Wakefield LM, Letterio JJ, Chen T, Danielpour D, Allison R, et al. (1995) Transforming growth factor-beta1 circulates in normal human plasma and is unchanged in advanced metastatic breast cancer. *Clinical cancer research* 1: 129–136.
65. Sohda S, Arinami T, Hamada H, Yamada N, Hamaguchi H, et al. (1997) Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. *J Med Genet* 34: 525–526.
66. Grandone E, Margaglione M, Colaizzo D, Cappucci G, Paladini D, et al. (1997) Factor V Leiden, C>T MTHFR polymorphism and genetic susceptibility to preeclampsia. *Thromb Haemost* 77: 1052–1054.
67. Wang XM, Wu HY, Qiu XJ (2013) Methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism and risk of preeclampsia: an updated meta-analysis based on 51 studies. *Arch Med Res* 44: 159–168.
68. Higgins J, Thompson SG (2002) Quantifying heterogeneity in a meta-analysis. *Statistics in medicine* 21: 1539–1558.